

# Acetylcholine Receptors (Muscarinic)

## Key References

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## Overview

Muscarinic acetylcholine receptors are members of the superfamily of G protein-coupled receptors (GPCRs). They are relatively abundant and mediate the diverse actions of acetylcholine in the CNS, as well as throughout non-nervous tissues innervated by the parasympathetic nervous system. Five genes (m1-m5) encode muscarinic receptor proteins exhibiting the rhodopsin-like structural motif containing seven transmembrane domains. They show strong sequence homology with each other and with related GPCRs within the transmembrane spanning domains, but each receptor also has unique amino acid sequences located at the amino end (extracellular), and in the third intracellular loop (I3). As with other biogenic amine receptors, much of the effector coupling specificity of these receptors resides in their intracellular domains, in particular the I3 loop.

The five muscarinic receptor subtypes are referred to as M<sub>1</sub>-M<sub>5</sub>. The odd-numbered receptors (M<sub>1</sub>, M<sub>3</sub>, M<sub>5</sub>) couple efficiently, through G<sub>q/11</sub>, to activate phospholipase C, which initiates the phosphatidylinositol turnover response. This leads to inositol trisphosphate-mediated release of calcium from the endoplasmic reticulum and to diacylglycerol-mediated activation of protein kinase C. As a consequence, depending on cell type, other cellular effectors may become activated subsequent to the stimulation of phosphatidylinositol turnover. In smooth muscle, muscarinic receptor (M<sub>3</sub>) activation of the phosphatidylinositol turnover response leads to elevation in cellular calcium and contraction. In glandular tissue, M<sub>3</sub> receptor-mediated phosphatidylinositol turnover leads to hormone secretion. In brain, activation of post-synaptic

M<sub>1</sub> or M<sub>3</sub> receptors often mediates "slow" neuronal excitability. One mechanism for this involves inhibition of calcium-regulated potassium channels, and this leads to an inhibition of the after-hyperpolarization phase of the neuronal action potential. Stimulation of post-synaptic muscarinic receptors themselves may not directly lead to action potentials, but commonly the activation of these muscarinic receptors enhances the neuron's response to excitatory input (this is sometimes called "neuromodulation"). Cortical and hippocampal muscarinic receptors transduce cholinergic input from the basal forebrain, in circuits believed to be important in the attentional aspects of cognition. In these brain areas, the predominant receptor subtypes are M<sub>1</sub>, M<sub>3</sub> and M<sub>4</sub>. The striatum also expresses a mixture of muscarinic receptor subtypes, but the M<sub>4</sub> subtype predominates. The M<sub>2</sub> subtype is expressed at low levels in the telencephalon, most notably in the basal forebrain, and at relatively higher levels in the brainstem. The M<sub>5</sub> subtype is expressed in brain at very low levels with a limited distribution.

The even-numbered muscarinic receptors (M<sub>2</sub>, M<sub>4</sub>) inhibit adenylyl cyclase activity via activation of the G<sub>i</sub> class of G proteins. The M<sub>2</sub> and M<sub>4</sub> muscarinic receptors also activate G protein-coupled potassium channels, which leads to hyperpolarization of the plasma membrane of excitable cells. In nervous tissue, M<sub>2</sub> and M<sub>4</sub> receptors appear to frequently inhibit neuronal firing, and one or both of these subtypes are found on axon terminals where they inhibit neurotransmitter release (autoreceptors or heteroreceptors). The M<sub>2</sub> muscarinic receptor inhibits adenylyl cyclase in smooth muscle and, as a consequence, opposes

the effects of adrenergic innervation. In cardiac tissue, M<sub>2</sub> muscarinic receptors activate G protein-coupled potassium channels to hyperpolarize the muscle, contributing to the slowing of the heart rate.

Recent developments in muscarinic receptor biology include advances in the study of allosterism and in the coupling of these receptors to phosphokinase pathways. It has been known for decades that certain drugs can inhibit agonist action via sites other than the agonist binding site. Recently, the ability of some allosteric agents (e.g. brucine) to potentiate agonist action at muscarinic receptors has been demonstrated. This is analogous to the use of benzodiazepines to potentiate chloride flux in response to GABA at the GABA<sub>A</sub> receptor. This discovery suggests that new therapeutic approaches involving muscarinic receptor stimulation may soon be possible.

