Structural and pharmacological validation of allosteric sites at the M_5 Muscarinic acetylcholine receptor – a target for CNS disorders

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G protein-coupled receptors (GPCRs) are preeminent drug targets accounting for a third of approved medicines. Despite this success, the discovery of new drugs that selectively target GPCRs has been a challenge due to many GPCRs being activated by similar types of ligands. It is now well-appreciated that GPCRs contain allosteric sites, which are binding sites that are distinct, but conformationally linked to the endogenous/orthosteric binding site. A key feature of allosteric modulators is their capacity to specifically bind to one GPCR subtype due to allosteric sites being less conserved. This ability allows them to circumvent the challenge associated with targeting the conserved orthosteric-binding site found on closely related receptors. The muscarinic acetylcholine receptors (mAChRs) are a five-membered (M_1-M_5) subfamily of Class A GPCRs that are an exemplary example of therapeutically relevant GPCRs that can be selectively targeted by allosteric ligands. The mAChR subtypes play a critical role in neurological functioning, and M_5R knockout mice suggest a physiological role in CNS disorders. Although there is strong data supporting the M_5 mAChR as a potential therapeutic target, further clinical research has been hindered due to a lack of selective drug-like molecules for the receptor. Thus, researchers have focused on finding allosteric ligands that selectivity modulate the M_5 mAChR. One such ligand is VU6007678 which preferentially binds to the M_5 mAChR and acts as a positive allosteric modulator (PAM). In our study, we report a 1.9 Å cryo-EM structure of M_5 mAChR bound to the orthosteric ligand ACh and the selective PAM VU6007678. Interestingly, VU6007678 does not bind to the prototypical mAChR allosteric site located near the extracellular surface of the receptor. Instead, the PAM binds to an allosteric site positioned near the intracellular surface and interacts with a key activation motif of the receptor. In addition, the allosteric site is located outside of the receptor transmembrane bundle at the interface with the lipid bilayer. To validate the binding site of VU6007678 we designed alanine point mutations of key interacting residues and tested the activity of VU6007678 in binding and functional assays. Overall, our study has identified a new mAChR allosteric site that may be useful for the design of selective allosteric modulators.

References:

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