



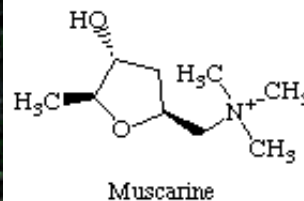
# MBC 3320 Acetylcholine

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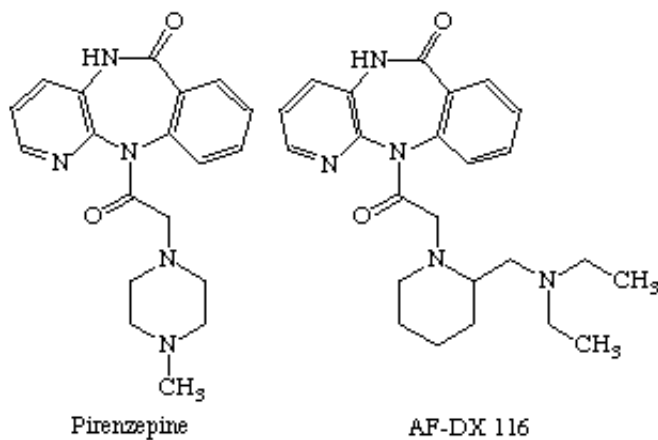
## Muscarinic receptors

Acetylcholine and carbamylcholine can bind to both muscarinic and nicotinic receptors, yet the responses elicited by activating each receptor differ in several ways. Muscarinic responses are slower, may produce excitation or inhibition and involve second messenger systems, rather than the direct opening of an ion channel. Muscarinic receptors are G protein-coupled receptors and mediate their responses by activating a cascade of intracellular pathways. Muscarine is the prototypical muscarinic agonist and derives from the fly agaric mushroom *Amanita muscaria*. Like acetylcholine, muscarine contains a quaternary nitrogen important for action at the anionic site of the receptor (an aspartate residue in transmembrane domain III). Most muscarinic agonists obey the "rule of five" atoms from the quaternary ammonium moiety to the terminal atom.



Muscarinic receptors are found in the parasympathetic nervous system. Muscarinic receptors in smooth muscle regulate cardiac contractions, gut motility and bronchial constriction. Muscarinic receptors in exocrine glands stimulate gastric acid secretion, salivation and lacrimation. Muscarinic receptors also are found in the superior cervical ganglion where they can produce at least two physiologically distinct responses. In addition, muscarinic receptors are found throughout the brain, including the cerebral cortex, the striatum, the hippocampus, thalamus and brainstem.

In general the classical muscarinic antagonists such as atropine recognize a single class of binding sites as determined in binding assays. In the 1980's, several selective muscarinic antagonists were identified. Pirenzepine was very useful in the characterization of M<sub>1</sub> muscarinic receptors, while AF-DX 116 was used to identify M<sub>2</sub> receptors in the heart. M<sub>3</sub> receptors are found in smooth muscle and in both exocrine glands (e.g., lacrimal glands) and endocrine glands (e.g., pancreas). Muscarinic agonists bind heterogeneously to receptors in both the brain and peripheral nervous system.



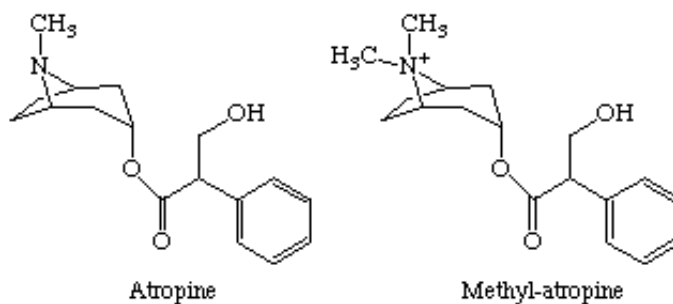
In the late 1980's, molecular cloning techniques identified five different subtypes of muscarinic receptors. Each receptor shares common features including specificity of binding for the agonists acetylcholine and carbamylcholine and the classical antagonists atropine and quinuclidinyl benzilate. Each receptor subtype couples to a second messenger system through an intervening G-protein.  $M_1$ ,  $M_3$  and  $M_5$  receptors stimulate phosphoinositide metabolism while  $M_2$  and  $M_4$  receptors inhibit adenylate cyclase. The tissue distribution differs for each subtype.  $M_1$  receptors are found in the forebrain, especially in the hippocampus and cerebral cortex.  $M_2$  receptors are found in the heart and brainstem while  $M_3$  receptors are found in smooth muscle, exocrine glands and the cerebral cortex.  $M_4$  receptors are found in the neostriatum and  $M_5$  receptor mRNA is found in the substantia nigra, suggesting that  $M_5$  receptors may regulate dopamine release at terminals within the striatum. The structural requirements for activation of each subtype remain to be elucidated.

