College of Pharmacy
Organic Pharmaceutical Chemistry II

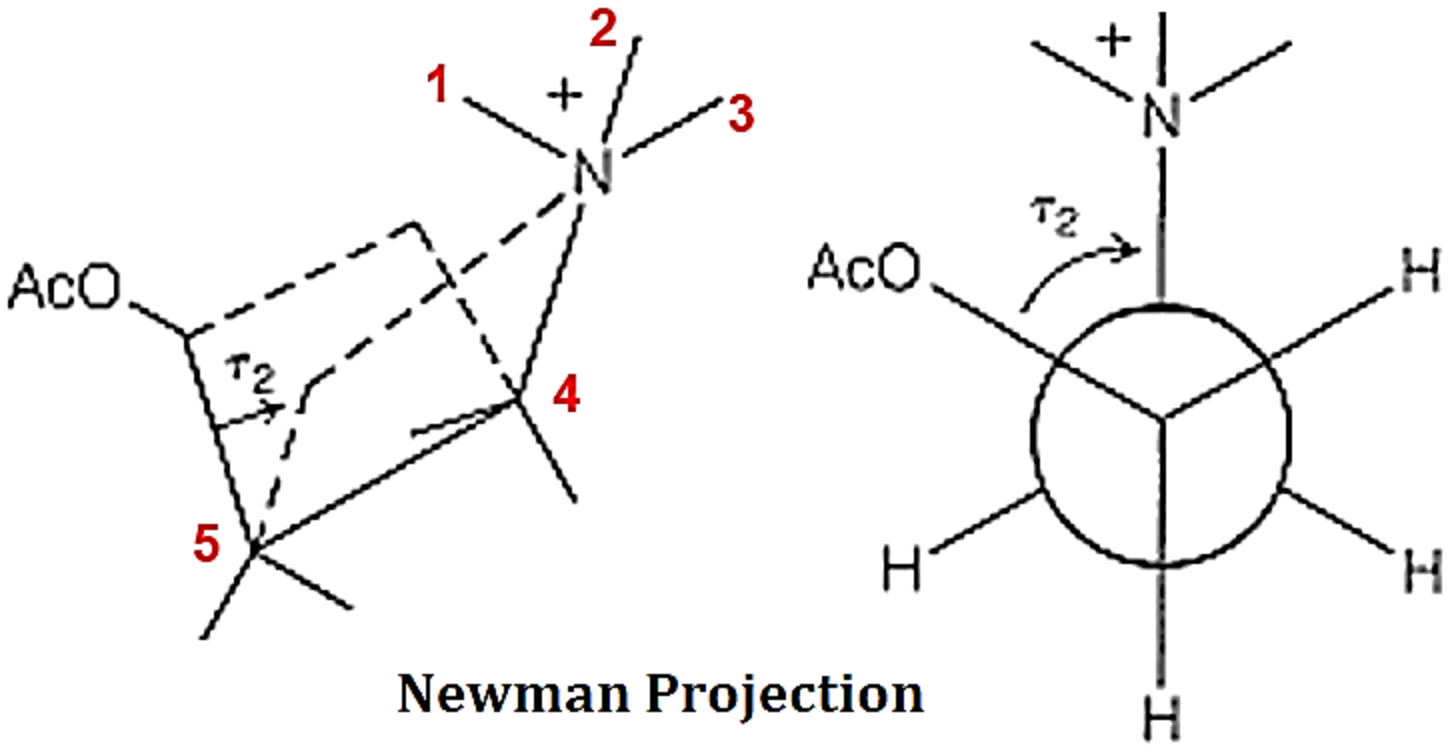
Lec2: Cholinergic agonists; stereochemistry and structure-activity relationships (SAR); products, & Cholinesterase inhibitors

Cholinergic Agonists

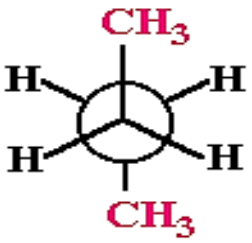
Cholinergic Stereochemistry:

- Three techniques have been used to study the conformational properties of ACh and other cholinergic chemicals:
    1. *X-ray crystallography.*
    2. *Nuclear Magnetic Resonance (NMR).*
    3. *Molecular modeling by computation.*
- Each of these methods may report the spatial distribution of atoms in a molecule in terms of torsion angles.

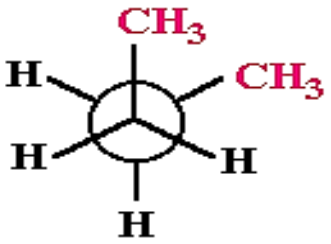
- A torsion angle is defined as the angle formed between two planes, for example, by the O-C5-C4-N atoms in Ach.
- The torsion angle **(O-C-C-N)** determines the spatial orientation of the cationic head of ACh to the ester group.



