Doctor 021

PHARMACOLOGY

Doctor :Dr.Hamza

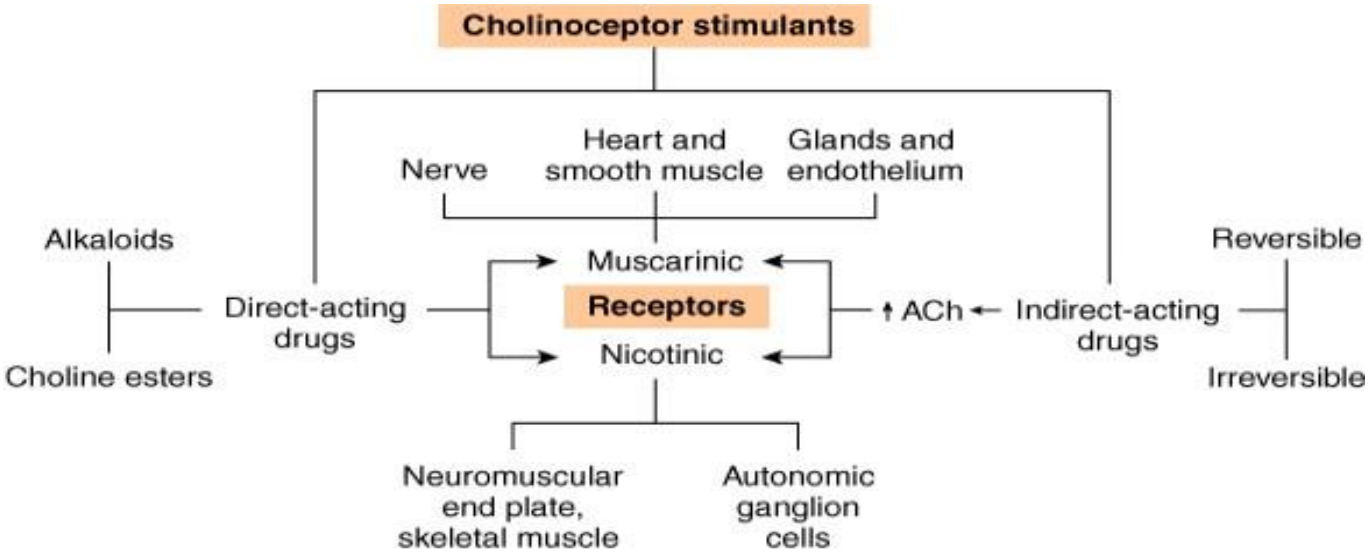
CHOLINOCEPTOR ACTIVATING & CHOLINESTERASE INHIBITING DRUGS

◆ Cholinoceptors stimulants include drugs that act on:

1. Muscarinic receptors (which are present in the nerves , heart , smooth muscles , glands and endothelium)
2. Nicotinic receptors (which are present in the neuromuscular end plate , skeletal muscle and autonomic ganglion cells)

◆ There are two types of drugs that are cholinoceptor stimulants:

1. Indirect acting drug 2. Direct acting drugs .



Source: Katzung BG, Masters SB, Trevor AJ: *Basic & Clinical Pharmacology, 11th Edition:* http://www.accessmedicine.com

1. INDIRECT ACTING DRUGS:

They perform their job by inhibiting Acetylcholinesterase (ACE), which is a very powerful enzyme; it hydrolyzes Acetylcholine (Ach) within milliseconds of its release. Inhibiting this enzyme increases the con. of ACh at the receptor site thus prolonging its effect (high effect). The drugs don't act directly on the receptor but they inhibit ACE therefore producing an effect similar to the activation of the cholinergic receptors directly.

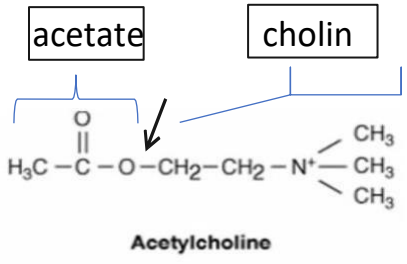
◆ There are two types of indirect acting drugs:

1. Reversible: Inhibit ACE for a short period of time (mins to hours).
2. Irreversible: Very toxic, they permanently inactivate the enzyme

1. DIRECT ACTING DRUGS:

A. Choline esters (which is Ach..we also have some drugs that are considered synthetic choline esters , like Carbachol, Methacholine..etc)

B. Alkaloids (eg.muscarine and nicotine)



◆ This is the structure of **Ach**,

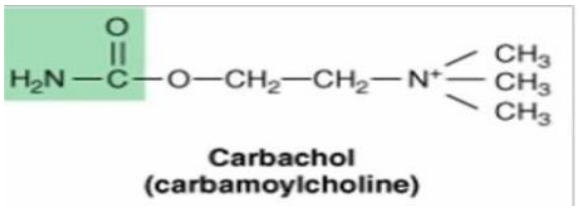
Note that the arrow points at the ester linkage

◆ ACh is not clinically useful by itself,

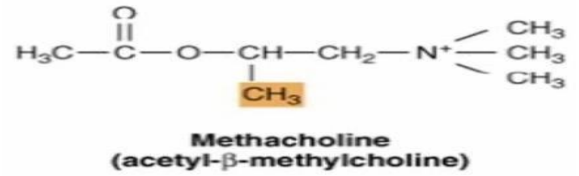
since you can't use it as a drug because it'll be degraded the moment it's injected as it has a very short lifetime. If you want to see the effect of Ach you have to give it by constant intravenous infusion (administration directly into a vein). The response obtained depends on the infusion rate.