### Muscarinic Acetylcholine Receptors

	$M_1$	$M_2$	$M_3$	$M_4$	$M_5$
Distribution	Cortex, hippocampus	Heart	Exocrine glands, GI tract	Neostriatum	Substantia nigra
Antagonists	Pirenzepine	AF-DX 116	pF-HHSiD		
Agonists	Xanomeline, CDD-0097				
G protein	$G_{\alpha q/11}$	$G_{\alpha i/o}$	$G_{\alpha q/11}$	$G_{\alpha i/o}$	$G_{\alpha q/11}$
Intracellular response	Phospholipase $C\beta$	Adenylyl cyclase inhibition	Phospholipase $C\beta$	Adenylyl cyclase inhibition	Phospholipase $C\beta$

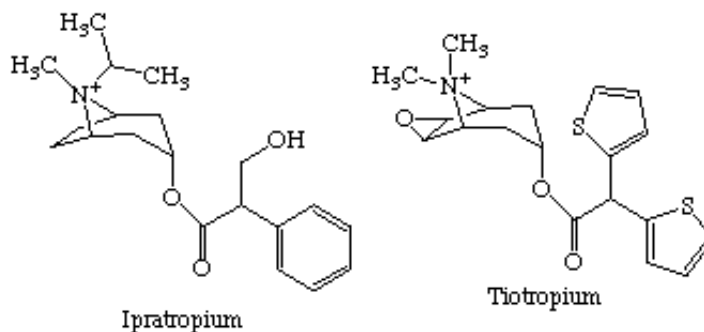
### Muscarinic antagonists

Muscarinic antagonists such as scopolamine and atropine are among the oldest known molecules, originally derived from natural sources. They are both alkaloids (natural, nitrogenous organic bases, usually containing tertiary amines) from the nightshade plant *Atropa belladonna*. The presence of an N-methyl group on atropine or scopolamine changes the activity of the ligand, possibly by preventing a close interaction between the ligand and the membrane or lipophilic sites on the receptor. The methyl group also prevents the penetration into the brain.



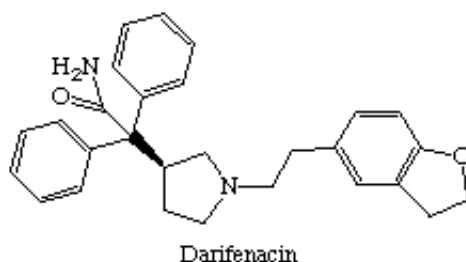
The potent anticholinergics are used to control the secretion of saliva and gastric acid, slow gut motility, and prevent vomiting. They also have a limited therapeutic use for the treatment of Parkinson's disease. In large doses however, the muscarinic antagonists with tertiary amines have severe central effects, including hallucinations and memory disturbances.

In recent years, the quaternary muscarinic antagonist ipratropium has been used in the treatment of chronically obstructed pulmonary disorder as an adjunct to  $\beta_2$  agonist therapy.  $M_3$  muscarinic receptors mediate bronchoconstriction in the airways. Muscarinic antagonists such as ipratropium and the long-lasting tiotropium are effective bronchodilators.



