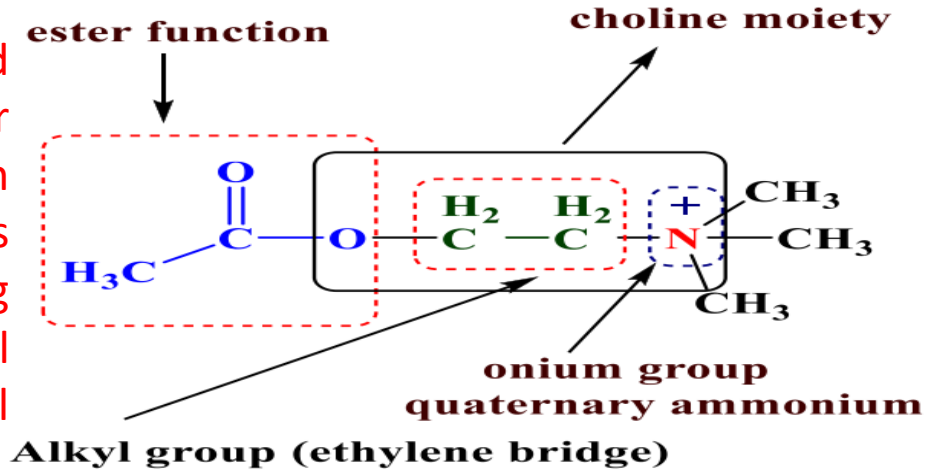
Structure-Activity Relationships (SAR)

1- Direct acting agonists
2- Indirect-acting agonists

Design of Cholinergic Agonists: Structural Modification of Acetylcholine. Alterations on the molecule may be divided into four categories:

Although muscarinic receptors have been cloned and the amino acid sequences are known, their three-dimensional structures remain unresolved. Thus, it is not possible to use this information alone to design specific drug molecules. Scientists still use pharmacological and biochemical tests to determine optimal structural requirements for activity.



The onium group is essential for intrinsic activity and contributes to the affinity of the molecule for the receptors, partially through the binding energy and partially because of its action as a detecting and directing group.

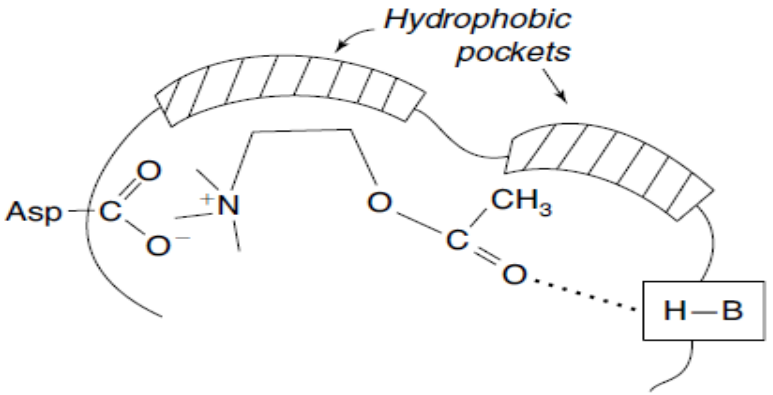
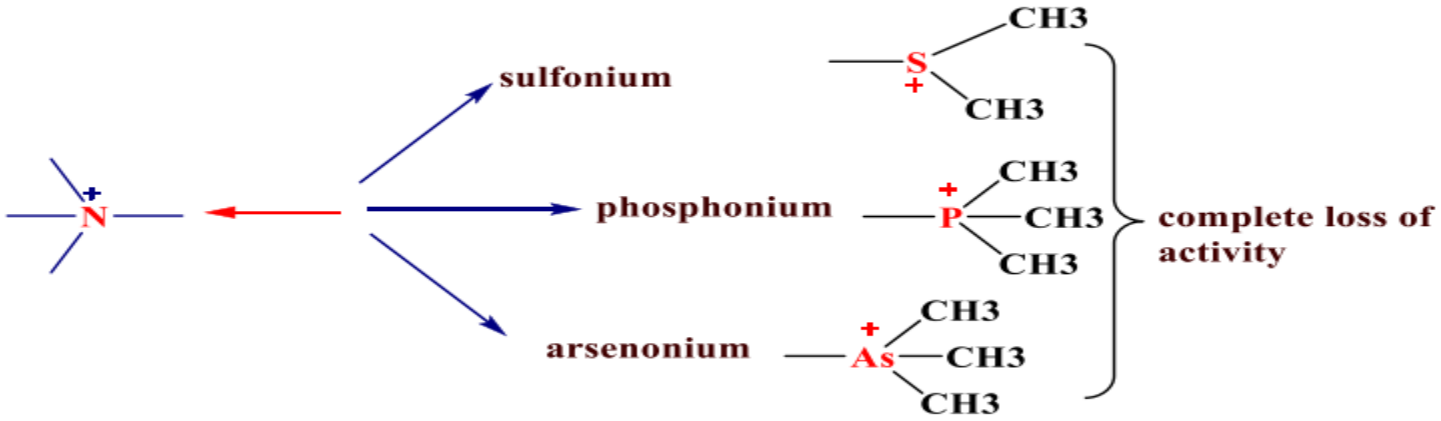
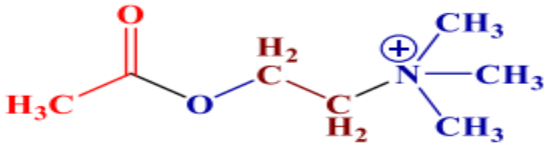


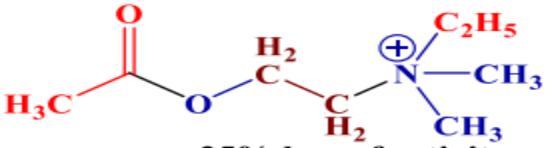
Figure 17.11 ● Hypothetical structure of the muscarinic receptor.