◆ **Acetylcholine's structure** can be modified to produce new drugs that function better clinically. **Examples**:

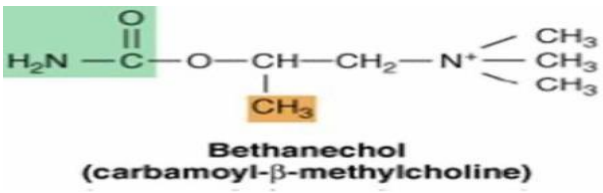
1. **Carbachol**: acetyl group is substituted with an amide group.



2. **Methacholine**: methyl group is added.



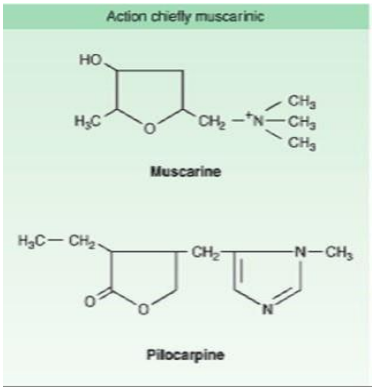
3. **Bethanechol**: a combination of the drugs above; A methyl group is added, and the acetyl group is substituted with an amide group.

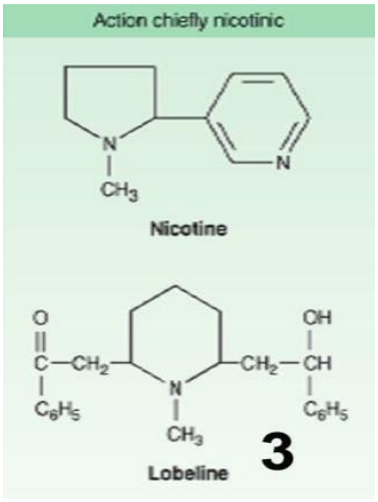


◆ **The effect of ACE on the drugs, and the effect of the drugs on muscarinic and nicotinic receptor**

Choline Ester	Degree of effect		
	by ACE	Muscarinic Receptors	Nicotinic Receptors
Acetylcholine	Extremely affected	Highly affects	Highly affects
	++++	+++	+++
Methacholine	Slightly affected	Extremely affects	No effect
	+	++++	
Carbachol	Not affected	Moderately affects	Highly affects
		++	+++
Benthanechol	Not affected	Moderately affects	No effect
		++	

◆ Methacholine has an extreme effect on the muscarinic receptors (remember , both words start with M)





◆ **Alkaloids :-**

Muscarine :

Muscarinic agonist

Pilocarpine :

Muscarinic agonist

Nicotine

Comes from tobacco(natural source)(nicotinic agonist)

Lobeline

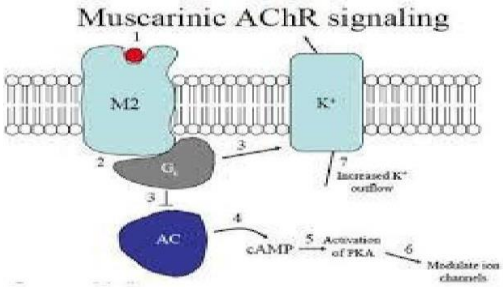
Comes from plant called lobelia (natural source) (nicotinic ago)

MECHANISM OF ACTION

◆ MUSCARINIC TRANSMISSION IN THE HEART

· The M2 receptor is the most abundant receptor in the heart

· Mechanism:



◆ **Ach activates M2 receptors which release Gi proteins**

◆ **Gi protein stimulates K+ channels to open**

◆ **This results in an outflux of K+ ions causing hyperpolarization**; the inside of the cell becomes more negative, so it is difficult to make depolarization inhibition effect on heart → (we need a stronger action potential to depolarize)

◆ **the voltage-dependent opening of pacemaker Na+ channels is shifted to more negative potentials** making them harder to open. Thus, we get less action potentials per unit time and so less contractility and heart rate

◆ ◆ **The phosphorylation of L-type Ca+2 channels is reduced**

M2R stimulates Gi protein → Open K+ channel → Hyperpolarization → ↓HR & ↓ force of contraction.