Newman Projection

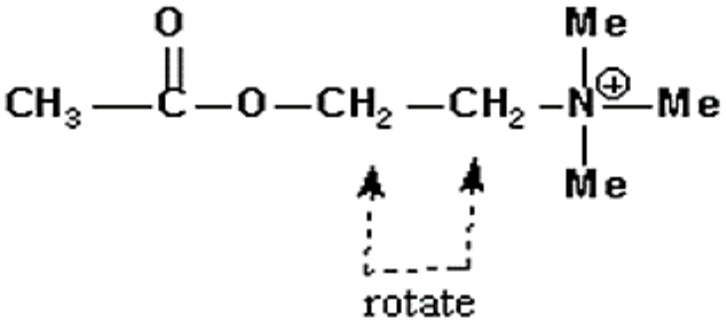


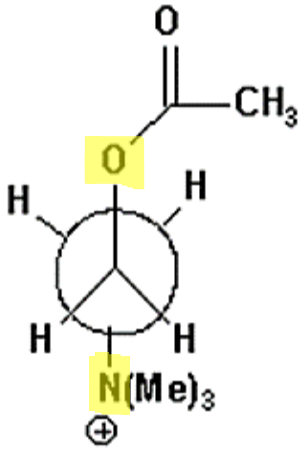
Anti

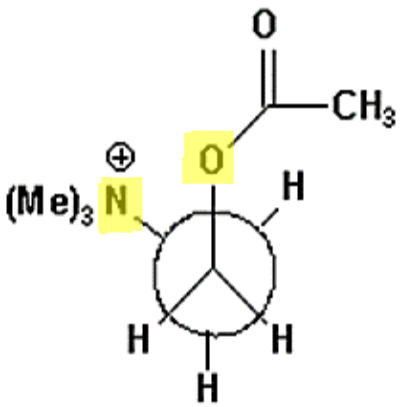


Gauche

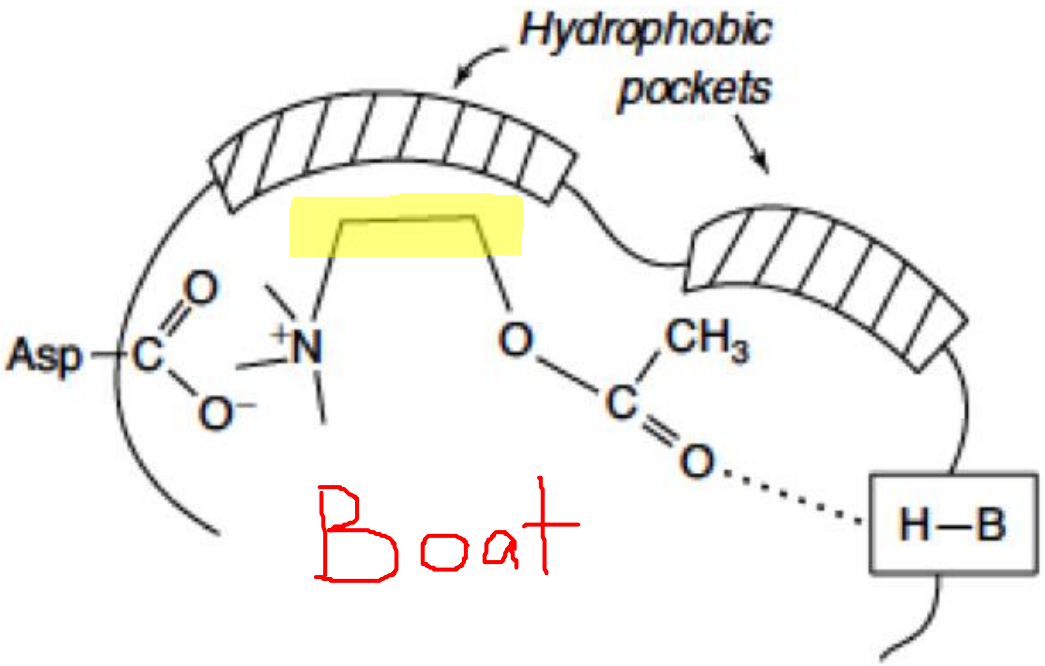
acetylcholine





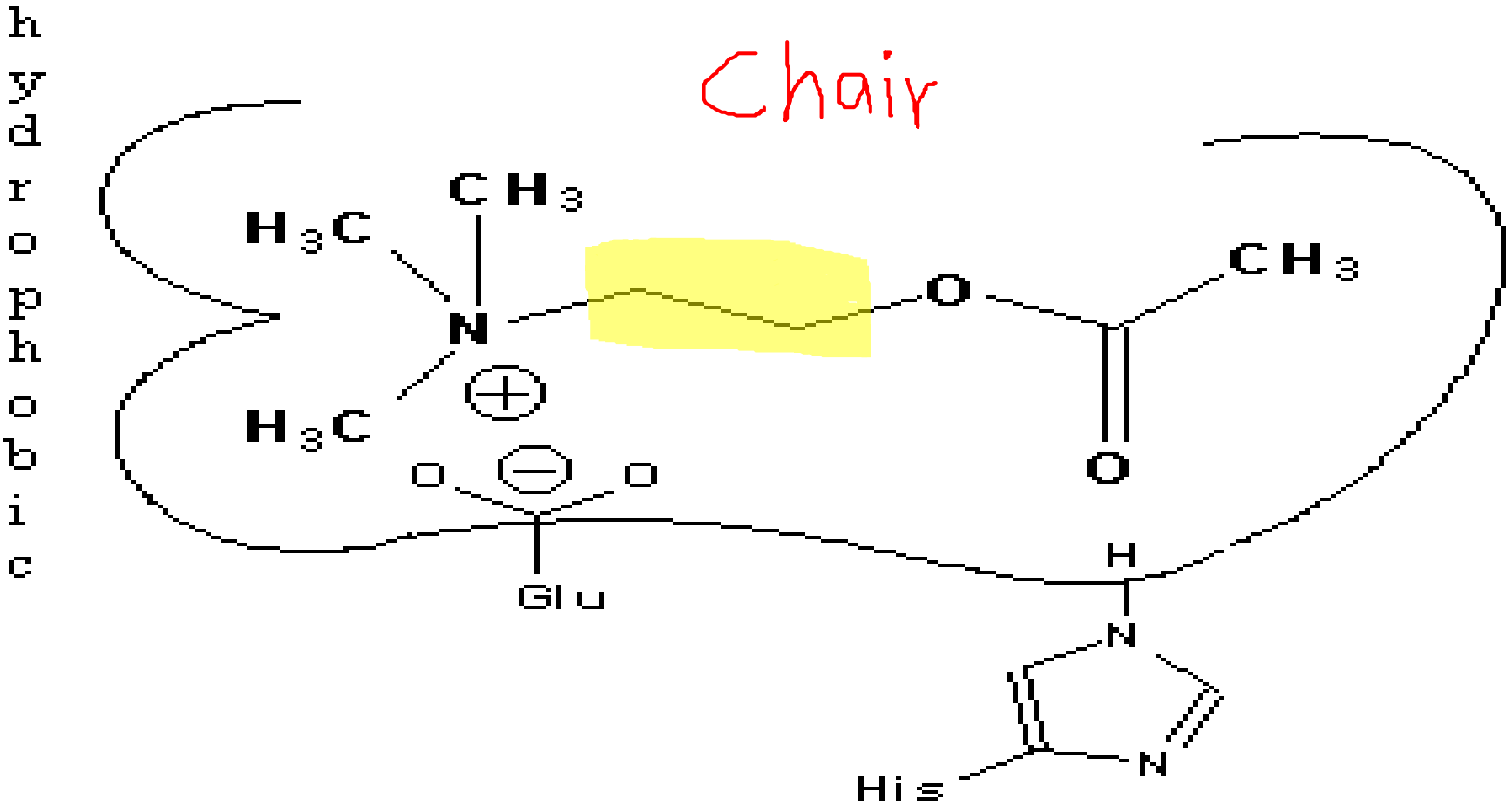


Structure–Activity Relationships (SAR)



Hypothetical structure of the muscarinic receptor.

Structure–Activity Relationships (SAR)



- **Hypothetical structure of nicotinic receptor.**

ACh - SAR

- Scientists still use pharmacological and biochemical tests to determine optimal structural requirements for activity.
- ACh is a relatively simple molecule.
- The chemistry and ease of testing for ACh biological activity have allowed numerous chemical derivatives to be made and studied.
- Alterations on the molecule may be divided into three categories: the *onium group*, the *ester* function, and the *choline* moiety.
- The ACh contain three core groups:

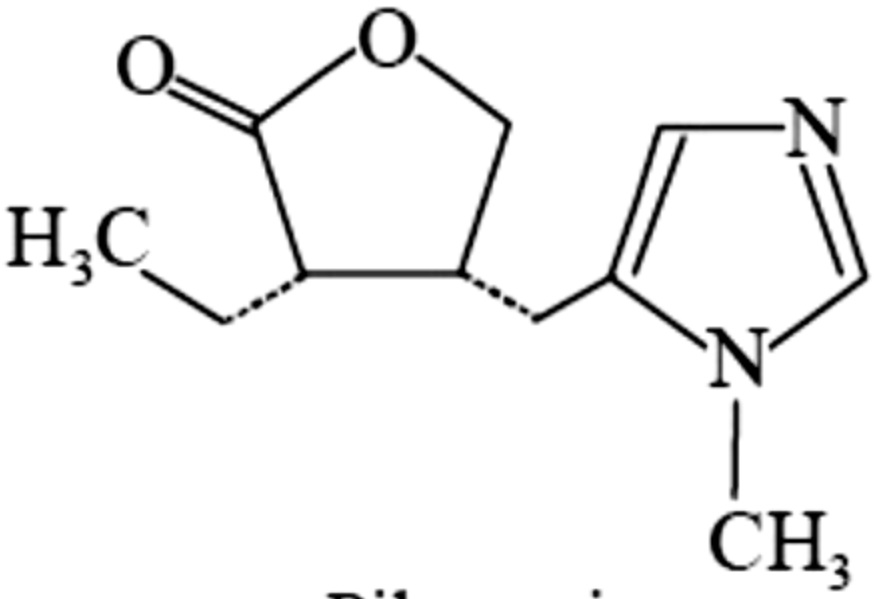
    1. *The onium group.*
    2. *The ester function.*
    3. *The choline moiety.*

The onium group

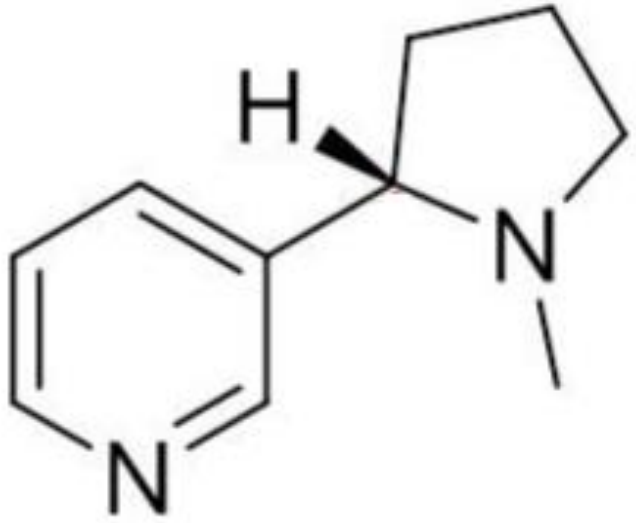
- Is essential for intrinsic activity and contributes to the affinity of the molecule for the receptors, partially through the binding energy and partially because of its action as a detecting and directing group.
- Molecular modeling data show the binding site to be:
    i. *A negatively charged aspartic acid residue in the third of the seven transmembrane helices of the muscarinic receptor.*
    ii. *Hydrophobic pockets are located in helices 4, 5, 6, and 7 of the muscarinic receptor.*

The onium group

- Binds to the negatively charged aspartic acid residue in the third of the seven transmembrane helices of the muscarinic receptor.
- The trimethylammonium group is the optimal functional moiety for activity, although some significant exceptions are known (e.g., pilocarpine and nicotine).
- 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.



Pilocarpine



Nicotine

The ester group

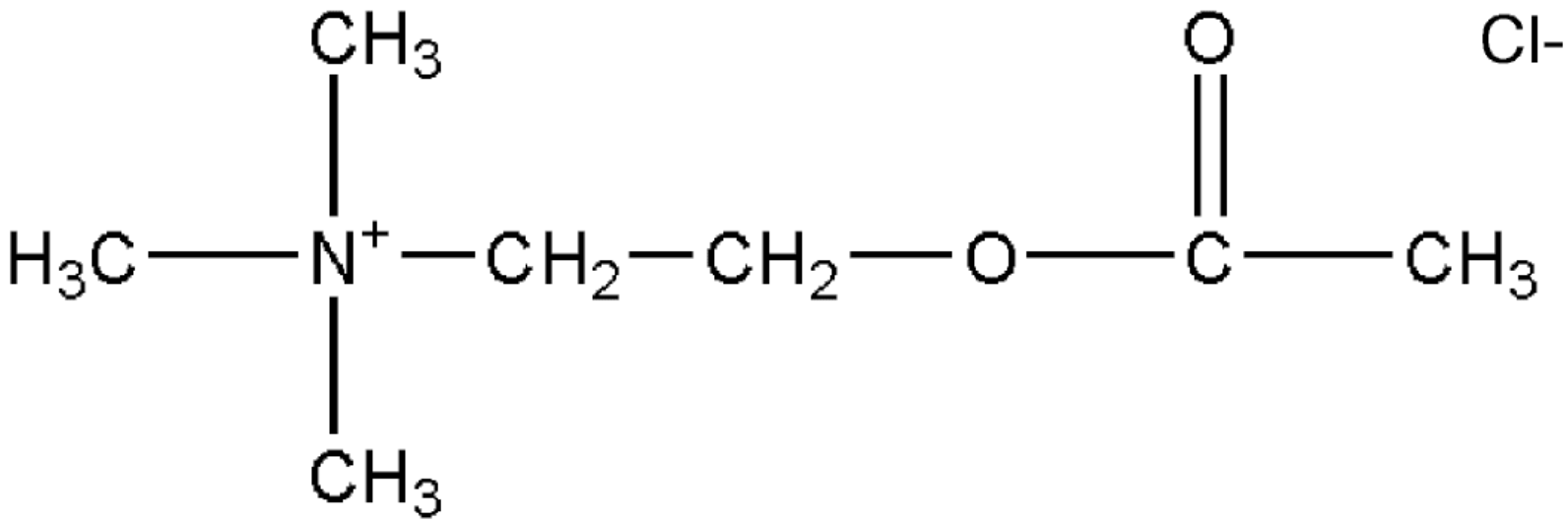
- Contributes to the binding of the compound to the muscarinic receptor because of hydrogen bond formation with threonine and asparagine residues.
- The presence of the acetyl group in ACh is not as critical as the size of the molecule.
- Studying a series of alkyltrimethylammonium salts revealed that for maximal muscarinic activity, the quaternary ammonium group should be followed by a chain of five atoms; this has been referred to as the *five-atom rule.*
- *This rule suggest that the should be no more than five atoms between the nitrogen atom and the terminal hydrogen atom for maximal muscarinic activity.*
- Shortening or lengthening the chain of atoms that separates the ester group from the onium moiety reduces muscarinic activity.