Molecular modeling data show the binding site to be a negatively charged aspartic acid residue in the third of the seven transmembrane helixes of the muscarinic receptor. Hydrophobic pockets are located in helices 4, 5, 6, and 7 of the muscarinic receptor

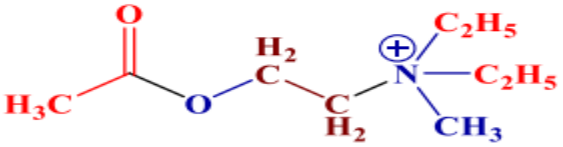
Ammonium Group(-$N^+(CH_3)_3$)

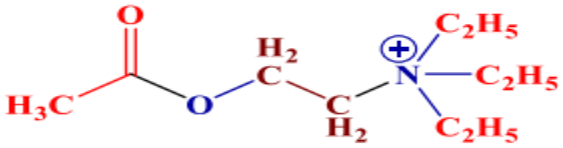




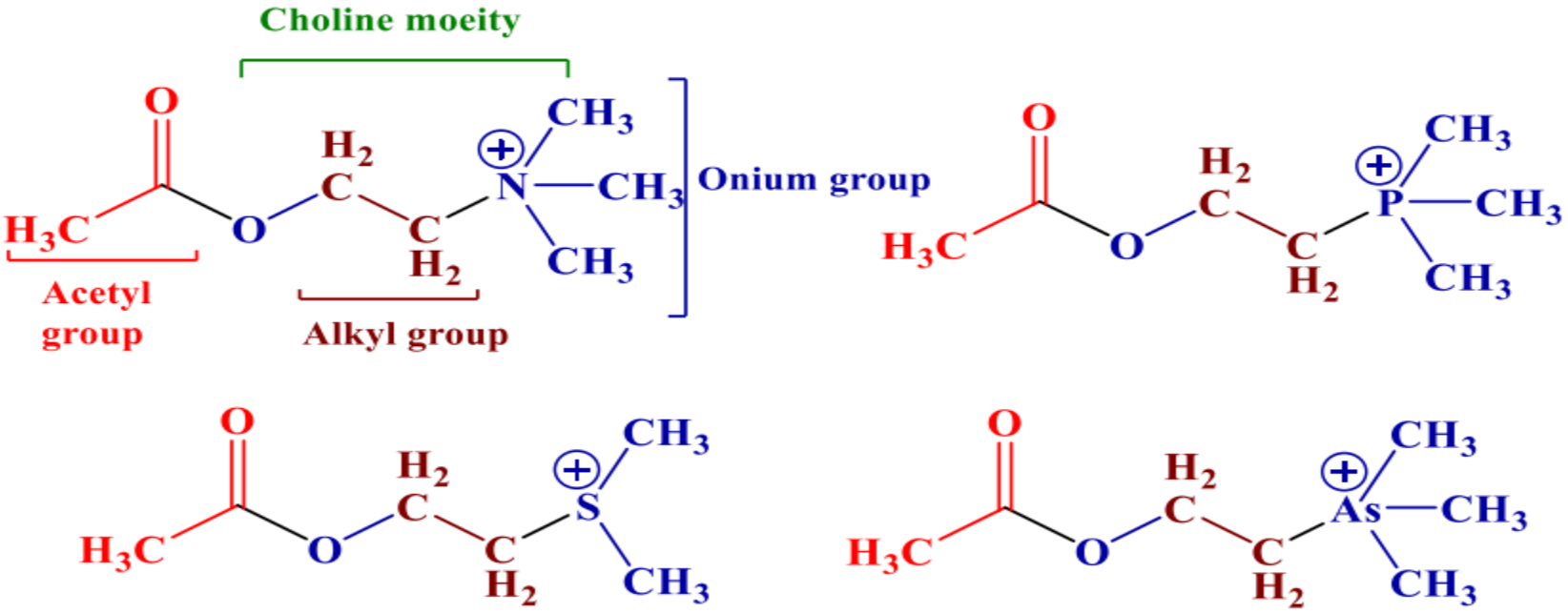


25% loss of activity





complete loss of all activity

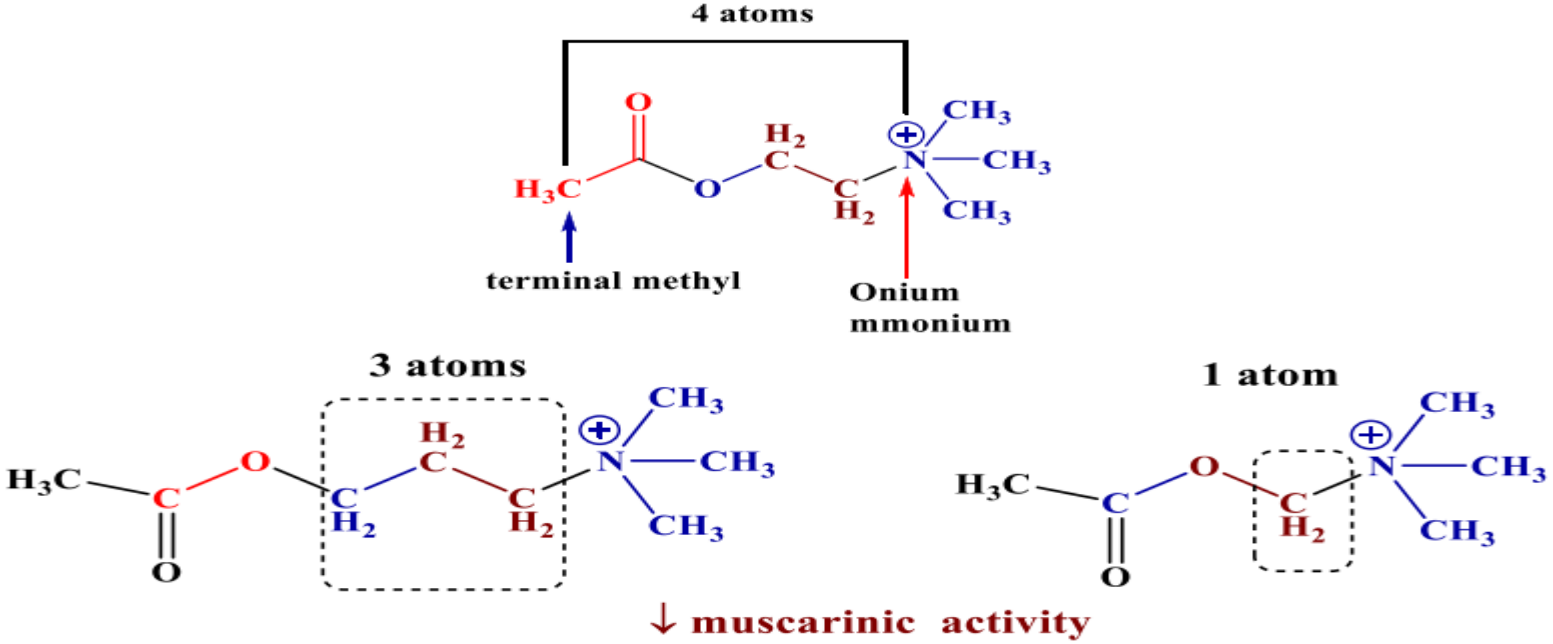

Choline moeity
Onium group
Acetyl group
Alkyl group

Phosphonium, sulfonium, arsenonium isosteres, or substituents larger than methyl on the nitrogen increase the size of the onium moiety, produce diffusion of the positive charge, and interfere sterically with proper drug–receptor interaction, resulting in decreased activity

The ester group in ACh contributes to the binding of the compound to the muscarinic receptor because of hydrogen bond formation with threonine and asparagine residues at the receptor site

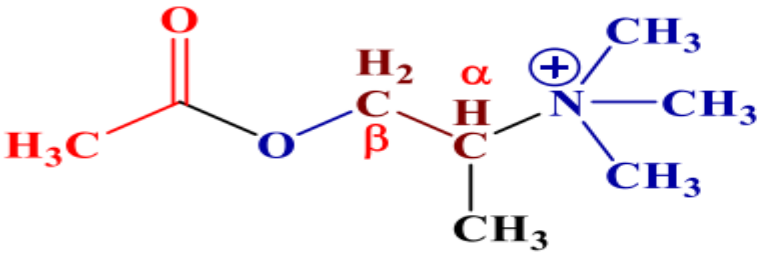
The presence of the acetyl group in ACh is not as critical as the size of the molecule.