Gi protein also inhibits adenylyl cyclase AC:

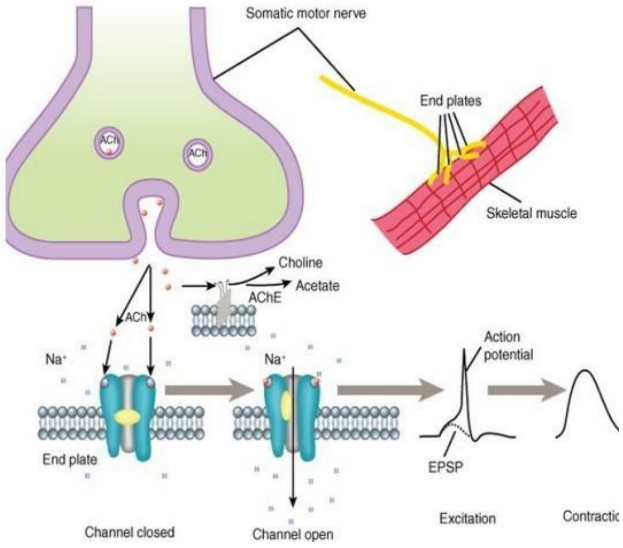
M2R stimulates Gi protein→↓adenylyl cyclase→ ↓cAMP formation

→↓phosphorylation of L-type Ca2+ channels →↓ their permeability to Ca+

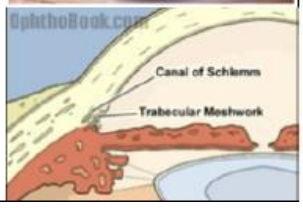
↓Heart rate & ↓ force of contraction

NICOTINIC TRANSMISSION AT NEUROMUSCULAR JUNCTIONS

1. **Released Ach binds to and opens the Na+ nicotinic receptors**
2. **This allows Na+ to flow into the cell, producing an excitatory postsynaptic potential (EPSP).**
3. **The EPSP depolarizes the muscle membrane, generating an action potential and triggering contraction.**
4. **After Ach dissociates, it is metabolized into choline and acetate by the extracellular Acetylcholinesterase (ACE).**



EFFECTS OF DIRECT-ACTING CHOLINOCEPTOR STIMULANTS:

Eye		
Sphincter muscle of iris (circular muscle)	Contraction – stimulation of miosis	
Ciliary muscle	• Contraction (for near vision) • Facilitation of Aqueous humor outflow into the canal of Schlemm.	

◆ Ciliary muscles have ligaments attached to the eye lenses and are affected parasympathetically only. When they are stimulated, they contract, pulling the ligaments, which causes the shape of lenses to change. In this case the lenses focus on near objects enabling the vision of near objects like when reading.

◆ Aqueous humor is the liquid inside the Eye

◆ In cases of glaucoma (increase intraocular pressure), these miotic (cholinergic) drugs can help to reduce intraocular pressure

Heart	
SA node	Decrease in rate (negative chronotropy)
Atria	Decrease in contractile strength (negative inotropy)
Atrioventricular node	• Decrease in conduction velocity (negative dromotropy) • Increase in refractory period.
Ventricles	Small decrease in contractile strength (because it is only sympathetic)

◆ in the negative dromotropy we have Less impulses pass through AV node (from atria to ventricles)

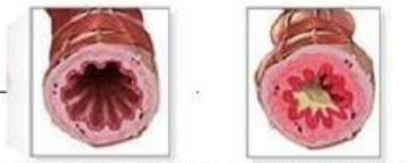
◆ refractory period: when no impulses can pass from the atria to the ventricle

◆ the ventricles have small amount of muscarinic receptors and that's why their contractile strength decreases a little bit (Symp < parasymp)

Blood vessels	
Arteries + Veins	Dilation via **Nitric Oxide**

◆ when an exogenous parasympathetic drug (injected) binds to their muscarinic receptors , they release NO (nitric oxide), which causes the vessel smooth muscles to relax, resulting in vasodilation (although they are activated by symp nerves and not innervated by parasympathetic nerves , they have muscarinic receptors)

Lung	
Bronchial muscle	Contraction (Bronchoconstriction)
Bronchial glands	Stimulation - increase bronchial secretion
GI tract	
Motility	Increase
Sphincter	Relaxation
Secretion	Stimulation
Urinary bladder	
Detrusor	Contraction
Trigone and sphincter	Relaxation (voiding of urine)
Glands	
Sweat, salivary, lacrimal, nasopharyngeal	↑ Secretion



Normal bronchiole Asthmatic bronchiole

◆ Detrusor: a muscle which forms a layer of the wall of the bladder.

◆ Bronchoconstriction resembles the state of asthmatic attack.