The choline moiety

- An α substitution on *the choline moiety* decreases both nicotinic and muscarinic activity, although muscarinic activity is decreased to a greater extent.
- Nicotinic activity is decreased to a greater degree by substitution on the β carbon.
- Therefore, acetyl-α-methylcholine, although less potent than ACh, has more nicotinic than muscarinic activity, whereas acetyl-β-methylcholine (methacholine) exhibits more muscarinic than nicotinic activity.
- Methacholine has a muscarinic activity almost equivalent to that of acetylcholine.

Products

- *Acetylcholine Chloride:*



Acetylcholine Chloride

Acetylcholine Chloride:

- ACh chloride exerts a 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.

Acetylcholine Chloride:

- Its cardiac-depressant effect results from:
    i. *A negative chronotropic effect that causes a decrease in heart rate.*
    ii. *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.

Acetylcholine Chloride:

- 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.

Methacholine Chloride

- Methacholine chloride, is the acetyl ester of β-methylcholine.
- 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.

Methacholine Chloride

- 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.

Carbachol

- Like ACh, carbachol is 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

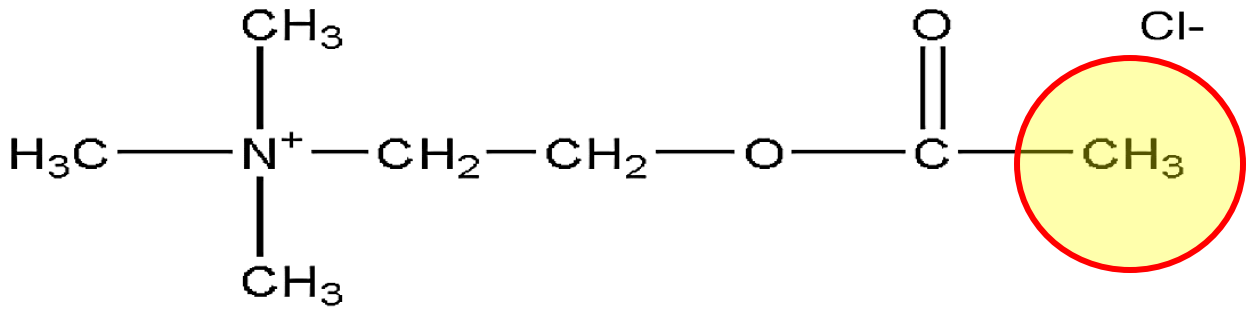
- Carbachol forms a **carbamyl ester** in the active site of AChE, which is hydrolyzed more **slowly** than an **acetyl ester.**
- This slower hydrolysis rate reduces the amount of free enzyme and prolongs the duration of ACh in the synapse.
- Carbachol also stimulates the autonomic ganglia and causes 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 M_2 receptors.

Carbachol

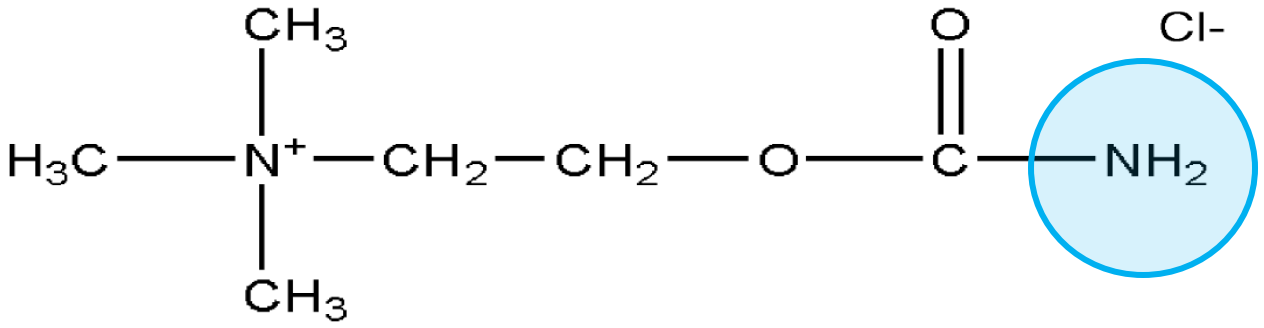
- Carbachol is a miotic and has been used to reduce the intraocular tension of glaucoma when a response cannot be obtained with pilocarpine or neostigmine.
- Carbachol differs chemically from ACh in its stability to hydrolysis.
- The carbamyl group of carbachol decreases the electrophilicity of the carbonyl and, thus, can form resonance structures more easily than ACh can.

Carbachol

- The result is that carbachol is less susceptible to hydrolysis and, therefore, more stable in aqueous solutions.



Acetylcholine Chloride



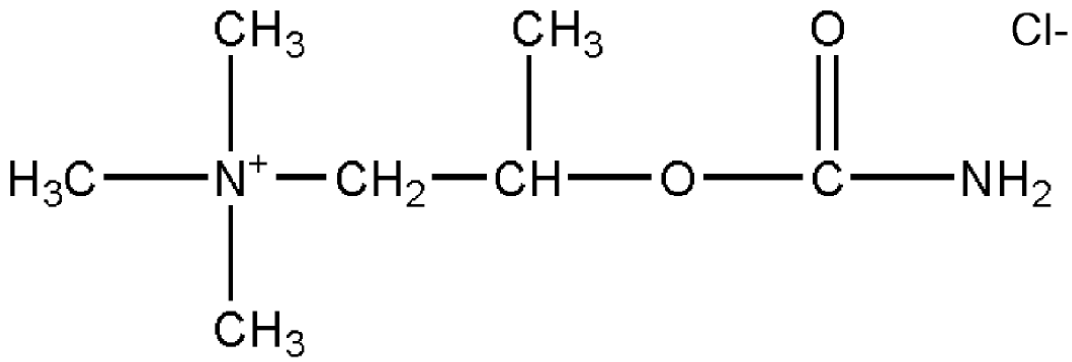
Carbachol Chloride

Bethanechol Chloride

- Is 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.
- Bethanechol is inactivated more slowly by AChE in vivo than is methacholine (β-methyl substitution).
- The main use of bethanechol chloride is in the relief of urinary retention and abdominal distention after surgery.

Bethanechol Chloride

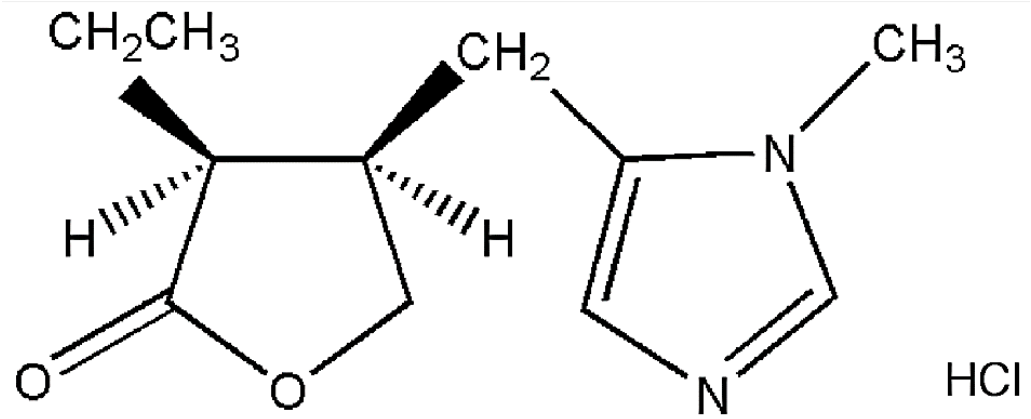
- The drug is used orally and by subcutaneous injection.
- It must never be administered by intramuscular or intravenous injection because of the danger from cholinergic overstimulation and loss of selective action.



Bethanechol Chloride

Pilocarpine Hydrochloride

- Pilocarpine monohydrochloride is the hydrochloride of an alkaloid obtained from the dried leaflets of Pilocarpus jaborandi or P. microphyllus, in which it occurs to the extent of about 0.5% together with other alkaloids



Pilocarpine Hydrochloride

Pilocarpine Hydrochloride

- Pilocarpine is a nonselective agonist on the muscarinic receptors.
- Despite this, it reportedly acts on M_3 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.

Pilocarpine Hydrochloride

- These effects are valuable in the treatment of glaucoma.
- The pupil constriction and spasm of the ciliary muscle reduce intraocular tension by establishing better drainage of ocular fluid through the canal of Schlemm, located near the corner of the iris and cornea.
- Pilocarpine is used in treating glaucoma.

Cholinesterase inhibitors

Cholinesterase Inhibitors

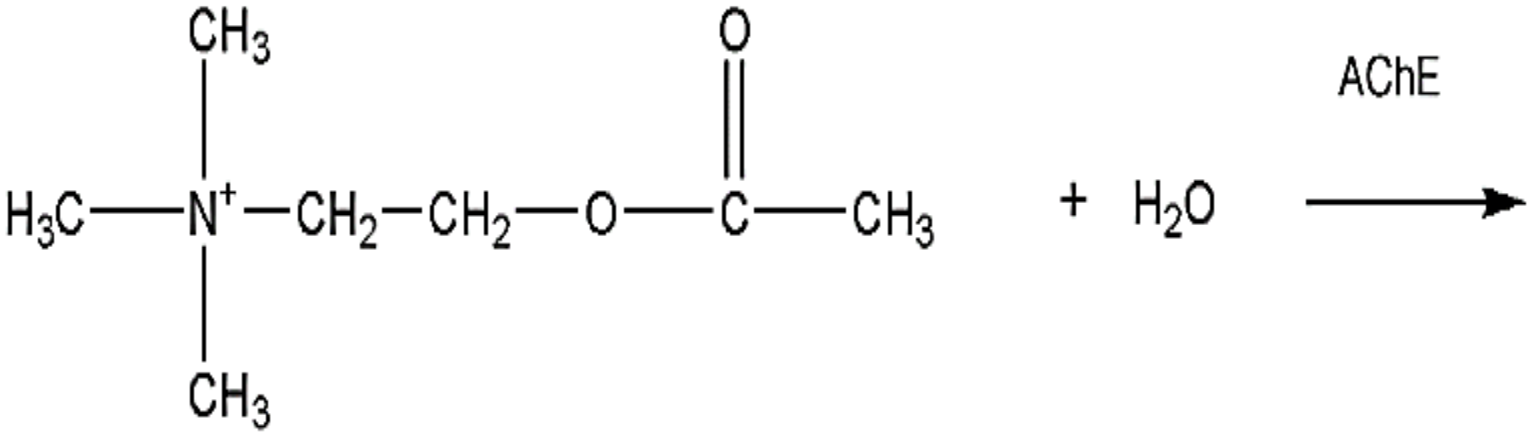
- These inhibitors are indirect-acting cholinergic agonists.
- There are two types of cholinesterases in humans, AChE and butyrylcholinesterase (BuChE).
- The cholinesterases differ in their location in the body and their substrate specificity.