2-Ethylene bridge. Acts as a "perfect spacer", the result show that for muscarinic activity, Should be no more than four atoms between the ammonium and the terminal methyl group, otherwise a loss of activity. (i.e.ammonium group should be followed by a chain of five atom, this has been referred to as the five atoms rules.

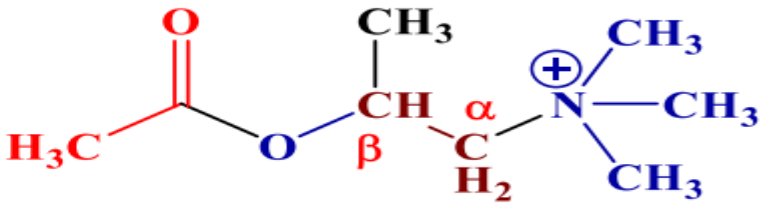


B- An α substitution on the choline moiety decreases both nicotinic and muscarinic activity, but muscarinic activity is decreased to a greater extent.

C- An β substitution on the choline moiety decreases both nicotinic and muscarinic activity, but nicotinic activity is decreased to a greater extent.



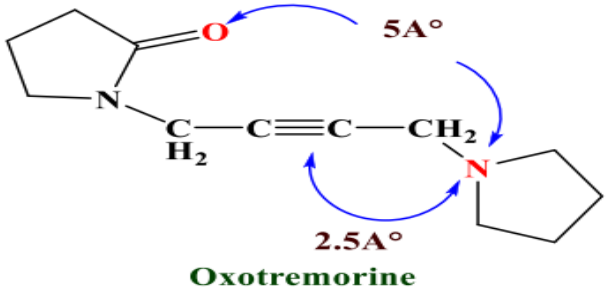
Acetyl-α-methylcholine



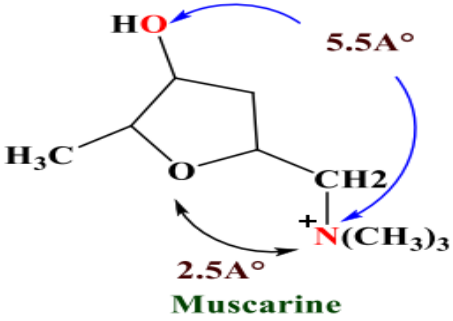
Acetyl-β-methylcholine (Methacholine)

D- Hydrolysis by AChE is more affected by substitutions on the β than the α carbon. The hydrolysis rate of racemic acetyl β-methylcholine is about 50% of that of Ach ; racemic acetyl α- methylcholine is hydrolyzed about 90% as fast.

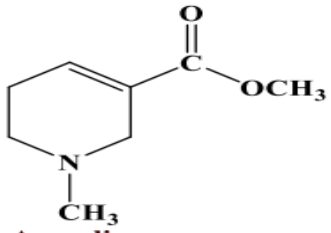
3- Ester Group. The ester group in ACh contributes to the binding of the compound to the muscarinic receptor because of hydrogen bond formation with threonine and asparagine residues at the receptor site.