ORGAN SYSTEM EFFECTS

The effect of these drugs on the system as a whole: ·

Cardiovascular system (M2)

(The effect of Ach depends on the infusion rate of Ach)

◆ **Intravenous (IV) infusions of low doses of Ach will mostly affect the blood vessels.**

1. **Vasodilation**
2. This causes a **reduction in blood pressure (Hypotension)**
3. Which evokes the baroreceptor **reflex**
4. Resulting in an **increase in heart rate** to restore blood pressure to normal levels

◆ **Intravenous (IV) infusions of larger doses of Ach produce**

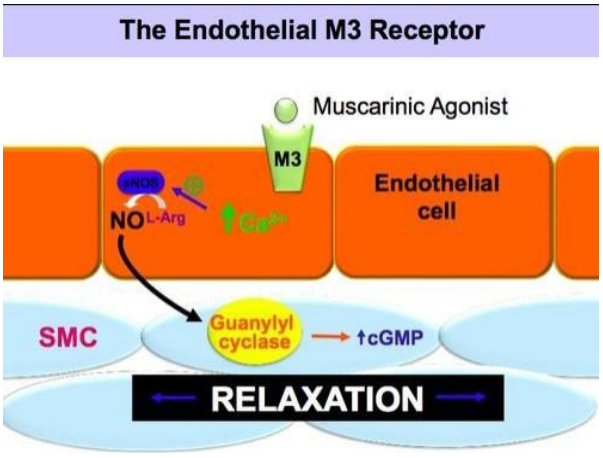
1. **Bradycardia (↓HR):** The direct effect of Ach on the M2 receptors overrides the Baroreceptor reflex thus reducing the heart rate
2. **Decreases in the AV node conduction velocity**
3. **Hypotension** due to vasodilation of blood vessels
4. **Decrease the contractility of atrium (mostly) & ventricle (minorly)**

■ **The direct slowing of sinoatrial rate & atrioventricular conduction is often opposed by reflex sympathetic discharge, elicited by the decrease in blood pressure and** …thus the heart rate might increase .

Mechanism of Muscarinic stimulation (M3 receptors)

***IV injection of muscarinic agonists produces marked vasodilation**:

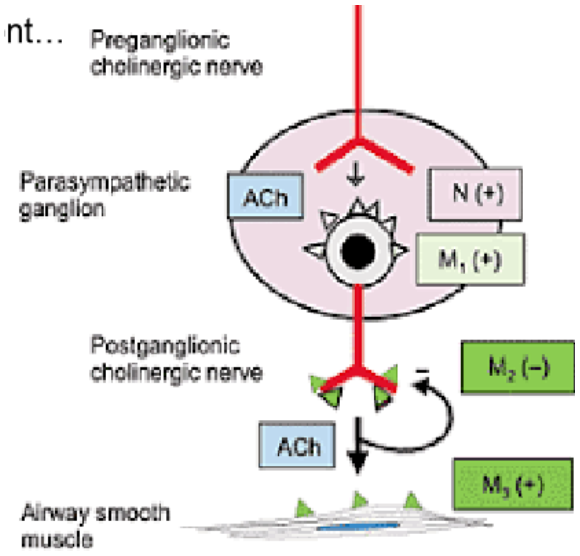
1. **Muscarinic agonists** bind to their receptors (M3)
2. This stimulates the **release of nitric oxide (NO) from the endothelial cells.**
3. **NO diffuses to adjacent vascular smooth muscle activating guanylyl cyclase**
4. Guanylyl cyclase **increases** intercellular **cGMP** concentration **resulting in muscle relaxation**



Pilocarpine: (An unexpected effect)

◆ **Natural alkaloid that may produce hypertension** – unexpected-**(vasoconstriction) after a brief initial** -expected - **hypotension (vasodilation).**

◆ **The longer-lasting hypertensive Effect is due to sympathetic ganglionic activation caused by activation of ganglionic M1 receptors, which elicit slow excitatory postsynaptic potentials. This effect, like the hypotensive effect, can be blocked by atropine, an antimuscarinic drug.**



◆ Autonomic ganglia have mainly nicotinic receptors, but they also have muscarinic receptors

Respiratory System:

◆ **Bronchoconstriction and increase in bronchial secretion** which resembles the acute attacks of bronchial asthma

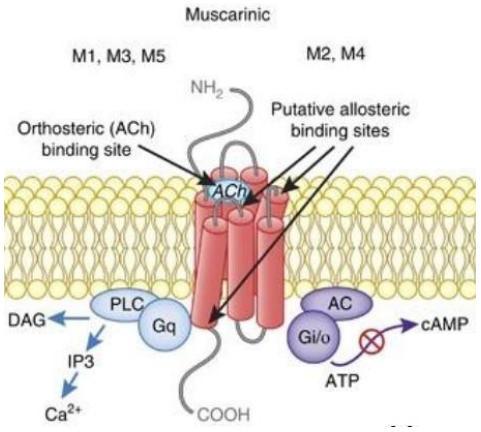
Gastrointestinal Tract:

Effects**:**

- **Increases the secretory and motor activity of the gut**
- **Salivary and gastric glands are strongly stimulated**, thus increasing the acidity of the stomach (in the past they used to give high doses of atropine to patients with peptic ulcer, which caused a lot of unpleasant side effects)
- **Peristaltic activity is increased**
- **Most sphincters are relaxed**

◆ **The M3 receptors** are the major receptors that **are required for direct activation of smooth muscle contraction.**

These receptors work through DAG and IP3, leading to increase in calcium ion concentration, which increases contractility in the intestine.