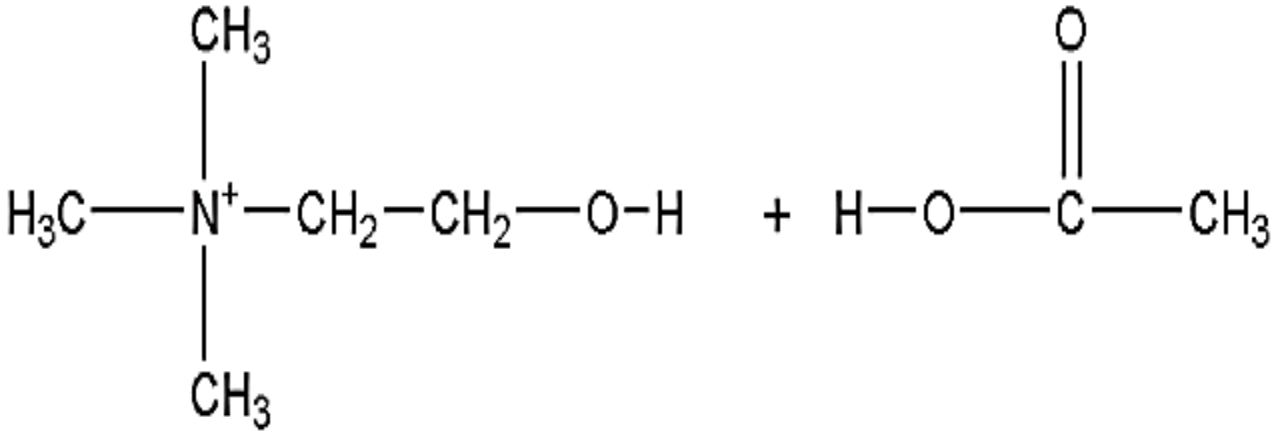
AChE inhibitors

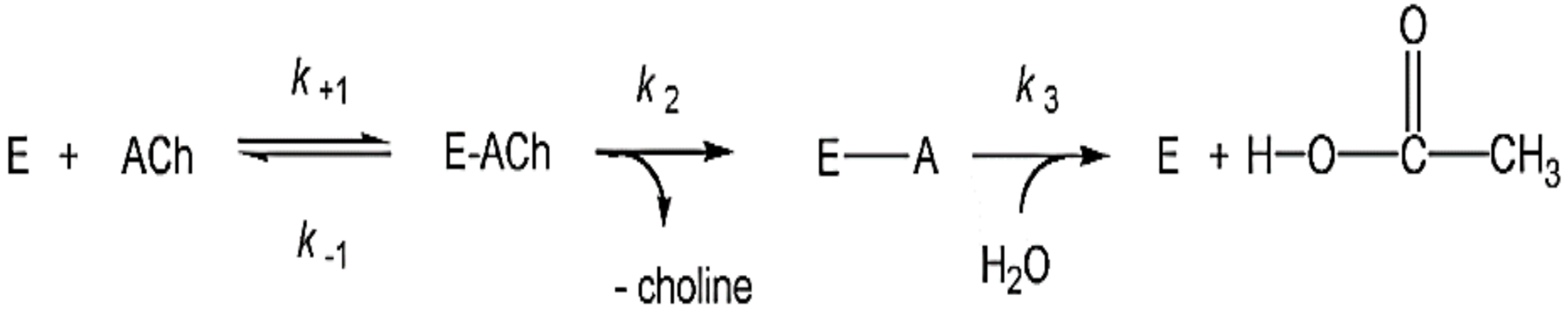
- AChE is associated with the outside surface of glial cells in the synapse and catalyzes the hydrolysis of ACh to choline and acetic acid.
- Inhibition of AChE prolongs the duration of the neurotransmitter in the junction and produces pharmacological effects similar to those observed when ACh is administered.

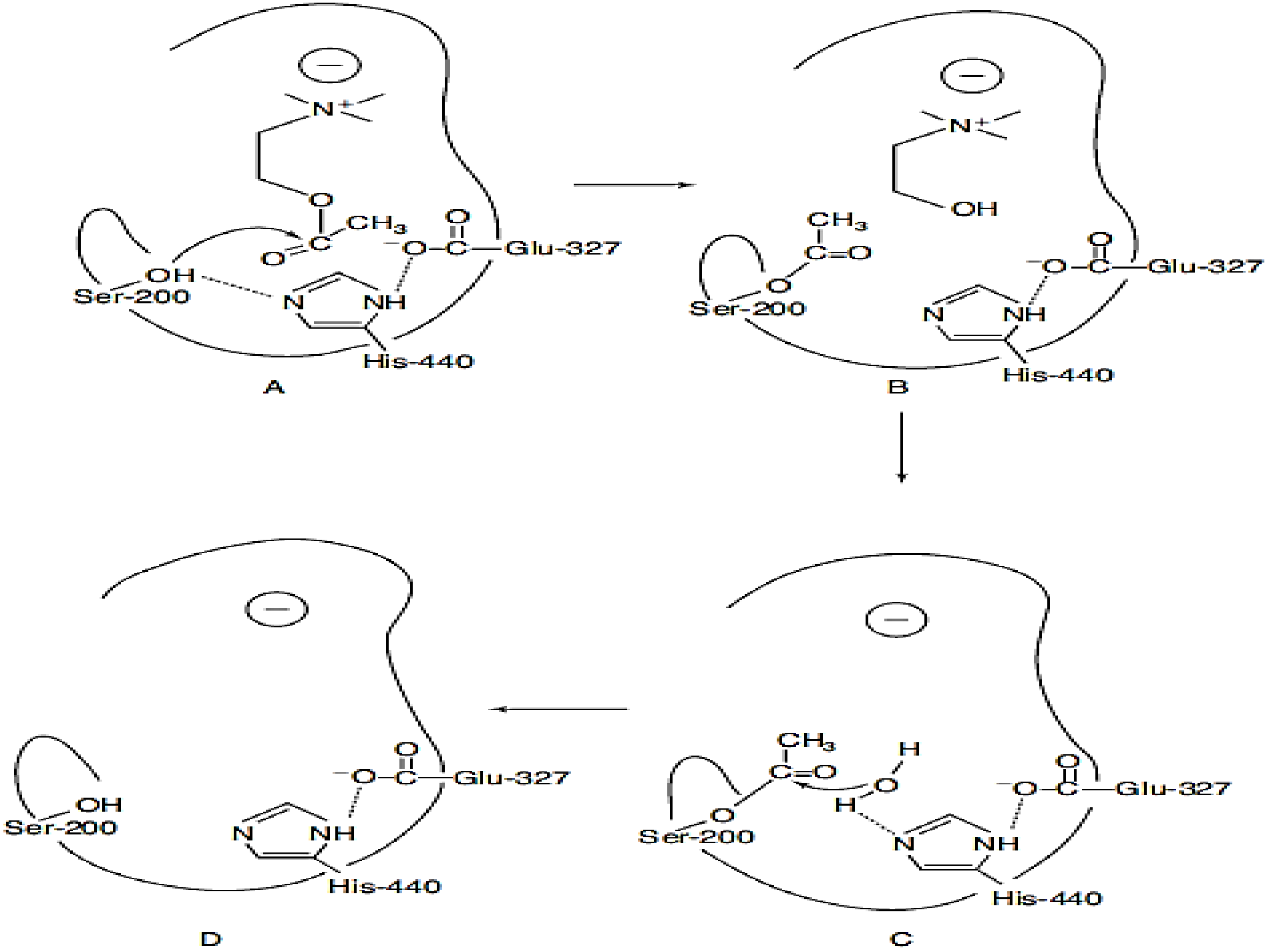
AChE inhibitors

- AChE inhibitors have been used in the treatment of myasthenia gravis, atony in the GI tract, and glaucoma.
- They have also been used as agricultural insecticides and nerve gases.
- More recently, they have received attention as symptomatic drug treatments in patients suffering from Alzheimer disease.