Oxotremorine



Muscarine



Arecoline

Characterization of muscarinic receptors can now be extended beyond the pharmacological observations on organ systems (e.g., smooth muscle, heart) to determine structure–activity relationships.

Antagonists with high affinity for one receptor and a low affinity for the other four receptor types are very few, however, and many antagonists bind to several subtypes with equal affinity.

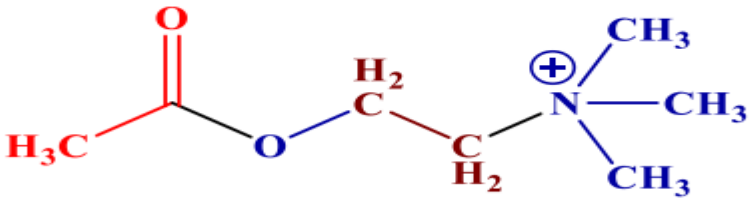
Receptor	***High affinity***	***Low affinity***	***Distinguish***
M1	pirenzepine	Af-DX 116	himbacine
M4	pirenzepine		
M1		Methoctramine, polymethylenetetramine,	
M2	Methoctramine, polymethylenetetramine, Af-DX 116 (bind) gallamine (bind)		same
M3	4-diphenylacetoxy-N-methylpiperidine (4-DAMP) hexahydrosiladifenidol (HHSiD)		also M1 and M2

Cholinergic Drugs and Related Agents

Direct acting cholinergic agents (Agonist)

Products

1- Acetylcholine Chloride



Acetylcholine chloride
Cholinechloride acetate
powerful stimulant effect on the parasympathetic nervous system

Attempts have been made to use it as a cholinergic agent, but its duration of action is too short for sustained effects, because of rapid hydrolysis by esterases and lack of specificity when administered for systemic effects.

It is a cardiac depressant and an effective vasodilator. Stimulation of the vagus and the parasympathetic nervous system produces a tonic action on smooth muscle and induces a flow from the salivary and lacrimal glands.

Its cardiac-depressant effect results from (a) a negative chronotropic effect that causes a decrease in heart rate and (b) a negative inotropic action on heart muscle that produces a decrease in the force of myocardial contractions.

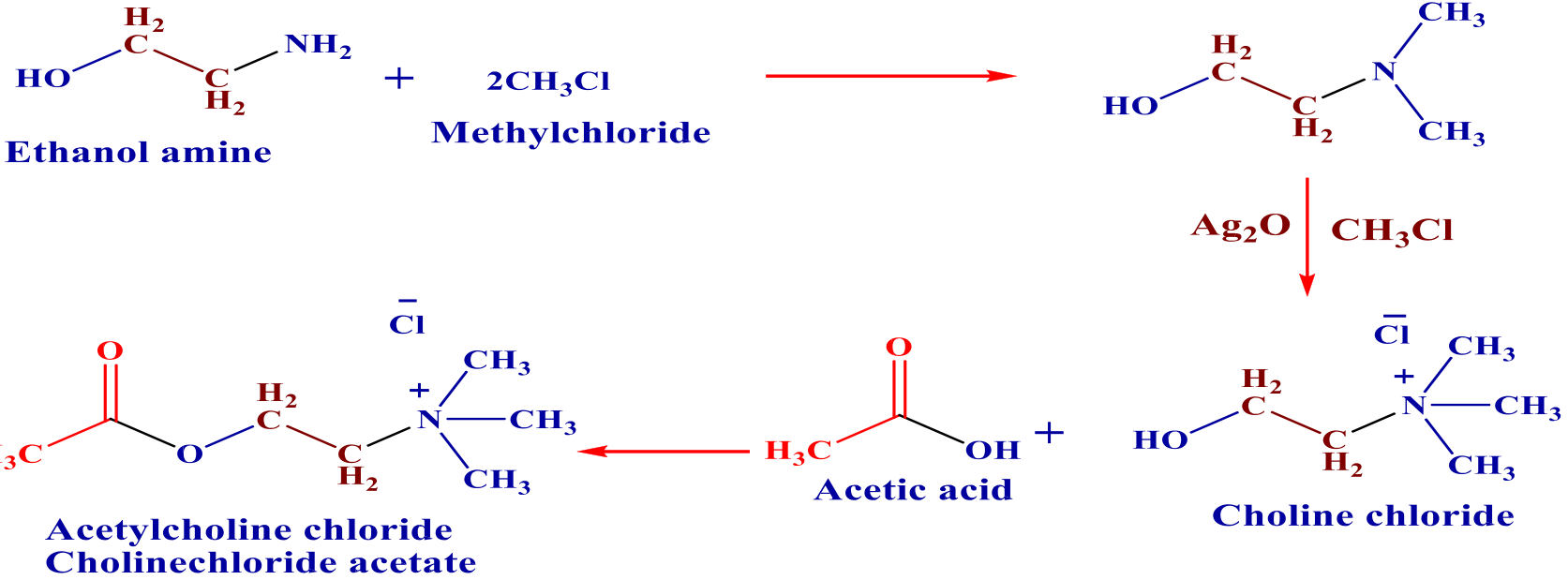
The vasodilatory action of ACh is primarily on the arteries and the arterioles, with distinct effect on the peripheral vascular system. Bronchial constriction is a characteristic side effect when the drug is given systemically.

One of the most effective antagonists to the action of ACh is atropine, a nonselective muscarinic antagonist. Atropine blocks the depressant effect of ACh on cardiac muscle and its production of peripheral vasodilation (i.e., muscarinic effects) but does not affect the skeletal muscle contraction (i.e., nicotinic effect) produced.