◆ **The M2 receptors cause inhibition of cAMP formation**, therefore **relaxation caused by sympathomimetic drugs** results
◆ Sympathetic system works through activation of cAMP
◆ M1 ,3 and 5 have similar effects (M3 is in the GI)
◆ M2 and M4 have similar effects (M2 is in the GI)

Genitourinary Tract:

◆ **Stimulate the detrusor muscle and relax the trigone and sphincter muscles of the bladder, thus promoting voiding.**
◆ **The function of M2 and M3 receptors in the urinary bladder is the same as in intestinal smooth muscle:**
◆ M3 receptors are required for the direct activation of smooth muscle contraction
◆ M2 receptors reduce cAMP formation & relaxation caused by sympathomimetic drugs
◆ **The human uterus is not sensitive to muscarinic agonists**

Miscellaneous Secretory Glands

◆ **Muscarinic agonists stimulate secretion of sweat, lacrimal, and nasopharyngeal Glands**

Central Nervous System:

◆ **The CNS contains both muscarinic and nicotinic receptors. The brain is richer in muscarinic sites** (receptors) **while the spinal cord contains more nicotinic sites** (receptors)
· Most directly acting muscarinic agents don't penetrate the blood brain barrier, but Pilocarpine does; because it's an alkaloid tertiary amine
◆ **Pilocarpine is used to induce chronic epilepsy in rats, to examine different treatments (M1 stimulation in the brain)** (when they want to try new antiepileptic drugs they need to make epileptic mice first)
◆ **Presynaptic nicotinic receptors regulate the release of several neurotransmitters** (in physiological concentrations).
◆ **In high concentrations nicotine induces tremor, emesis (vomiting), and stimulation of the respiratory center**.
◆ **At even higher levels, nicotine causes convulsions & fatal coma**

Autonomic ganglia:

◆ CVS

In the **CVS** (cardiovascular system), **the effects of nicotine are chiefly sympathomimetic** (act on the sympathetic ganglia) because blood vessels don't have parasympathetic nerve (only sympathetic)

** **Nicotine causes**:

Tachycardia which may alternate with bradycardia mediated by vagal discharge (due to baroreceptors) **and Hypertension.**

(It increases the vagus nerve activity)

◆ GIT and urinary tracts:

The effects are parasympathomimetic: nausea, vomiting, diarrhea, and voiding of urine.

Prolonged exposure to nicotine may result in depolarizing blockade of the ganglia, and the ganglia won't be stimulated again (is considered stimulant (small conc.) and depressant (high conc.))

◆ Neuromuscular Junction:

- **Nicotine applied directly stimulates nicotinic receptors producing contractile responses that vary from disorganized fasciculations** (each muscle fiber contracts on its own and they're not synchronized) **to strong contraction of entire muscles**.
- Nicotine is a stimulant in low concentrations and inhibitor (blocker) at high concentrations
- Under high concentrations, **nicotine causes rapid development of depolarization blockade.**
- **Transmission blockade persists even when the membrane has repolarized** (so even if the membrane got back to the potential it had before the drug acted on it, the blockade persists because desensitization occurred)
- **This latter phase of block is manifested as flaccid paralysis of skeletal muscle**

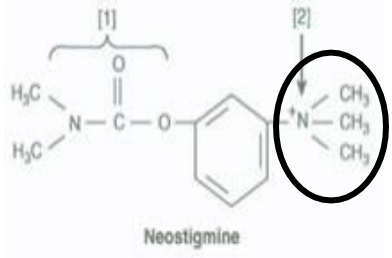
Indirect-Acting Cholinomimetics:

They inhibit the effect of Ach-esterase enzyme and increase conc of Ach

Reversible Cholinesterase Inhibitors:

1. **Neostigmine**: is the most commonly used drug, and **it's an ester composed of carbamic acid and a phenol bearing a quaternary ammonium group**.

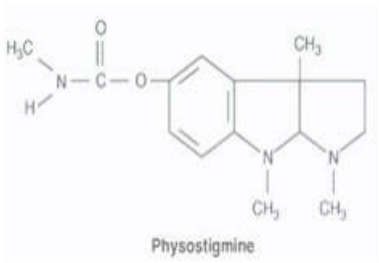
*in the circle you can see the quaternary ammonium Group (have +ve charge on the nitrogen which renders the compound more water soluble, which affects penetration and absorption)



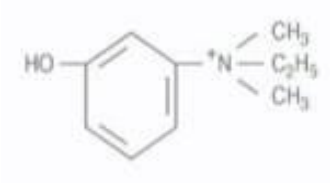
2. **Physostigmine**: **a naturally occurring carbamate**, it's an alkaloid from a plant called Calabar bean, and **is a tertiary amine.**

--Is highly lipid soluble, gets absorbed very well, penetrates the blood brain barrier and its effect is central and peripheral.