Besides their coupling to the well-established phosphatidylinositol and cyclic AMP effector systems, it is becoming increasingly clear that muscarinic receptors also couple to, or intersect with, signaling pathways which involve sequential activation of serine/threonine protein kinases, from which modulation of gene expression can result. For example, muscarinic receptors can activate certain MAP kinase pathways. Some components of the phosphokinase pathways that could conceivably be modulated by muscarinic receptors *in vivo* have the potential to enhance cell survival by upregulation of certain protection systems and/or blockade of apoptosis, or modulation of learning and memory.

## Acetylcholine Receptors (Muscarinic)

CURRENTLY ACCEPTED NAME	M <sub>1</sub> ( <a href="#">M-194</a> )	M <sub>2</sub> ( <a href="#">M 4560</a> )	M <sub>3</sub> ( <a href="#">M-176</a> )	M <sub>4</sub> ( <a href="#">M 4810</a> )	M <sub>5</sub> ( <a href="#">M-179</a> )
MOLECULAR BIOLOGY CLASSIFICATION	m1	m2	m3	m4	m5
STRUCTURAL INFORMATION	460 aa (human)	466 aa (human)	590 aa (human)	479 aa (human)	532 aa (human)
SUBTYPE SELECTIVE AGONISTS <sup>a</sup>	McN-A-343 ( <a href="#">C 7041</a> ) (ganglion) Pilocarpine ( <a href="#">P 6503</a> ) (relative to M <sub>3</sub> and M <sub>5</sub> ) L-689,660 Xanomeline CDD-0097 ( <a href="#">C-258</a> )	Bethanechol ( <a href="#">C 5259</a> ) (relative to M <sub>4</sub> )	L-689,660	McN-A-343 ( <a href="#">C 7041</a> ) (relative to M <sub>2</sub> )	None known
SUBTYPE SELECTIVE ANTAGONISTS <sup>a</sup>	Pirenzepine ( <a href="#">P 7412</a> ) Telenzepine ( <a href="#">T-122</a> )	Methoctramine ( <a href="#">M-105</a> ) AF-DX 116 AF-DX 384 Gallamine ( <a href="#">G 8134</a> ) (non-competitive) Himbacine ( <a href="#">H 4904</a> ) Triptiramine	Hexahydro-sila-difenidol <i>p</i> -Fluorohexahydro-sila-difenidol ( <a href="#">H-127</a> ) 4-DAMP ( <a href="#">D-104</a> )	Tropicamide ( <a href="#">T 9778</a> ) Himbacine ( <a href="#">H 4904</a> ) AF-DX 384	None known
RECEPTOR SELECTIVE AGONISTS	Bethanechol ( <a href="#">C 5259</a> ) Metoclopramide ( <a href="#">M 0763</a> ) Muscarine ( <a href="#">M 6532</a> ) Pilocarpine ( <a href="#">P 6503</a> ) Oxotremorine M ( <a href="#">O-100</a> )	Bethanechol ( <a href="#">C 5259</a> ) Metoclopramide ( <a href="#">M 0763</a> ) Muscarine ( <a href="#">M 6532</a> ) Pilocarpine ( <a href="#">P 6503</a> ) Oxotremorine M ( <a href="#">O-100</a> )	Bethanechol ( <a href="#">C 5259</a> ) Metoclopramide ( <a href="#">M 0763</a> ) Muscarine ( <a href="#">M 6532</a> ) Pilocarpine ( <a href="#">P 6503</a> ) Oxotremorine M ( <a href="#">O-100</a> )	Bethanechol ( <a href="#">C 5259</a> ) Metoclopramide ( <a href="#">M 0763</a> ) Muscarine ( <a href="#">M 6532</a> ) Pilocarpine ( <a href="#">P 6503</a> ) Oxotremorine M ( <a href="#">O-100</a> )	Bethanechol ( <a href="#">C 5259</a> ) Metoclopramide ( <a href="#">M 0763</a> ) Muscarine ( <a href="#">M 6532</a> ) Pilocarpine ( <a href="#">P 6503</a> ) Oxotremorine M ( <a href="#">O-100</a> )
RECEPTOR SELECTIVE ANTAGONISTS	Scopolamine ( <a href="#">S 1875</a> ) QNB, (±) ( <a href="#">C-002</a> ) QNB, R(-) ( <a href="#">C-003</a> ) Atropine ( <a href="#">A 0257</a> )	Scopolamine ( <a href="#">S 1875</a> ) QNB, (±) ( <a href="#">C-002</a> ) QNB, R(-) ( <a href="#">C-003</a> ) Atropine ( <a href="#">A 0257</a> )	Scopolamine ( <a href="#">S 1875</a> ) QNB, (±) ( <a href="#">C-002</a> ) QNB, R(-) ( <a href="#">C-003</a> ) Atropine ( <a href="#">A 0257</a> )	Scopolamine ( <a href="#">S 1875</a> ) QNB, (±) ( <a href="#">C-002</a> ) QNB, R(-) ( <a href="#">C-003</a> ) Atropine ( <a href="#">A 0257</a> )	Scopolamine ( <a href="#">S 1875</a> ) QNB, (±) ( <a href="#">C-002</a> ) QNB, R(-) ( <a href="#">C-003</a> ) Atropine ( <a href="#">A 0257</a> )
SIGNAL TRANSDUCTION MECHANISMS	G <sub>q/11</sub> (increase IP <sub>3</sub> /DAG) NO	G <sub>i</sub> (cAMP modulation) ↑K <sup>+</sup> (G)	G <sub>q/11</sub> (increase IP <sub>3</sub> /DAG) NO	G <sub>i</sub> (cAMP modulation) ↑K <sup>+</sup> (G)	G <sub>q/11</sub> (increase IP <sub>3</sub> /DAG) NO
RADIOLIGANDS OF CHOICE	[ <sup>3</sup> H]-Pirenzepine [ <sup>3</sup> H]-Telenzepine [ <sup>3</sup> H]-QNB	[ <sup>3</sup> H]-AF-DX 384 [ <sup>3</sup> H]-QNB	[ <sup>3</sup> H]-4-DAMP [ <sup>3</sup> H]-QNB	[ <sup>3</sup> H]-AF-DX 384 [ <sup>3</sup> H]-QNB	[ <sup>3</sup> H]-QNB [ <sup>3</sup> H]-NMS