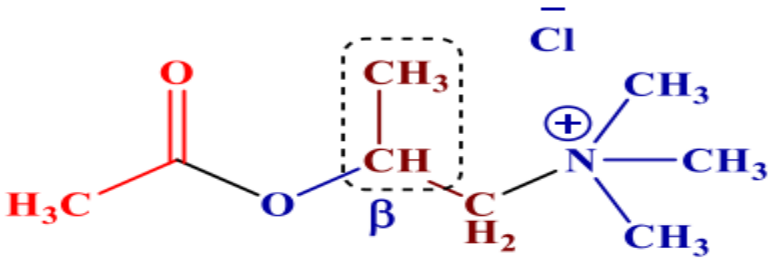
ACh chloride is a hygroscopic powder that is available in an admixture with mannitol (as it is poorly absorbed from the intestines. As a medication, it is used to decrease pressure in the eyes) **to be dissolved in sterile water for injection shortly before use.**

It is a short-acting miotic when introduced into the anterior chamber of the eye and is especially useful after cataract surgery during the placement of sutures. When applied topically to the eye, it has little therapeutic value because of poor corneal penetration and rapid hydrolysis by AChE.

Synthesis of ACh



2-Methacholine Chloride



Methacholine chloride
Acetyl-β-methylcholine
(2-hydroxypropyl)trimethylammonium chloride acetate

Unlike ACh, methacholine has sufficient stability in the body to give sustained parasympathetic stimulation. This action is accompanied by little (1/1,000 that of ACh) or no nicotinic effect.

Methacholine can exist as (S) and (R) enantiomers. Although the chemical is used as the racemic mixture, its muscarinic activity resides principally in the (S)-isomer. The (S)/(R) ratio of muscarinic potency for these enantiomers is 240:1.

(+)-Acetyl-(S)-B-methylcholine is hydrolyzed by AChE, whereas the (R)(-)-isomer is not.

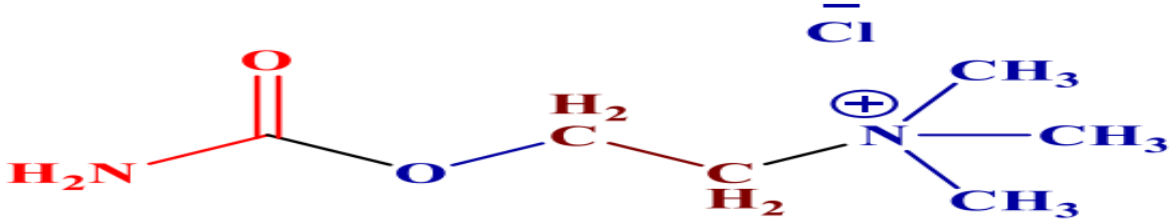
The hydrolysis rate of the (*S*)(+)-isomer is about 54% that of ACh.

(-)-Acetyl-(R)-b-methylcholine weakly inhibits AChE and slightly reinforces the muscarinic activity of the (S)(+)-isomer in the racemic mixture of acetyl-B-methylcholine.

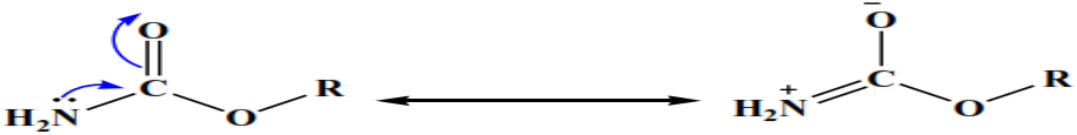
In the hydrolysis of the acetyl a- and B-methylcholines, the greatest stereochemical inhibitory effects occur when the choline is substituted in the B-position. This also appears to be true of organophosphorous inhibitors. The (R)(-)- and (S)(+)-isomers of acetyl--methylcholine are hydrolyzed at 78% and 97% of the rate of ACh, respectively.

Methacholine slight chloride occurs as colorless or white crystals or as a white crystalline powder. It is odorless or has a odor and is very deliquescent. It is freely soluble in water, alcohol, or chloroform, and its aqueous solution is neutral to litmus and bitter. It is hydrolyzed rapidly in alkaline solutions.

3- Carbachol chloride



Carbacol chloride
Choline chloride carbamate



Carbachol differs chemically from ACh in its stability to hydrolysis. The carbamyl group of carbachol decreases the electrophilicity of the carbonyl and, thus, can form resonance structures more easily than ACh can. The result is that carbachol is less susceptible to hydrolysis and, therefore, more stable in aqueous solutions.

nonspecific in its action on muscarinic receptor subtypes. The pharmacological activity of carbachol is similar to that of ACh.