--which makes it very dangerous and poisonous drug, that's why it's not used for systemic uses at all except in certain cases.



3.**Edrophonium**: **not an ester but it binds to the active site of the enzyme** (acetylcholinesterase) by electrostatic and hydrogen bonds.



Metabolism of Acetylcholine

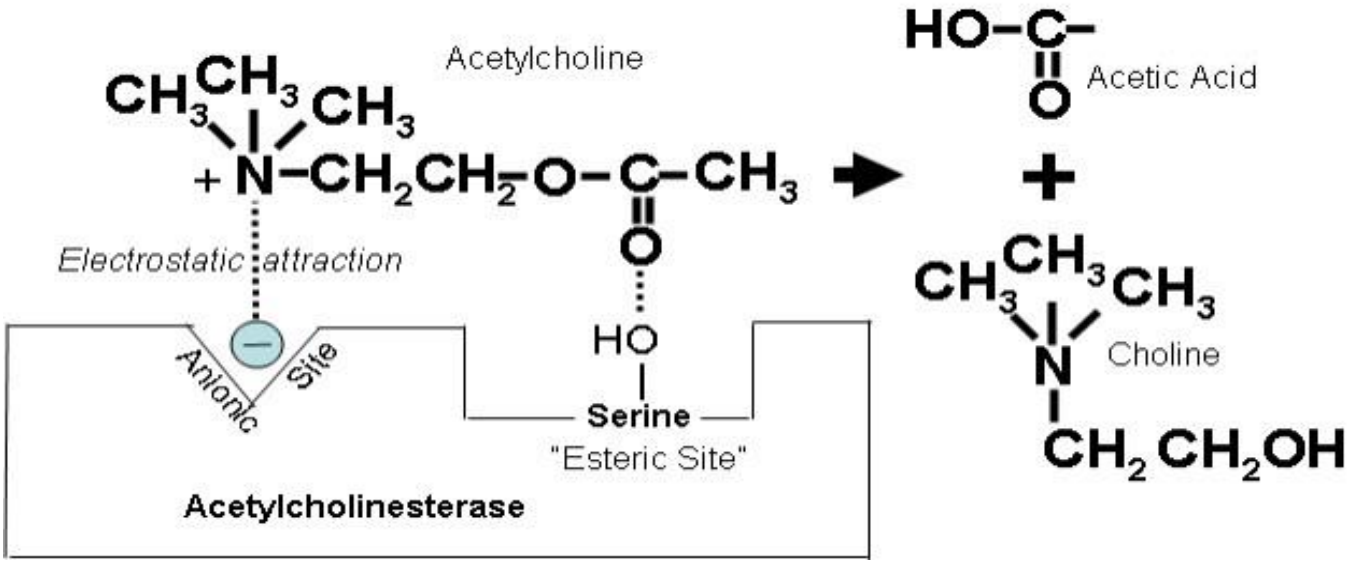
*Acetylcholine esterase is a macromolecule that contains many active units, and each unit has many site

*The active site of acetylcholinesterase comprises 2 subsites, the anionic site (has a negative charge) and the esteric site (contains AA serine) and the distance between these 2 subsites is the same as the distance between the positively charged nitrogen and the carbonyl carbon of acetylcholine

*Ach is normally broken down by the enzyme acetylcholinesterase to acetic acid and choline

* Since acetylcholine is positively charged because of the quaternary ammonium group, an electrostatic attraction happens between the positive charge of acetylcholine and the negative charge of the anionic site.

* This brings Ach very close to the active unit , and the alignment between them allows the hydroxyl group of serine to interact with the oxygen of the carbonyl group forming a hydrolysable covalent bond, and then a hydrolysis reaction(breaking the ester bond) is catalyzed by the esteric site to form choline and acetic acid .
* This process occurs very fast within milliseconds



◆ **The positively charged nitrogen in the acetylcholine molecule is attracted to the ionic site on acetylcholinesterase, and hydrolysis is catalyzed at the esteric site to form choline and acetic acid.**

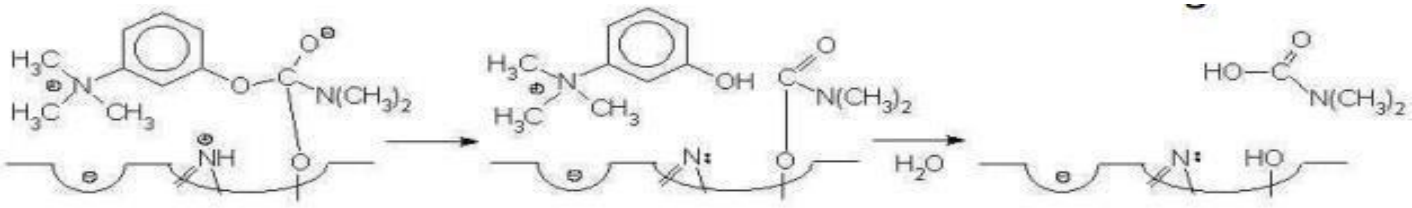
*Watch this to understand better
https://www.youtube.com/watch?v=8Wluv1kKGUM