### Abbreviations

**AF-DX 116:** 11-((2-((Diethylamino)methyl)-1-piperidinyl)acetyl)-5,11-dihydro-6-pyrido[2,3-b][1,4]benzodiazepin-6-one

**AF-DX 384:** 5,11-Dihydro-11-[2-[2-((N,N-dipropylaminomethyl)piperidin-1-yl)ethylamino]-carbonyl]6H-pyrido[2,3-b][1,4]benzodiazepin-6-one

**CDD-0097:** 5-Propargyloxycarbonyl-1,4,5,6-tetrahydropyrimidine

**4-DAMP:** 4-Diphenylacetoxy-N-methylpiperidine methiodide

**L-689,660:** 1-Azabicyclo[2,2,2]octane,3-(6-chloropyrazinyl)maleate

**McN-A-343:** 4-(N-[3-Chlorophenyl]carbamoxyloxy)-2-butynyltrimethylammonium chloride

**NMS:** N-Methylscopolamine

**QNB:** Quinuclidinyl- $\alpha$ -hydroxydiphenylacetate; Quinuclidinylbenzylate

### FOOTNOTES

<sup>a</sup> Absolute selectivity for any muscarinic agent has so far not been achieved. The compounds listed are relatively, but not absolutely selective. Variations in their potencies/affinities may occur as a result of numerous factors, including tissue/species differences, variations in receptor densities and differences in the receptor/effector coupling efficiency. The reader is directed to the literature for detailed information concerning the pharmacological specificity of these compounds.