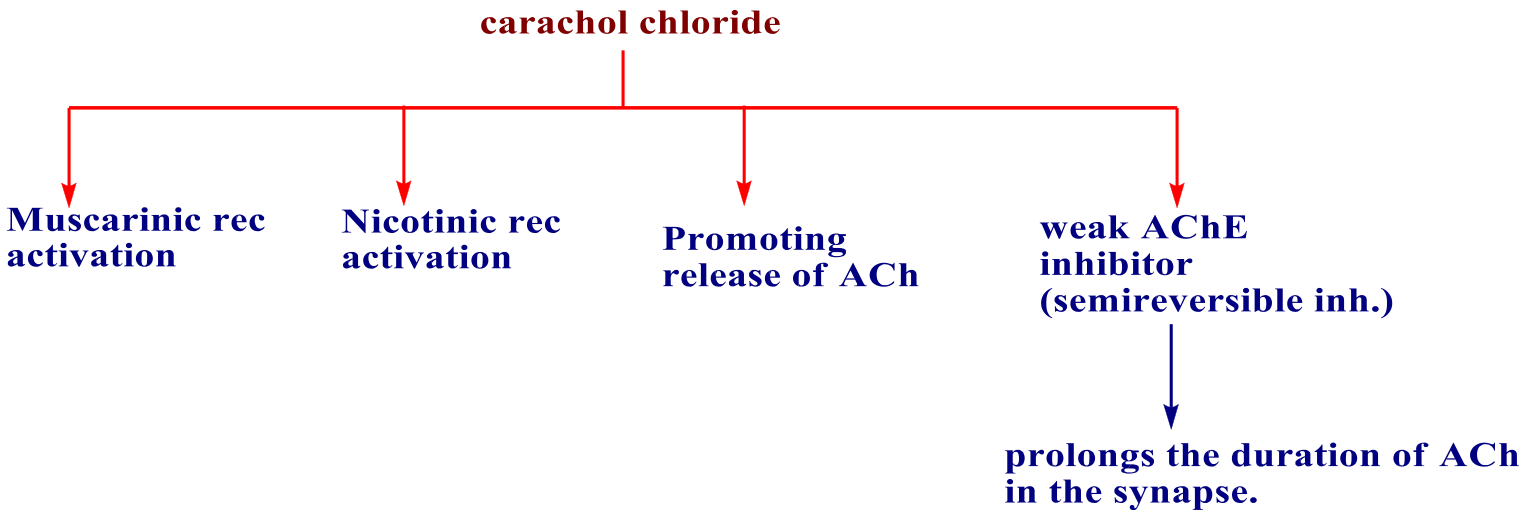
It is an ester of choline and thus possesses both muscarinic and nicotinic properties by cholinergic receptor stimulation.

It can also act indirectly by promoting release of ACh and by its weak anticholinesterase activity.

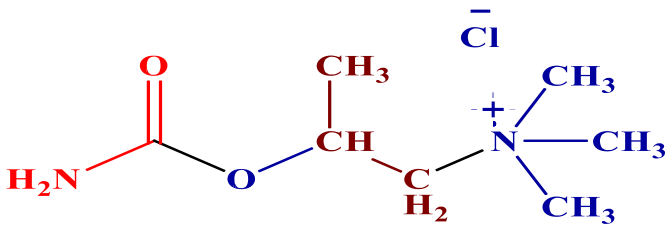
Carbachol forms a carbamyl ester in the active site of AChE, which is hydrolyzed more slowly than an acetyl ester. This slower hydrolysis rate reduces the amount of free enzyme and prolongs the duration of ACh in the synapse.

Carbachol also stimulates the autonomic ganglia and causes contraction of skeletal muscle but differs from a true muscarinic agent in that it does not have cardiovascular activity despite the fact that it seems to affect M2 receptors

Carbachol is a miotic and has been used to reduce the intraocular tension of glaucoma when a response cannot be obtained with pilocarpine or neostigmine (a cholinesterase inhibitor used in the treatment of myasthenia gravis and to reverse the effects of muscle relaxants such as gallamine and tubocurarine. Neostigmine, unlike PHYSOSTIGMINE, does not cross the blood-brain barrier.).


carachol chloride
Muscarinic rec activation
Nicotinic rec activation
Promoting release of ACh
weak AChE inhibitor (semireversible inh.)
prolongs the duration of ACh in the synapse.

4- Bethanechol chloride



Bethanechol
β-methylcholinechloride carbamate
(2-hydroxypropyl)trimethylammonium chloride carhamate
carbamylmethylcholine chloride (Urecholine)

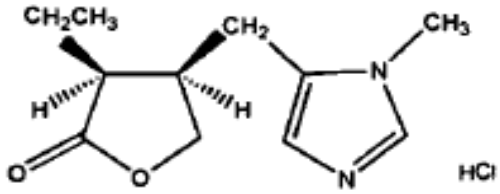
nonspecific in its action on muscarinic receptor subtypes but appears to be more effective at eliciting pharmacological action of M_3 receptors.

It has pharmacological properties similar to those of methacholine. Both are esters of -methylcholine and have feeble nicotinic activity. Bethanechol is inactivated more slowly by AChE in vivo than is methacholine. It is a carbamyl ester and is expected to have stability in aqueous solutions similar to that of carbachol.

The main use of bethanechol chloride is in the relief of urinary retention and abdominal distention after surgery.

The drug is used orally and by subcutaneous injection. It must never be administered by intramuscular or intravenous injection because of the danger from cholinergic overstimulation and loss of selective action.

5- Pilocarpine Hydrochloride.



Pilocarpine hydrochloride

It occurs as colorless, translucent, odorless, faintly bitter crystals that are soluble in water (1:0.3), alcohol (1:3), and chloroform (1:360).

Pilocarpine is a nonselective agonist on the muscarinic receptors. Despite this, it reportedly acts on M3 receptors in smooth muscle to cause contractions in the gut, trachea, and eye

In the eye, it produces pupillary constriction (miosis) and a spasm of accommodation. These effects are valuable in the treatment of glaucoma.

Pilocarpine is used as a 0.5% to 0.6% solution (i.e., of the salts) in treating glaucoma. Systemic effects include copious sweating, salivation, and gastric secretion.

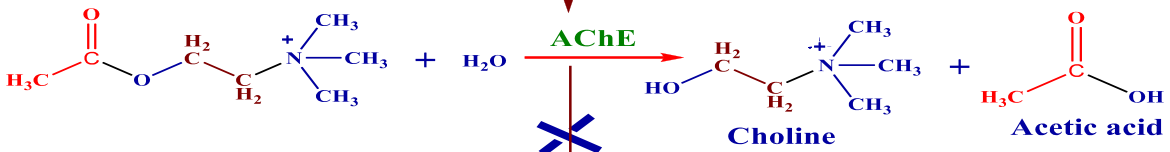
Indirect acting cholinergic agents (agonists)

Cholinesterases in humans

Differ in their location in the body and their substrate specificity.