◆ ◆ Inhibitors of acetylcholinesterase :

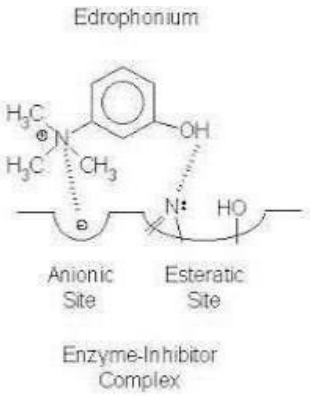
Neostigmine:

◆ It undergoes a two-step hydrolysis sequence similar to acetylcholine, it inhibits acetylcholinesterase by interacting with it in the same way acetylcholine does (the distance between N and C is also similar to acetylcholine), by forming an ionic bond and a hydrolysable covalent bond at the esteric site, but there is a difference in their second hydration process after the hydrolysis of ester bond.
◆ The covalent bond of neostigmine of the esteric site is more resistant to the hydrolysis reaction (last step which is the second hydration after hydration of ester bond) which makes it prolonged by 30 minutes to 6 hours, and during this time acetylcholinesterase cannot metabolize other acetylcholine molecules , and when the bond is broken , the enzyme can resume its activity .

Stabilized by an ionic bond at the anionic site and a hydrolyzable covalent bond at the esteratic site, e.g.

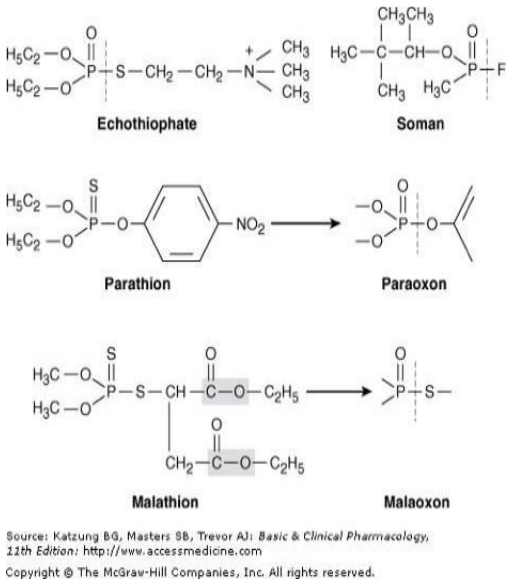


Edrophonium



It isn't an ester but it can still bind to acetylcholinesterase and inhibit it by forming an **ionic bond at the anionic site and a weak hydrogen bond at the esteric site** thus stabilizing it briefly and then it gets detached, this enzyme inhibitor complex doesn't involve a covalent bond and is short lived (2-10 minutes

Irreversible Cholinesterase Inhibitors:



*They are also known as Organophosphates (composed of pentavalent phosphorus).

*These drugs are very dangerous, examples include nerve gases .

Here are a few examples:

Echothiophate

Soman (it is a toxic nerve agent)

Parathion → Paraoxon (insecticide)

Malathion → Malaoxon (insecticide)

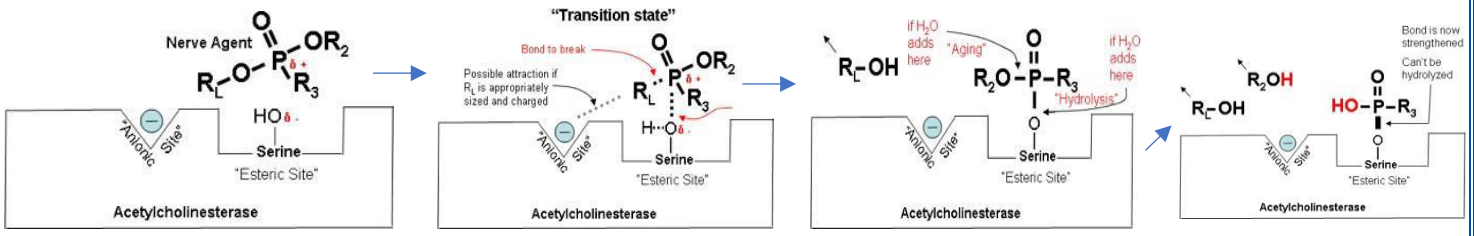
The dashed lines indicate the bond that is hydrolyzed in binding to the enzyme. The shaded ester bonds in malathion represent the points of detoxification of the molecule in mammals and birds.