AChE
+ H2O




E + ACh
k +1
k -1
E-ACh
k 2
- choline
E—A
k 3
H2O
E +


A
B
C
D
Ser-200
His-440
Glu-327

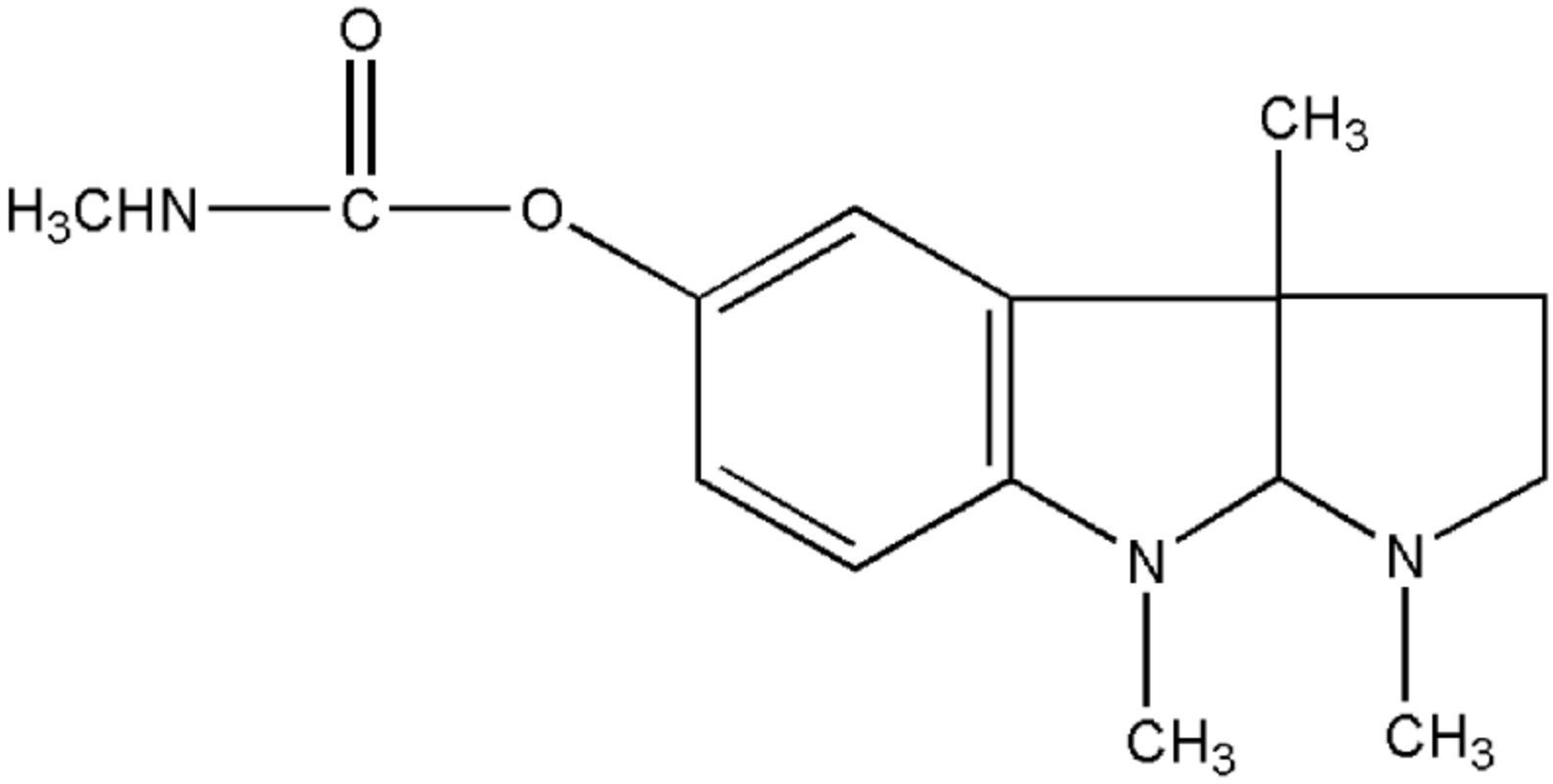
BuChE (pseudocholinesterase)

- Is located in human plasma.
- It has catalytic properties similar to those of AChE.
- The substrate specificity is broader, and it may hydrolyze dietary esters and drug molecules in the blood.

Reversible Inhibitors:

- Physostigmine, USP:
- Physostigmine is an alkaloid obtained from the dried ripe seed of Physostigma venenosum.
- Hydrolysis does take place, however, the physostigmine is inactivated.
- Solutions are most stable at pH 6 and should never be sterilized by heat.

Physostigmine:



Physostigmine

Neostigmine Bromide:

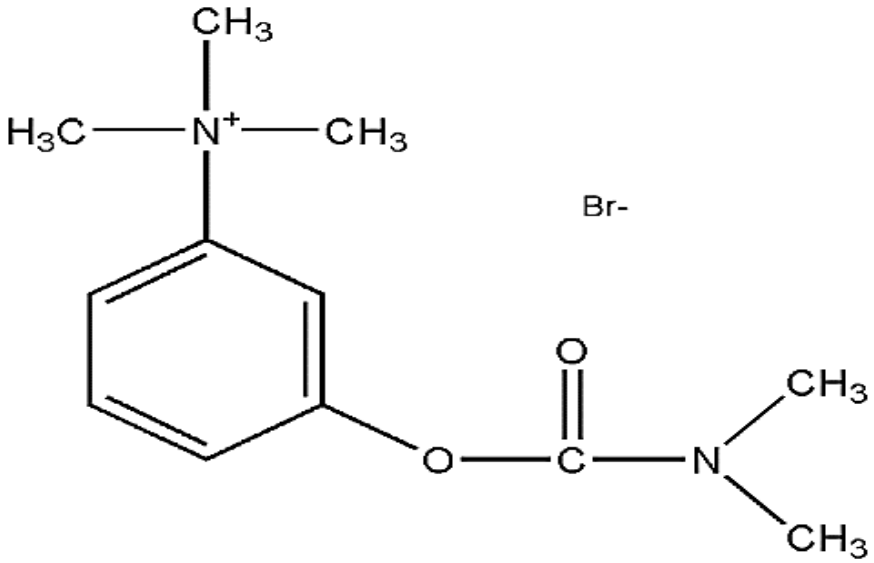
- Neostigmine bromide is used as an antidote to nondepolarizing neuromuscular blocking drugs and in the treatment of myasthenia gravis.
- Neostigmine has a mechanism of action quite similar to that of physostigmine.
- It effectively inhibits cholinesterase at a concentration about 10^{-6} M.
- Skeletal muscle is also stimulated by neostigmine, a property that physostigmine does not have.

Neostigmine Bromide:

- The uses of neostigmine are similar to those of physostigmine but differ in exhibiting greater miotic activity, fewer and less unpleasant local and systemic manifestations, and greater chemical stability.
- The most frequent application of neostigmine is to prevent atony of the intestinal, skeletal, and bladder musculature.

Neostigmine Bromide:

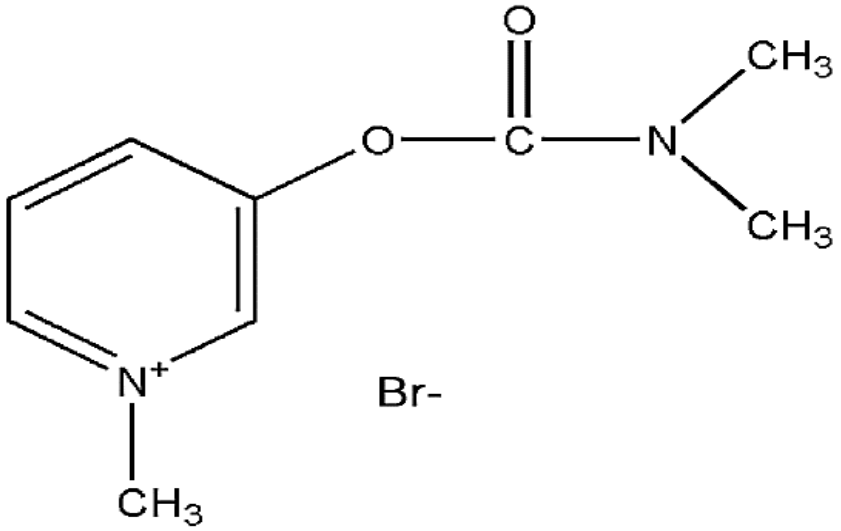
- An important use is in the treatment of myasthenia gravis, a condition caused by an autoimmune mechanism that requires an increase in ACh concentration in the neuromuscular junction to sustain normal muscular activity.



Neostigmine Bromide

Pyridostigmine Bromide:

- Pyridostigmine bromide (Mestinon®) is about one fifth as toxic as neostigmine.
- It appears to function in a manner similar to that of neostigmine and is the most widely used anticholinesterase agent for treating myasthenia gravis.