- **AChE** — Associated with the outside surface of glial cell in the synaps
- **butyrylcholinesterase (BuChE)** (pseudocholinesterase) — Located in → Human plasm → hydrolyze dietary ester and drug molecule in the blood

Cholinesterases are catalyzes the hydrolysis of ACh to choline and acetic acid



inhibition of AChE

prolongs the duration of the neurotransmitter in the junction and produces pharmacological effects similar to those observed when ACh is administered

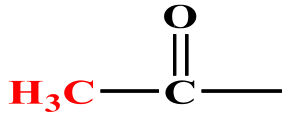
AChE inhibitors have been used in the treatment of

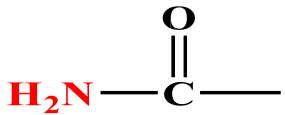
- myasthenia gravis
- atony in the GI tract
- glaucoma
- agricultural insecticides
- nerve gases
- Alzheimer's disease

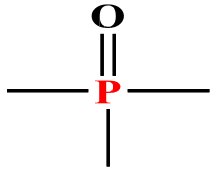
The initial step in the hydrolysis of ACh by AChE is a reversible enzyme–substrate complex formation. The association rate ($k+1$) and dissociation rate ($k-1$) are relatively large. The enzyme–substrate complex, EA–ACh, may also

form an acetyl-enzyme intermediate at a rate ($k2$) that is slower than either the association or dissociation rates. Choline is released from this complex with the formation of the acetyl-enzyme intermediate, EA. This intermediate is then hydrolyzed to regenerate the free enzyme and acetic acid. The acetylation rate, $k2$, is the slowest step in this sequence and is rate-limiting (see discussion that follow).

Three different chemical groupings may react with the esteratic site of AChE which includes-:

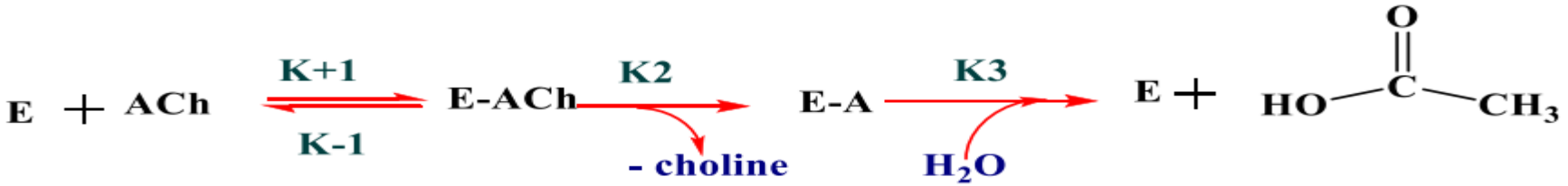
a- Acetyl group  reversible inhibitor

b- Carbamyl group  semireversible inhibitor

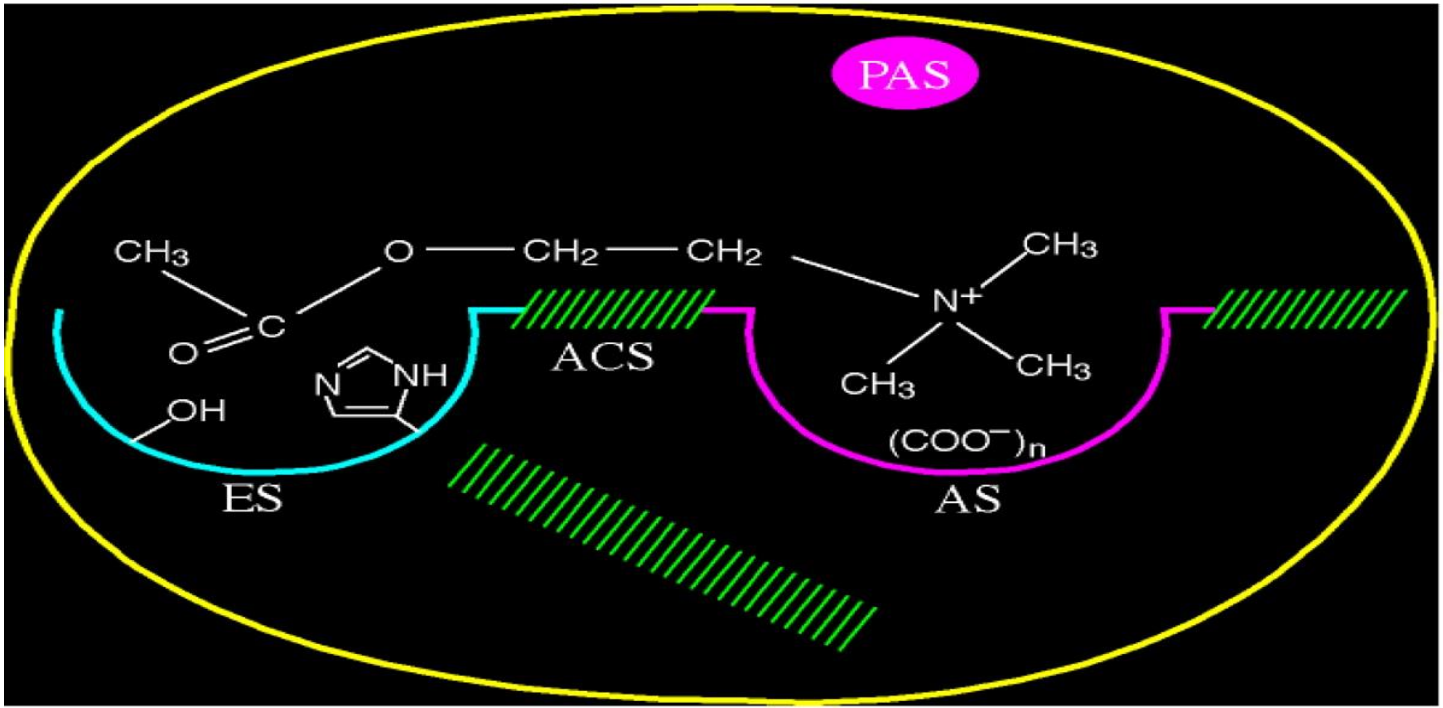
c- phosphoryl group  irreversible inhibitor

Although the chemical reactions are similar, the kinetic parameters for each type of substrate differ and result in differences between toxicity and usefulness.

