Structural Insights into Positive Allosteric Modulation at the M_4 muscarinic acetylcholine receptor

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Introduction. The M4 muscarinic acetylcholine receptor has emerged as a clinically validated target for the treatment of schizophrenia. There remains a major challenge in selectively activating this receptor due to the highly conserved orthosteric binding domain that is shared amongst the muscarinic receptor subtypes. A promising approach to overcome this hurdle, is to target allosteric sites of the M4R, which are spatially distinct from the orthosteric site and comprised of less-conserved residues. Understanding how positive allosteric modulators (PAMs) engage with and activate the M4R is crucial to facilitate drug discovery efforts.

Aims. Probe the structure-activity relationship of the M4R and a new generation PAM, XY6, to elucidate interactions that may underlie receptor engagement and positive allosteric modulation.

Methods. Using cryogenic electron microscopy, we determined an active state structure of the M4R in complex with its cognate Gi1 protein, and co-bound to acetylcholine and XY6 (2.4Å). We pharmacologically validated the interactions of XY6 at the M4R and determined how key residues contribute to the pharmacological profile of XY6. The structurally identified residues were replaced with alanine and stably expressed in cells lines (Flp-In CHO) for radioligand binding and BRET-based G protein activation assays.

Results. Mutations of residues Y89, F186 and W434 into alanine significantly impacted both the binding and functional abilities of XY6 at the M4R. Importantly, XY6 now represents the highest affinity M4R PAM and is the first to retain some activity at the aforementioned mutant residues.

Discussion. These findings suggest that all three aromatic residues are key contributors to XY6's allosteric modulation at the M4R. Critically, our study indicates that XY6 is less sensitive to mutagenic alterations likely due to its unique binding mode and improved properties. These exciting findings represent an important milestone in drug discovery efforts for novel PAM scaffolds, which can inform future structure-activity relationship studies to enhance the development of both tool and therapeutically beneficial M4R PAMs.