Structural insights into the subtype-selective antagonist binding to the M2 muscarinic receptor

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Human muscarinic receptor, M_2 is one of the five subtypes of muscarinic receptors belonging to the family of G protein-coupled receptors. Muscarinic receptors are targets for multiple neurodegenerative diseases. The challenge has been designing subtype selective ligands against one of the five muscarinic receptors. We report high resolution structures of a thermostabilized mutant M_2 receptor bound to a subtype selective antagonist AF-DX 384 and a non-selective antagonist NMS. The thermostabilizing mutation S110R in M_2 was predicted using a theoretical strategy previously developed in our group. Comparison of the crystal structures and pharmacological properties of the M_2 receptor shows that the Arg in the S110R mutant mimics the stabilizing role of the sodium cation, that is known to allosterically stabilize inactive state(s) of class A GPCRs. Molecular Dynamics simulations reveal that tightening of the ligand-residue contacts in M_2 receptor compared to M_3 receptor leads to subtype selectivity of AF-DX 384.