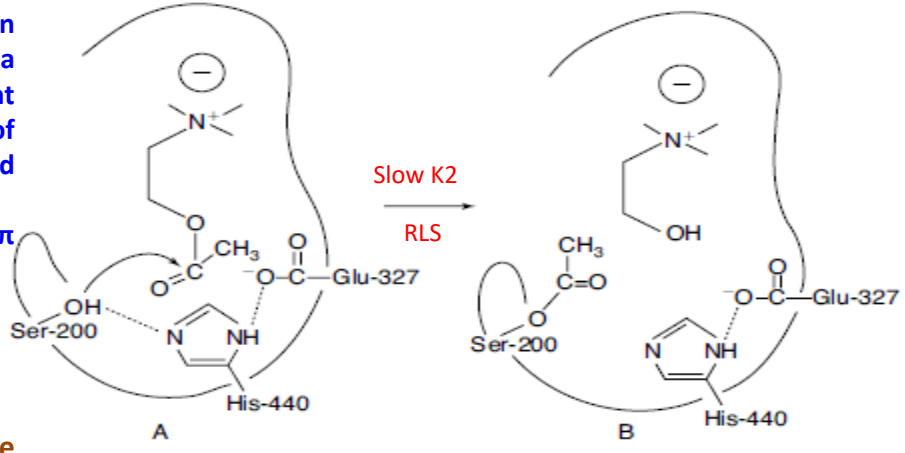
The hydrolysis of Ach by AChE



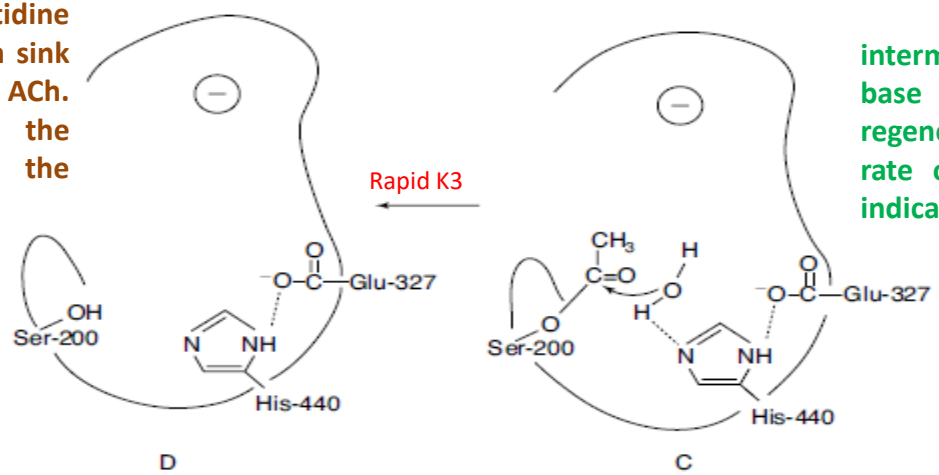
The active center of AChE consists of several major domains-:



The anionic site was believed to have been formed by the ɤ-carboxylate group of a glutamic acid residue, but more recent studies suggest that the aromatic moieties of tryptophan and phenylalanine residues bind the quaternary ammonium group of ACh in the anionic site through cation-π interactions



AChE attacks the ester substrate through a serine hydroxyl, forming a covalent acyl-enzyme complex. The serine is activated as a nucleophile by the glutamic acid and histidine residues that serve as the proton sink to attack the carbonyl carbon of ACh. Choline is released, leaving the acetylated serine residue on the enzyme. The acetyl-enzyme



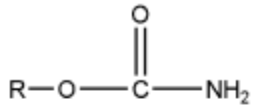
intermediate is cleaved by a general base catalysis mechanism to regenerate the free enzyme. The rate of the deacetylation step is indicated by k3.

Carbamates such as carbachol are also able to serve as substrates for AChE, forming a carbamylated enzyme intermediate (E–C). The rate of carbamylation (k2) is slower than the rate of acetylation. Hydrolysis (k3, decarbamylation) of the carbamyl-enzyme intermediate is 10^7 times slower than that of its acetyl counterpart. The slower hydrolysis rate limits the optimal functional capacity of AChE, allowing carbamate substrates to be semireversible inhibitors of AChE. In the mechanism above, k3 is rate-limiting. The rate k2 depends not only on the nature of the

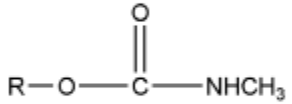
where CX = carbamylating substrate

where PX = phosphorylating substrate

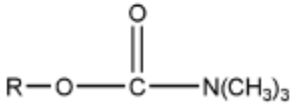
Organophosphate esters of selected compounds can also esterify the serine residue in the active site of AChE. The hydrolysis rate (*k*3) of the phosphorylated serine is extremely slow, and hydrolysis to the free enzyme and phosphoric acid derivative is so limited that the inhibition is considered irreversible. These organophosphorous compounds are used in the treatment of glaucoma, as agricultural insecticides, and, at times, as nerve gases in warfare and bioterrorism.



alcohol moiety of the ester but also on the type of carbamyl ester. Esters of carbamic acid are better



carbamylating agents of AChE than the methylcarbamyl



and dimethylcarbamyl analogs.[53]

Q/ What is the differences between nicotinic and muscarinic receptor?