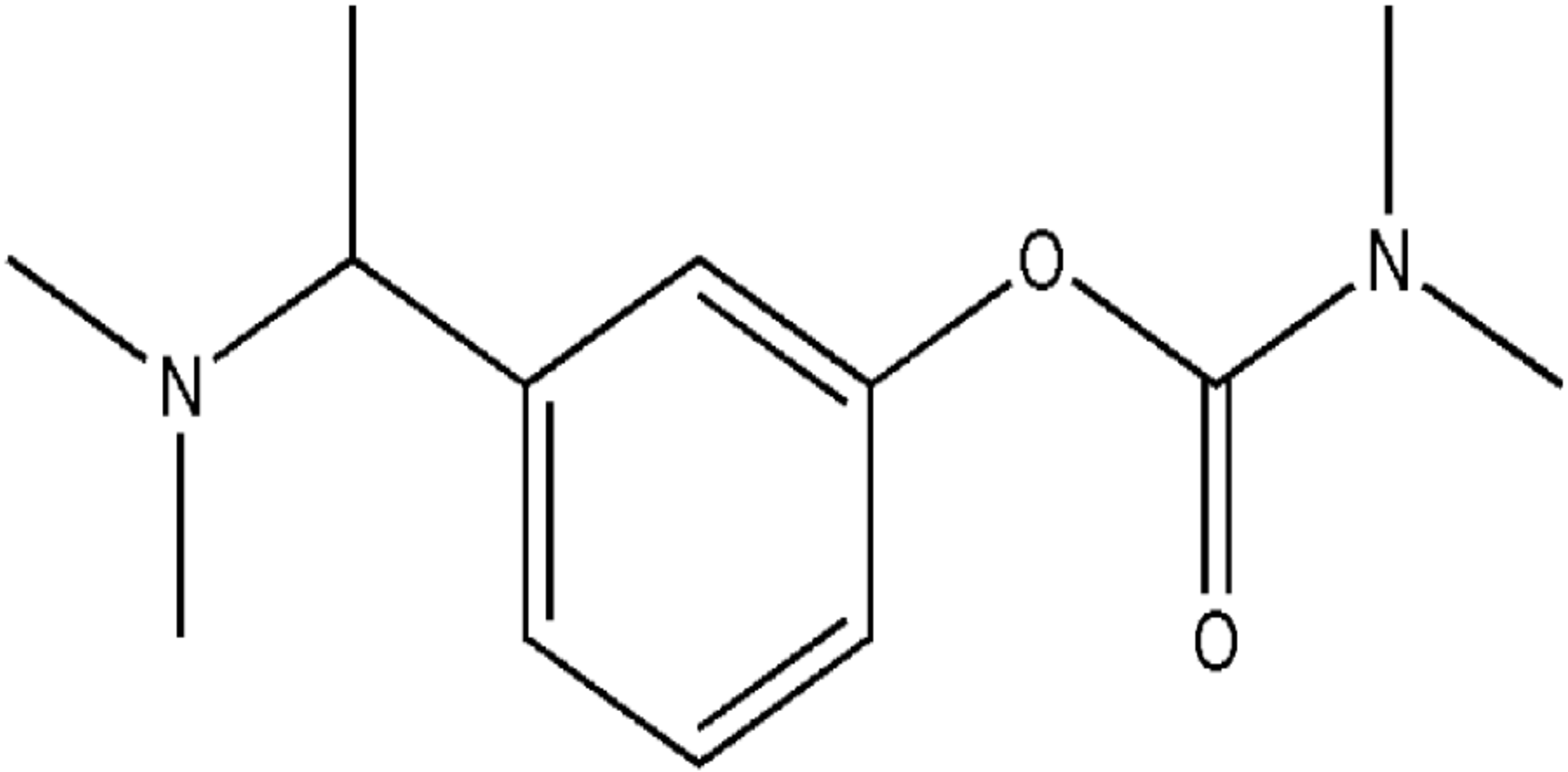


Pyridostigmine Bromide

Rivastigmine:

- Rivastigmine (Exelon®) is, a pseudo-irreversible noncompetitive carbamate, an inhibitor of AChE.
- Although the half-life is approximately 2 hours, the inhibitory properties of this agent last for 10 hours *because* of the slow dissociation of the drug from the enzyme.
- The Food and Drug Administration (FDA) approved its use in mild-to-moderate Alzheimer disease in April 2000.
- In July 2007, rivastigmine was granted approval for use in managing mild-to-moderate dementia associated with Parkinson disease.

Rivastigmine:



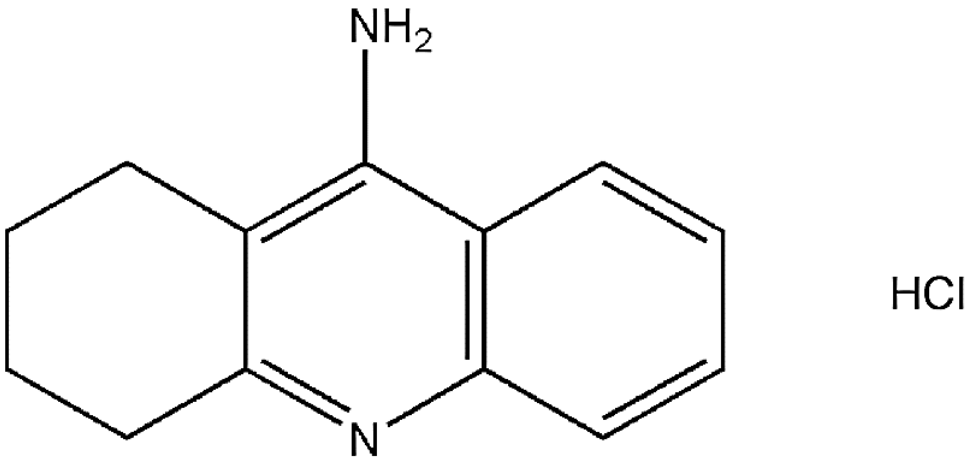
Rivastigmine

Tacrine Hydrochloride:

- Tacrine hydrochloride, is a reversible cholinesterase inhibitor that has been used in the treatment of Alzheimer disease for several years.
- The drug has been used to increase the levels of ACh in these patients on the basis of observations from autopsies (i.e. post-mortem examination) that concentrations of ChAT and AChE are markedly reduced in the brain, whereas the number of muscarinic receptors is almost normal.

Tacrine Hydrochloride:

- The use of the drug is not without controversy, as conflicting results on efficacy have been reported.
- The drug has been used in mild-to-moderate Alzheimer and dementia.



Tacrine Hydrochloride

Irreversible Cholinesterase Inhibitors:

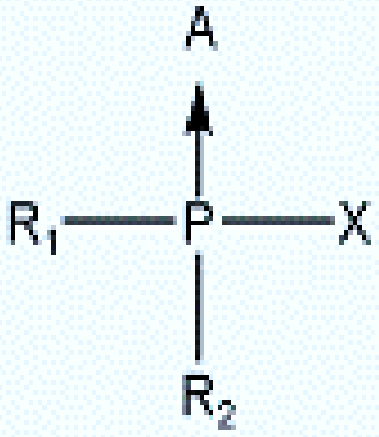
- Organophosphate esters of selected compounds can also esterify the serine residue in the active site of AChE.
- The hydrolysis rate of the phosphorylated serine is extremely slow, and hydrolysis to the free enzyme and phosphoric acid derivative is so limited that the inhibition is considered irreversible.
- These organophosphorous compounds are used in the treatment of glaucoma, as agricultural insecticides, and, at times, as nerve gases in warfare and bioterrorism.
- Finally, some have either been or are currently being evaluated for use against Alzheimer disease.

Irreversible Cholinesterase Inhibitors:

- Both AChE and BuChE are inhibited irreversibly by a group of phosphate esters that are highly toxic (LD50 for humans is 0.1–0.001 mg/kg).
- They permit ACh to accumulate at nerve endings and exacerbate ACh-like actions.
- The compounds belong to a class of organophosphorous esters.

Organophosphorous esters

- "A" is usually oxygen or sulfur but may also be selenium.
- When A is other than oxygen, biological activation is required before the compound becomes effective as an inhibitor of cholinesterases.



where R_1 = alkoxy
R_2 = alkoxyl, alkyl, or tertiary amine
X = a good leaving group
(e.g., F, CN, thiomalate, *p*-nitrophenyl)

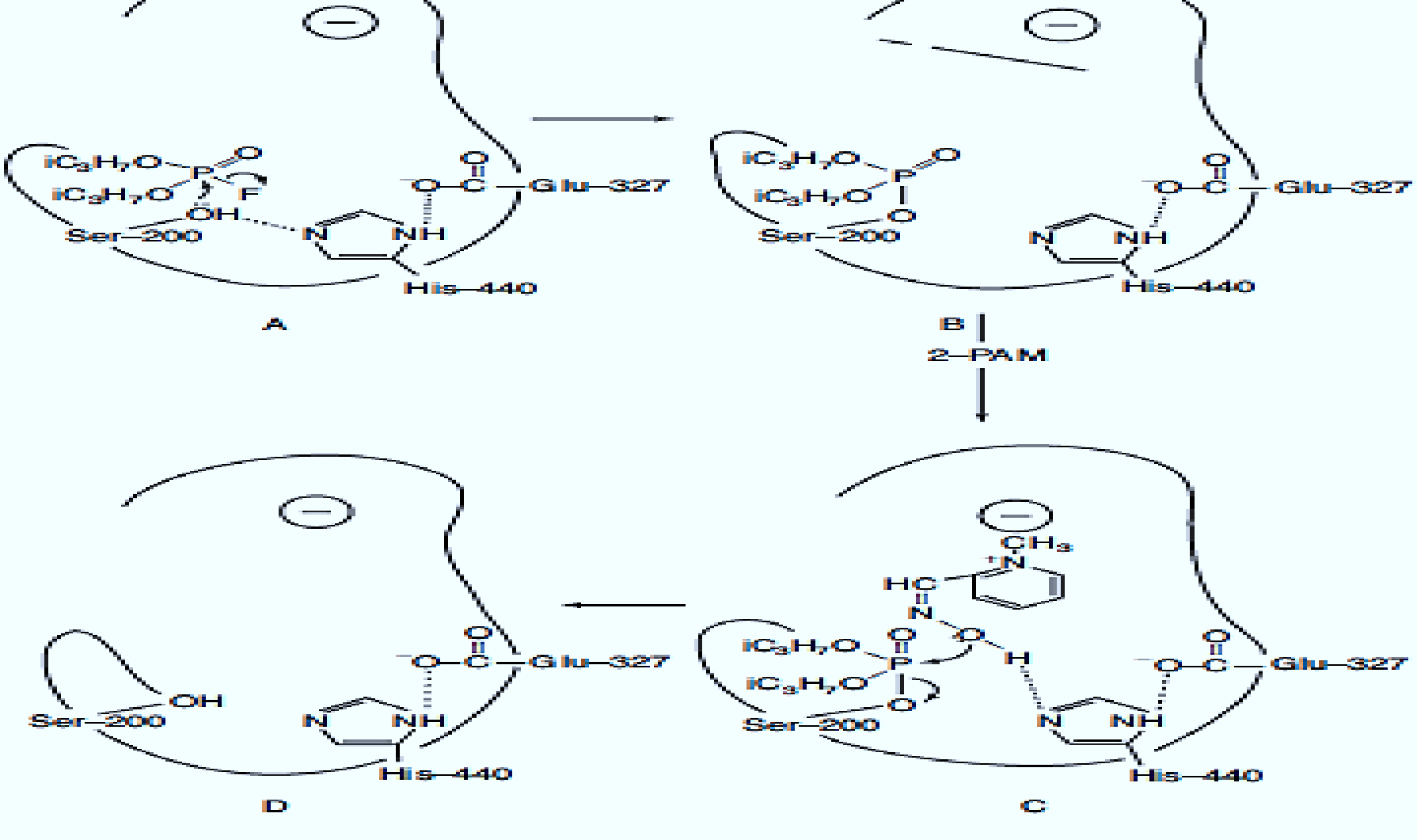
Inhibition of AChE

- Inhibition of AChE by organophosphorous compounds takes place in two steps:
  1. *Association of enzyme and inhibitor.*
  2. *The phosphorylation step.*

- Stereospecificity is mainly caused by interactions of enzyme and inhibitor at the esteratic site.
- The serine residue at the esteratic site forms a stable phosphoryl ester with the organophosphorous inhibitors.