** NOTE ;- The sulfur in Parathion and Malathion has to be replaced by oxygen in order for the drug to be active and so they are converted to Paraoxon and Malaoxon for them to kill the insects.

* The interaction between these organophosphates and acetylcholinesterase also depends on charges, the phosphorus atom in these organophosphates is partially

positive which directs the drug towards the active sites (partial negative charge) and breaks the bond between the phosphorus atom and the R group allowing it to leave as a free radical as well as forming a very strong bond (hydrolyzed after 100 hours!!) between the oxygen of serine and the same phosphorus atom.

*A phenomenon called Aging happens after few hours or few mins (this depends on the type of the drug), to prevent the hydrolysis of the P-O bond on the esteric site, and ultimately converting the inhibited enzyme into a non-reactive form (it further strengthens the phosphorus-enzyme bond) and another radical is released

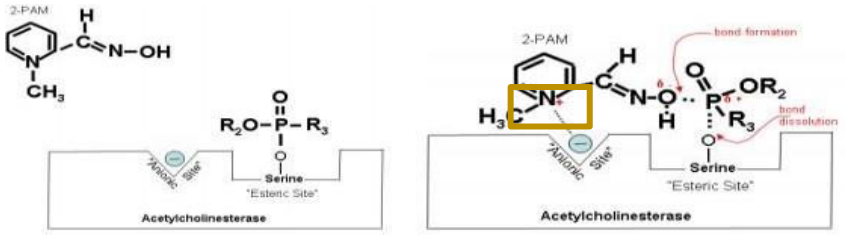


◆ **A cholinesterase inhibitor attaches to the serine hydroxyl group on ACh.E. This prevents acetylcholine from interacting with the cholinesterase enzyme and being broken down.**

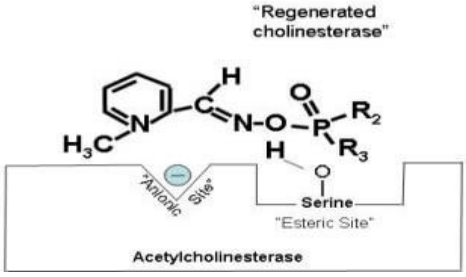
Now the enzyme is damaged and the body has to make a new one , these drugs are very toxic

Pralidoxime (2-PAM):

*can regenerate the enzyme if given before aging has taken place .



*it is able to break the phosphorus-enzyme bond making it a "cholinesterase regenerator" drug to counter organophosphate insecticide poisoning



*The attraction between the +ve charge (in the square) and the -ve charge in the anionic site brings 2- PAM close to the phosphorous moiety and this causes interaction between the 2 molecules resulting in breaking the phosphorus-enzyme bond and freeing the enzyme.

Absorption, Distribution and Metabolism:

◆ Absorption of the **quaternary carbamates** (like Neostigmine and Edrophonium) from the conjunctiva (a thin membrane that covers the front surface of the eye), skin, and lungs is poor, since their permanent charge renders them **relatively insoluble in lipids** (highly polar) **due to their positive charge** . **Thus, much larger doses are required for oral administration than for parenteral injection** and their distribution into the CNS is negligible as they can't penetrate the blood-brain barrier.

◆ On the other hand, **Physostigmine**, unlike the other reversible inhibitors, **is well absorbed from all sites and can be used topically in the eye, it is distributed into the CNS** (because it can pass through the blood-brain barrier so it has peripheral and central actions) **and is more toxic than the more polar quaternary carbamates.**

◆ **The carbamates metabolized by nonspecific esterases and by cholinesterase**.

◆ **The duration of their effect is determined chiefly by the stability of the inhibitor-enzyme complex** (the intermediate), **not by metabolism or excretion**.

◆ **Organophosphates are also well absorbed from the skin, lung, gut, and conjunctiva, thereby making them dangerous to humans and highly effective as insecticides.**

◆ **Parathion and Malathion must be activated in the body by conversion to the oxygen analogs** as we mentioned before.

THERAPEUTIC USES AND DURATIONS OF ACTION OF CHOLINESTERASE INHIBITORS

Endrophonium duration of action is relatively short because it doesn't form a stable covalent bond with the esteric site

Myasthenia gravis:muscle weakness

Ileus: is a term for the lack of peristaltic movement

Since physostigmine is toxic, it's only used topically in the eye to treat glaucoma (high intraocular presser)

Therapeutic Uses and Durations of Action of Cholinesterase Inhibitors

	Uses	Approximate Duration of Action
Alcohols		
• Edrophonium	Myasthenia gravis, ileus,	5–15 minutes
Carbamates and related agents		
• Neostigmine	Myasthenia gravis, ileus	0.5–2 hours
• Pyridostigmine	Myasthenia gravis	3–6 hours
• Physostigmine	Glaucoma	0.5–2 hours
• Ambenonium	Myasthenia gravis	4–8 hours
• Demecarium	Glaucoma	4–6 hours
Organophosphates		
• Echothiophate	Glaucoma	100 hours

GOOD LUCK

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