The possible use of presynaptic antagonists to increase acetylcholine levels has attracted some attention recently. Muscarinic autoreceptors resemble pharmacologically the  $M_2$  receptor found in the heart.  $M_2$  antagonists enhance acetylcholine release by blocking the feedback inhibition produced by the action of acetylcholine on presynaptic terminals.

$M_2$  antagonists also may be useful in the treatment of urinary incontinence. Darifenacin is an  $M_2$  antagonist that promotes urinary retention.

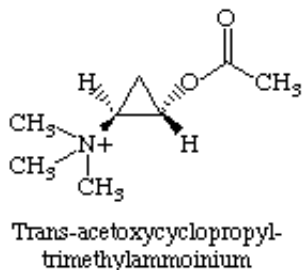


## Muscarinic agonists

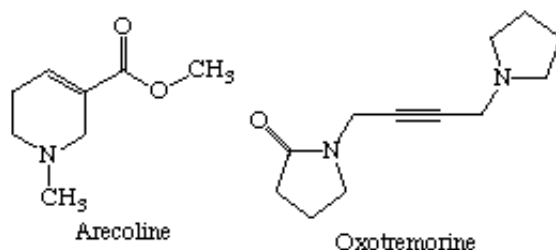
The ability for the quaternary ammonium group to fit into an anionic site on muscarinic receptors may be an important factor for the binding of a ligand to muscarinic receptors. For an example of the requirement of the

quaternary amine moiety, consider that dimethylaminoethylacetate (the tertiary form of acetylcholine) is 1000-fold less than acetylcholine, in part due to a lower affinity for the receptor.

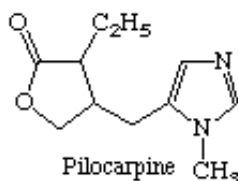
The molecule of acetylcholine is flexible and may form an infinite number of conformations from the extended to the quasi-ring structure. The three-membered ring of acetoxypropyl-trimethylammonium iodide demonstrates the concept that the extended form of acetylcholine contains the highest intrinsic activity. The trans isomer has much higher activity than the cis isomer which orients the ester and the quaternary amine together.



While the quaternary nitrogen is essential for eliciting full muscarinic responses with muscarinic agonists, there are a few potent muscarinic agents which contain tertiary amines (e.g., arecoline, oxotremorine and pilocarpine). They are potent both peripherally and centrally although they are of limited therapeutic value because of the wide range of cholinergic responses that they elicit. Oxotremorine is of interest because of its ability to produce tremors, thereby providing an early model for Parkinson's disease.



Simple tertiary amines do not show considerable potency for the receptor, but this can be counteracted if the rest of the molecule binds potently to the receptor (e.g., through an ester bioisostere). Oxotremorine fills this role with an amide group in a pyrrolidone ring as the nitrogen replaces oxygen in a hydrogen bond acceptor role. Arecoline (isolated originally from the betel nut) has a reversed ester acetylcholine profile, while pilocarpine has its ester in the cyclic form of a lactam ring, which may help increase the binding interaction. In general, it is important to have two sites for hydrogen bond acceptance in the ester isostere. The orientation of the ester isostere may be important for selective action as well.



The events associated with G protein-coupled receptor activation are as follows.

1. Agonist binds to the receptor, which has a high affinity for agonists at rest.
2. The binding of the agonist stabilizes a receptor conformation promotes receptor/ G protein coupling and allows GTP to exchange for GDP on the G protein  $\alpha$  subunit.

3. The binding of GTP leads to the dissociation of the G protein from the receptor, thereby lowering agonist affinity. The agonist then dissociates from the activated receptor.
  4. The G protein consists of three subunits ( $\alpha$ ,  $\beta$ , and  $\gamma$ ) which also dissociate. The  $\alpha$  subunit activates the appropriate second messenger system (e.g., phospholipase C for  $M_1$  receptors). The  $\beta$  and  $\gamma$  subunits can exert independent actions.
  5. The  $\alpha$  subunit is inactivated by the hydrolysis of GTP to form GDP by a GTPase intrinsic to the G protein (GTPase activity may be activated by other intracellular proteins called GTPase activating proteins [GAPs]).
  6. The  $\alpha$  subunit (with GDP bound) can then recombine with the  $\beta$  and  $\gamma$  subunits. The receptor is then in a high affinity state and ready for the binding of another agonist.
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## Alzheimer's disease

Alzheimer's disease is characterized by amyloid plaques and neurofibrillary tangles. Amyloid plaques contain deposits of  $\beta$ -amyloid, which is a 40-42 amino acid peptide derived from amyloid precursor protein.