Inhibition of AChE

- Insecticides and nerve gases are irreversible inhibitors of cholinesterases by forming a phosphorylated serine at the esteratic site of the enzyme.
- It is possible to reactivate the enzyme if action is taken soon after exposure to these poisons.
- Basically, insecticides must be toxic to insects and safe for humans.

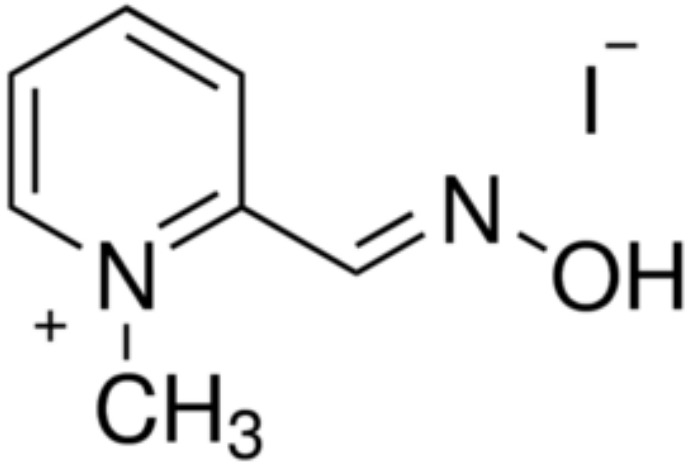


Phosphorylation and reactivation of cholinesterase. **A. Phosphorylation of serine by isofluorphate. B. Phosphorylated serine at esteratic site. C. Nucleophilic attack on phosphorylated residue by 2-PAM. D. free enzyme.**

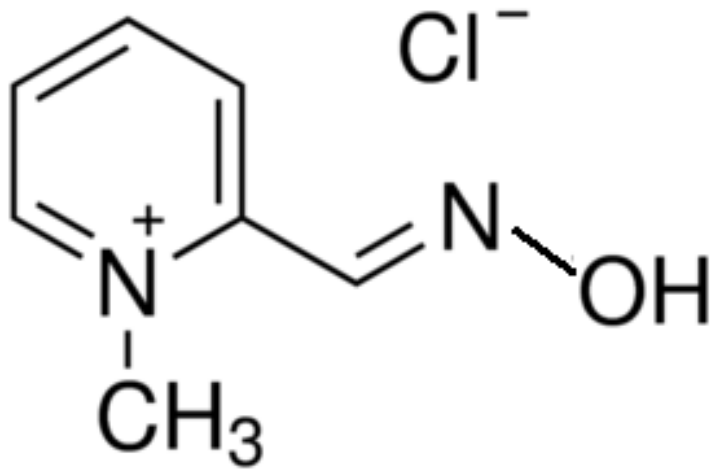
Cholinesterase reactivators

- *One of the most effective cholinesterase reactivators is pyridine-2-aldoxime methiodide (2-PAM)/ pralidoxime chloride.*
- *The biological half-life of pralidoxime chloride in humans is about 2 hours, and its effectiveness is a function of its concentration in plasma, which reaches a maximum 2 to 3 hours after oral administration.*
- *Pralidoxime chloride, a quaternary ammonium compound, is most effective by intramuscular, subcutaneous, or intravenous administration.*
- *Treatment of poisoning by an anticholinesterase will be most effective if given within a few hours.*

Cholinesterase reactivators



Pyridine-2-Aldoxime Methiodide (2-PAM)

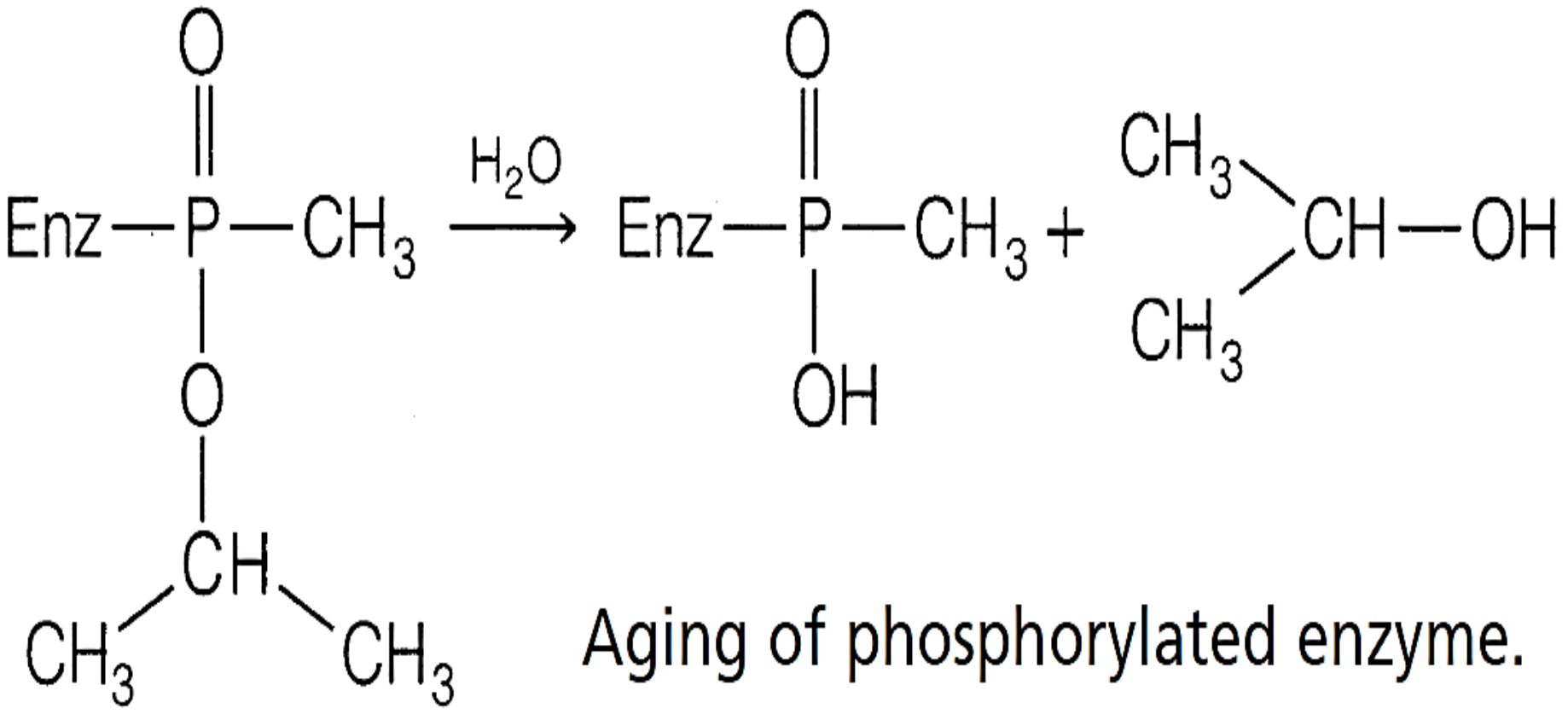


Pralidoxime Chloride.

Aging

- Cholinesterases that have been exposed to phosphorylating agents (e.g., sarin) become refractory to reactivation by cholinesterase reactivators.
- The process is called aging and occurs both in vivo and in vitro with AChE and BuChE.
- Aging occurs by partial hydrolysis of the phosphorylated moiety that is attached to the serine residue at the esteratic site of the enzyme.

Aging



Aging of phosphorylated enzyme.

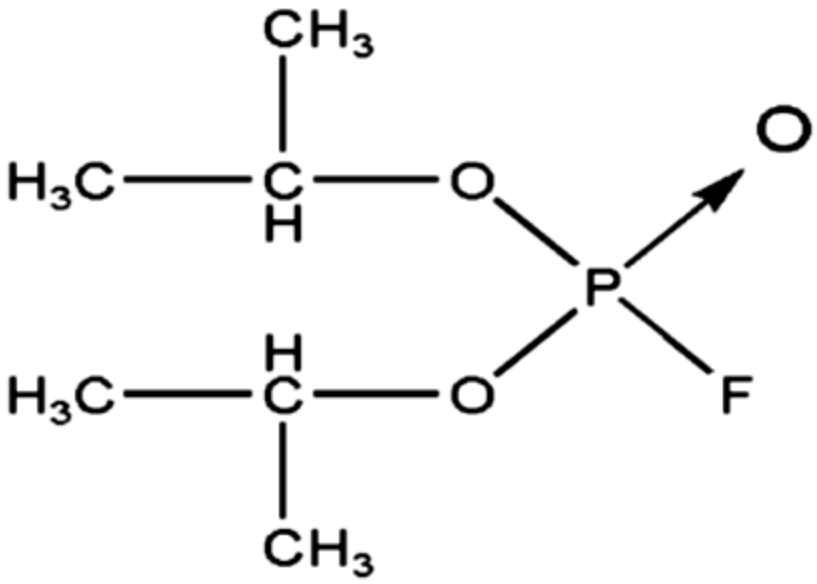
Aging

- The time it takes for a cholinesterase inhibitor bond to age varies. Some examples are:
  - Soman – 2 minutes
  - Sarin – 5 hours
  - VX – over 40 hours
- Early studies suggested that 2-PAM had to be given within 48 hours or aging would prevent it from working.
- However, it has been indicated that these studies were later proved to be flawed methodologically, and subsequent evidence suggests that 2-PAM can be effective long after 48 hours, **depending on** *the specific organophosphorous compound involved*.
- Furthermore, delayed onset of poisoning can result in delay of aging for days or even weeks.

Products

- **Isofluorphate, USP:**
- Isofluorphate is used in the treatment of glaucoma, however it becomes toxic in higher doses.
- The high lipophilicity of this drug resulted in ease of absorption via the intact skin.
- This recommends higher caution of the handling of isofluorphate.

- The toxicity of isofluorphate can be antagonized by atropine sulfate (antimuscarinic) as well as $MgSO_4$ to revert the action on nicotinic receptors.



