# Allosteric Modulation of Muscarinic Acetylcholine Receptors

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**Abstract:** Muscarinic acetylcholine receptors (mAChRs) are prototypical Family A G protein coupled-receptors. The five mAChR subtypes are widespread throughout the periphery and the central nervous system and, accordingly, are widely involved in a variety of both physiological and pathophysiological processes. There currently remains an unmet need for better therapeutic agents that can selectively target a given mAChR subtype to the relative exclusion of others. The main reason for the lack of such selective mAChR ligands is the high sequence homology within the acetylcholine-binding site (orthosteric site) across all mAChRs. However, the mAChRs possess at least one, and likely two, extracellular allosteric binding sites that can recognize small molecule allosteric modulators to regulate the binding and function of orthosteric ligands. Extensive studies of prototypical mAChR modulators, such as gallamine and alcuronium, have provided strong pharmacological evidence, and associated structure-activity relationships (SAR), for a "common" allosteric site on all five mAChRs. These studies are also supported by mutagenesis experiments implicating the second extracellular loop and the interface between the third extracellular loop and the top of transmembrane domain 7 as contributing to the common allosteric site. Other studies are also delineating the pharmacology of a second allosteric site, recognized by compounds such as staurosporine. In addition, allosteric agonists, such as McN-A-343, AC-42 and *N*-desmethylclozapine, have also been identified. Current challenges to the field include the ability to effectively detect and validate allosteric mechanisms, and to quantify allosteric effects on binding affinity and signaling efficacy to inform allosteric modulator SAR.

**Key Words:** Acetylcholine, allosteric interaction, G protein-coupled receptor, molecular modeling, muscarinic acetylcholine receptor, mutagenesis, radioligand binding, structure-activity studies, ternary complex model.

## INTRODUCTION

G protein-coupled receptors (GPCRs) account for 1 - 3% of the human genome, are abundantly expressed throughout the central nervous system (CNS) and periphery, and represent the major targets for approximately 30% of all medicines on the world market. However, current CNS-based GPCR drug discovery has a higher than average attrition rate with respect to translating fundamental research to the clinic [41]; this is likely due to two reasons, namely, an insufficient mechanistic understanding of the complexities of CNS GPCR-mediated signaling and a lack of selective pharmacological tools for targeting therapeutically relevant GPCRs. As a consequence there are many GPCR-based drug discovery programs aiming to develop more selective compounds, both as tools to probe GPCR biology and also as potential therapeutic leads. The traditional approach to GPCR-based drug discovery has been to focus on targeting that region of the receptor utilized by the receptor's endogenous ligand, i.e., the "orthosteric" site [80]. However, it is now recognized that GPCRs possess topographically distinct, allosteric binding sites, and that ligands that bind to these sites (allosteric modulators) offer tremendous potential for more selective and/or effective therapies than conventional orthosteric ligands. This brief review will focus on one of the best-studied families of GPCRs with respect to the phenomenon of allosteric modulation, namely, the muscarinic acetylcholine receptors.

## MUSCARINIC ACETYLCHOLINE RECEPTORS (mAChRs): A BRIEF OVERVIEW

The mAChRs belong to the Family A (rhodopsin-like) subclass of GPCRs. Pharmacological and genetic studies have identified five distinct mAChR subtypes, classed $M_1$-$M_5$. The $M_1$, $M_3$ and $M_5$ subtypes preferentially couple to the $G_{q/11}$ family of G proteins, resulting in phospholipase C activation, hydrolysis of inositol phosphates and mobilization of intracellular $Ca^{++}$ stores. In contrast, the $M_2$ and $M_4$ subtypes preferentially couple to the pertussis toxin-sensitive $G_{i/o}$ family of G proteins, resulting in the inhibition of adenylyl cyclase and subsequent cAMP formation. Although these generalizations speak to the best-characterized signaling pathways associated with the mAChRs, they should by no means be taken as absolutes. All five mAChR subtypes are known to couple promiscuously to multiple G proteins, usually in a cell background dependent manner, and have been linked to additional intracellular pathways, including activation of mitogen activated protein kinases, Rho GTPases, nitric oxide synthases, multiple phospholipases, and the modulation of a variety of potassium, calcium and chloride ion channels [58].

The mAChRs are widely distributed throughout the periphery and the CNS. Activation of peripheral mAChRs leads to increases in exocrine secretion, contraction of cardiac and smooth muscle (gastrointestinal tract and lungs), and reduced heart rate. Within the CNS, a far more complex array of physiological behaviors is thought to be mediated by the mAChRs, depending on their distribution and localization [13]. $M_1$ mAChRs are predominantly expressed post-synaptically in forebrain regions including the cerebral cortex, hippocampus and striatum [68, 69, 76, 80, 88]. These receptors have long been associated with cognitive deficits linked to neurodegenerative disorders, such as Alzheimer's disease, and as such selective agonists of the $M_1$ mAChR have been pursued as a potential avenue for treatment of dementia-related conditions [32]. The $M_2$ mAChR is located pre-synaptically on both cholinergic and non-cholinergic neurons [30, 88] in the brainstem, hypothalamus/thalamus, hippocampus, striatum and cortex [68, 69, 80], and generally serves an inhibitory function on the release of neurotransmitters. It has been suggested that enhancing synaptic ACh levels by selectively inhibiting $M_2$ autoreceptors may be beneficial in the treatment of psychosis and Alzheimer's disease, and an attractive alternative to the currently used cholinesterase inhibitors for the latter disorder [20]. $M_3$ mAChRs are expressed at relatively low levels in a number of regions including the cortex, striatum, hippocampus, hypothalamus/thalamus. These receptors have been particularly associated with appetite regulation, and the $M_3$ receptor is currently a potential target for treatment of obesity and other metabolic disorders [7, 34, 69, 109]. $M_4$ mAChRs are predominantly found presynaptically in the striatum, hippocampus, cortex and hypothalamus/thalamus [9, 69, 80]. There is the potential that $M_4$ mAChR selective antagonists may control tremor associated with Parkinson's disease, whilst agonists may be developed as analgesics, due to the regulation of neurotransmitter release in both cholinergic and non-cholinergic neurons [23, 113], and as novel antipsychotics, due to regulation of the dopaminergic system [1, 91]. Finally, $M_5$ mAChRs are discretely expressed at low levels in the brain, in particular in the ventral tegmental area [103, 110] as well as co-localised with $D_2$ dopamine receptors in the substantia nigra pars compacta [107]. They are also implicated in the control of vasodilatation of cerebral blood vessels [108]. $M_5$ mAChRs are associated with slow activation of dopaminergic neurons and sub-

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