Structural perspectives on activating the M4 muscarinic acetylcholine receptor

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Introduction. The M4 muscarinic acetylcholine receptor (M4R) is a therapeutically validated target for neuropsychiatric disorders such as schizophrenia. This G protein coupled receptor can be activated by several classes of ligands, including orthosteric, allosteric, and bitopic. Bitopic ligands contain two distinct pharmacophores, which are connected by a linker, allowing concomitant binding to the orthosteric and allosteric sites of the same receptor. These ligands, in theory, can stabilise distinct receptor conformations and consequently, different signalling properties. Understanding how novel bitopic ligands bind to and activate the M4R is necessary to facilitate drug discovery efforts.

Aim. Elucidate structural features of M4R activation by novel, rationally-design bitopic ligands.

Methods. We used cryo-electron microscopy to solve two active state structures of the M4R in complex with its cognate Gi1 protein, bound to distinctive bitopic ligands, MIPS4001 and MIPS4169. These compounds were synthesised in-house and contain the FDA-approved ligand, xanomeline joined to a well-characterised M4R positive allosteric modulator, MK-97, by polymethylene or polyamide linkers. We then pharmacologically validated the interactions between the M4R and these ligands through mutagenesis, whereby structurally identified residues were mutated to alanine and stably expressed in a mammalian cell system for functional experiments.

Results. The structures of the M4R bound to MIPS4001 and MIPS4169 were solved at global resolutions of 2.4 Å and 2.2 Å, respectively. These revealed, for the first time, full occupation of the canonical orthosteric and allosteric sites of the receptor by a single ligand. Whilst the conformation of the complexes are highly similar, there appears to be subtle differences in the extracellular loop regions of the receptor between the MIPS4001 and MIPS4169 bound states. We identified and validated several residues that are important in this region for ligand binding and receptor signalling through G protein activation and ERK1/2 phosphorylation assays.

Discussion. This study integrates structural biology, mutagenesis, and pharmacological characterisation to advance our mechanistic understanding of M4R activation and signalling. The validation of bitopic ligand engagement represents a novel approach in targeting the M4R and provides a foundation for future structure-guided drug design.