Isofluorphate

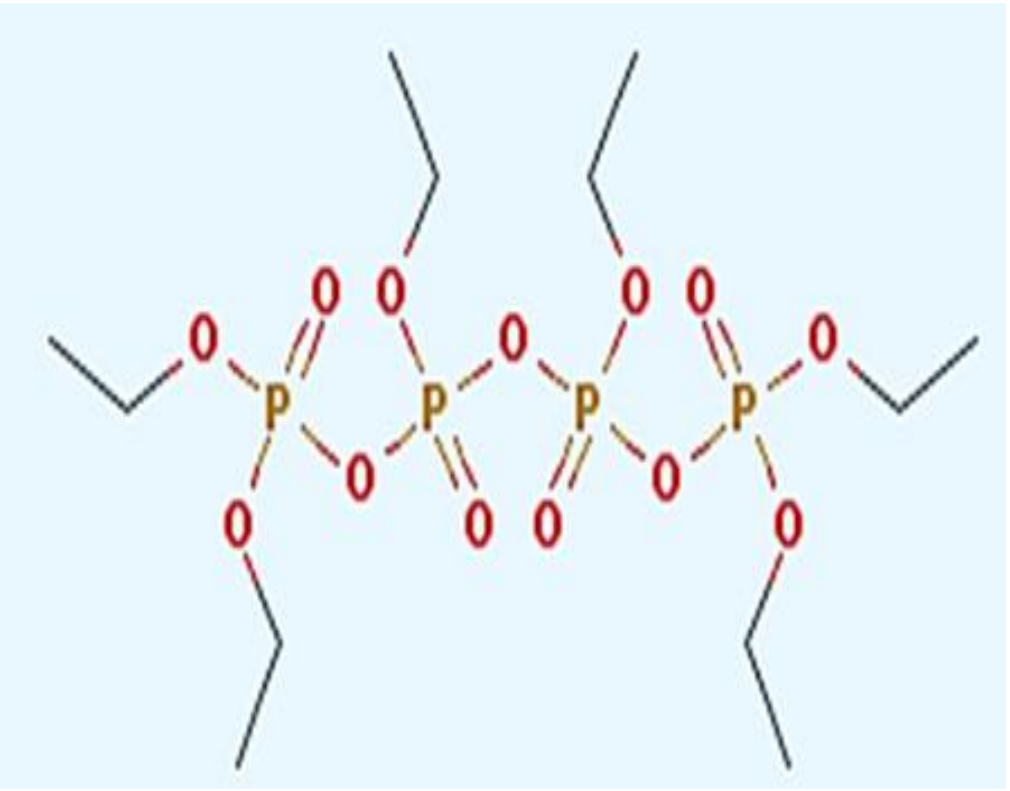
Echothiophate Iodide, USP:

- Echothiophate is a long-lasting cholinesterase inhibitor of the irreversible type.
- Unlike isofluorphate, Echothiophate is a quaternary salt, and when applied locally, its distribution in tissues is limited, which can be very desirable.
- It is used as a long-acting anticholinesterase agent in the treatment of glaucoma.

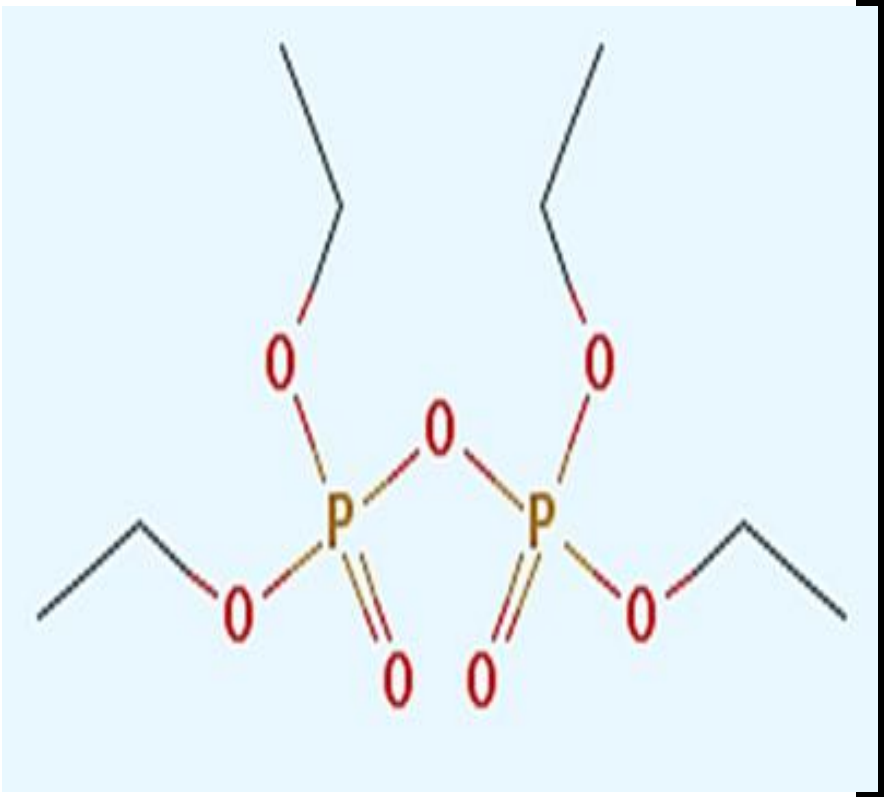
Hexaethyltetraphosphate (HETP) and Tetraethyl pyrophosphate (TEPP):

- HETP and TEPP are compounds that also show anticholinesterase activity.
- HETP was developed by the Germans during World War II and is used as an insecticide.
- When used as insecticides, these compounds have the advantage of being hydrolyzed rapidly to the relatively nontoxic; water-soluble compounds phosphoric acid and ethyl alcohol.
- Fruit trees or vegetables sprayed with this type of compound retain no harmful residue after a period of a few days or weeks, depending on the weather conditions.

Hexaethyltetraphosphate (HETP) and Tetraethyl pyrophosphate (TEPP):



(HETP)



(TEPP)

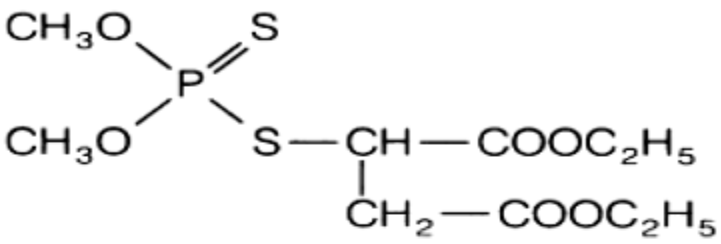
Malathion:

- Malathion is a water-insoluble phosphodithioate ester that has been used as an agricultural insecticide.
- Malathion is a poor inhibitor of cholinesterases.
- Its effectiveness as a safe insecticide is a result of the different rates at which humans and insects metabolize the chemical.
- Microsomal oxidation, which causes desulfuration, occurs slowly to form the phosphothioate (malaoxon), which is 10,000 times more active than the phosphodithioate (malathion) as a cholinesterase inhibitor.

Malathion:

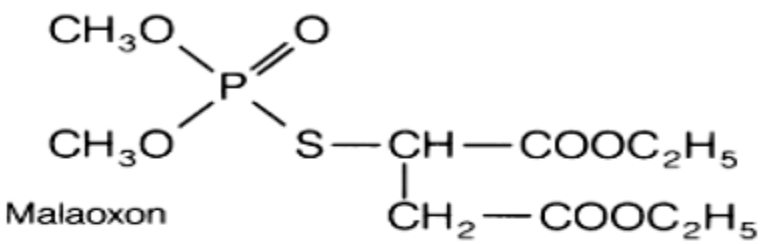
- Insects detoxify the phosphothioate by a phosphatase, forming dimethyl phosphorothioate, which is inactive as an inhibitor.
- Humans, however, can rapidly hydrolyze Malathion by a carboxyesterase enzyme, yielding Malathion acid, a still poorer inhibitor of AChE.
- Phosphatases and carboxyesterases further metabolize Malathion acid to dimethylphosphorothioate.
- The metabolic reactions are shown in the next slide:

Comparison of metabolism of malathion by mammals and insects.



Malathion (poor ChE inhibitor)

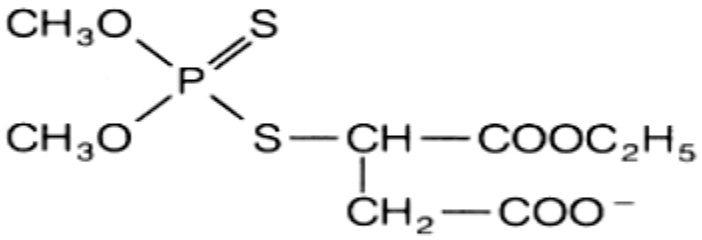
Activation
slow
Microsomal
oxid.



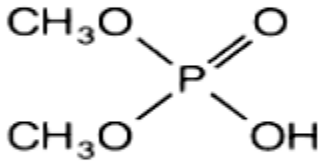
Malaoxon
(10,000 times more active as a ChE inhibitor)

Carboxyesterase
(rapid in mammals)

Phosphatase (insects)



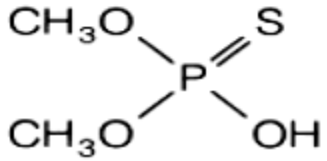
Malathion Acid (poor ChE inhibitor)



Dimethyl Phosphate
(inactive)

Phosphatases

Carboxyesterases



O,O-Dimethyl Phosphorothioate

Parathion:

- Parathion is used as an agricultural insecticide.
- It is decomposed at a pH above 7.5.
- Parathion is a relatively weak inhibitor of cholinesterase; however, enzymes present in liver microsomes and insect tissues convert parathion to paraoxon, a more potent inhibitor of cholinesterase.
- Parathion is also metabolized by liver microsomes to yield p-nitrophenol and diethylphosphate; the latter is inactive as an irreversible cholinesterase inhibitor.

References:

- Wilson and Gisvold's Textbook of Organic Medicinal And Pharmaceutical Chemistry, 12th Edition.