Neurofibrillary tangles contain a hyperphosphorylated  $\tau$  protein, which forms paired helical filaments. Alzheimer's disease is associated with a loss of cholinergic neurons which project from the basal forebrain to the cerebral cortex and the hippocampus. The loss of cholinergic neurons is progressive and results in profound memory disturbances and irreversible impairment of cognitive function.

The cause of Alzheimer's disease is unknown, yet several genes and gene products (proteins) have been implicated.

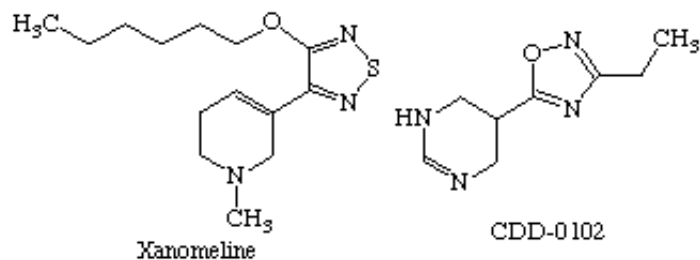
- Mutations in APP (a small percentage of all Alzheimer's patients)
  - Presenilin mutations (may promote the formation of  $\beta$ -amyloid)
  - Apolipoprotein E allele (E4 is associated with an increased risk of Alzheimer's disease)
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## Therapeutic approaches

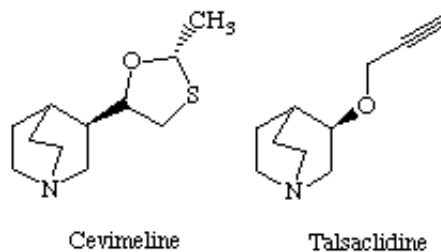
Amyloid precursor protein (APP) is a large protein that can be cleaved at three sites by the enzymes  $\alpha$ -,  $\beta$ - and  $\gamma$ -secretase. Cleavage at both the  $\beta$ - and  $\gamma$ -secretase sites leads to the formation of  $\beta$ -amyloid. Inhibitors of  $\beta$ - and  $\gamma$ -secretase might be useful in the treatment of Alzheimer's disease. Muscarinic agonists may prevent  $\beta$ -amyloid formation by activating  $\alpha$ -secretase. A vaccine (targeting  $\beta$ -amyloid) also is under development for Alzheimer's disease.

Recent efforts have focused on the development of centrally active muscarinic receptor agonists for the treatment of Alzheimer's disease. The rationale for therapy involves replacement of acetylcholine, which is depleted in Alzheimer's patients as the basal forebrain neurons degenerate. An ideal candidate for a drug would have several features including high CNS penetrance, high efficacy and selectivity for forebrain receptors and a low incidence of side effects.

The muscarinic agonist xanomeline is an arecoline derivative with very high affinity and selectivity for  $M_1$  muscarinic receptors. It contains a 1,2,5-thiadiazole ring, which is more stable than the ester found in arecoline. Talsaclidine is a quinuclidine derivative under development. In CDD-0102 a 1,2,4-oxadiazole moiety serves as a suitable ester isostere.



The muscarinic agonist cevimeline is a quinuclidine derivative useful in the treatment of xerostomia (dry mouth) associated with Sjogren's disease. Talsaclidine is another quinuclidine derivative under development for the treatment of Alzheimer's disease. Both compounds have shown some clinical utility in promoting the clearance of  $\beta$ -amyloid from the cerebrospinal fluid of Alzheimer's disease patients.



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## References

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2. The RBI Handbook of Receptor Classification and Signal Transduction. K.J. Watling. RBI. Third Edition, 1998.

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