12

Pharmacology

Doctor 2018 | Medicine | JU

◉ Sheet ○ Slides

Done By

Mohammad Abu Zalam

Contributed in the Scientific Correction

عبد الرحمن جبر

Contributed in the Grammatical Correction

عبدالله أسامة

Doctor

Dr. Hamzeh

❖ Indirect-Acting Cholinomimetics:

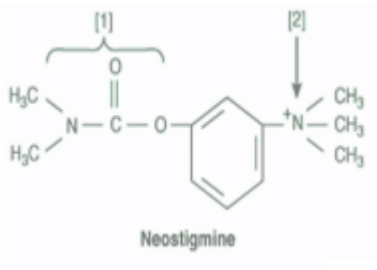
The overall mechanism of action here is to increase the concentration of endogenous Acetylcholine at cholinoceptors by inhibiting the enzyme that breaks it down.

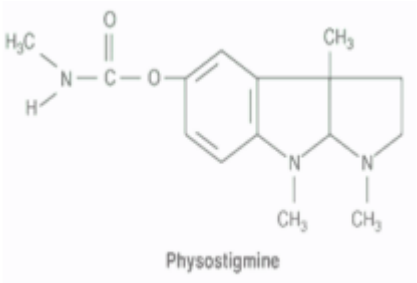
1. Reversible Cholinesterase Inhibitors:

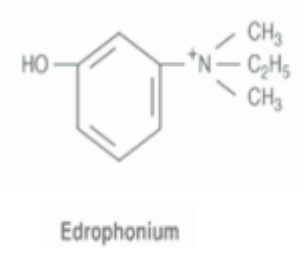
a. Neostigmine: an ester composed of carbamic acid [1] and a phenol bearing a quaternary ammonium group.

b. Physostigmine: a naturally occurring carbamate, it is a tertiary amine.

c. Edrophonium: not an ester but it binds to the active site of acetylcholinesterase







Acetylcholine is normally broken down by the enzyme acetylcholinesterase to acetic acid and choline.

The active site of acetylcholinesterase comprises 2 subsites, the anionic site (has a negative charge) and the esteratic 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.

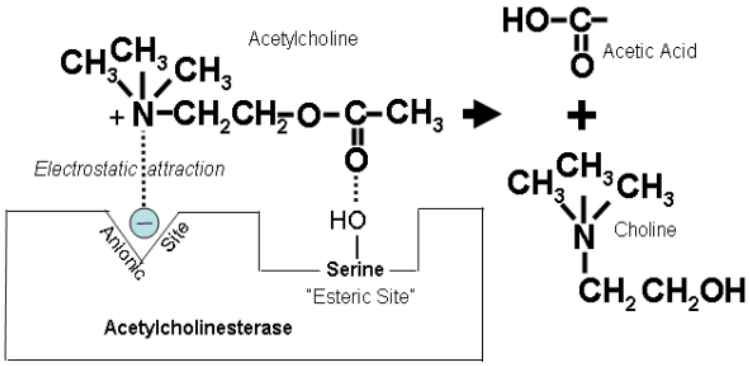
Since acetylcholine is positively charged because of the quaternary ammonium group, an electrostatic attraction happens between the positive charge of acetylcholine and the negative charge of the anionic site, and the alignment between them allows the hydroxyl group of serine to interact with the oxygen of the carbonyl group forming a hydrolysable covalent bond, and then a hydrolysis reaction is catalyzed by the esteratic site to form choline and acetic acid.

Neostigmine undergoes a two-step hydrolysis sequence similar to acetylcholine, it inhibits acetylcholinesterase by interacting with it in the same way acetylcholine does

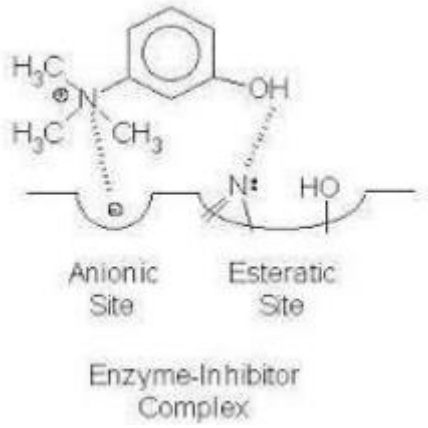
(the distance between N and C is also similar to acetylcholine), by forming an ionic bond and a hydrolysable covalent bond at the esteratic site, but there is a difference in their second hydration process after the hydrolysis of ester bond.

The covalent bond of neostigmine of the esteratic 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 can not metabolize other acetylcholine molecules.

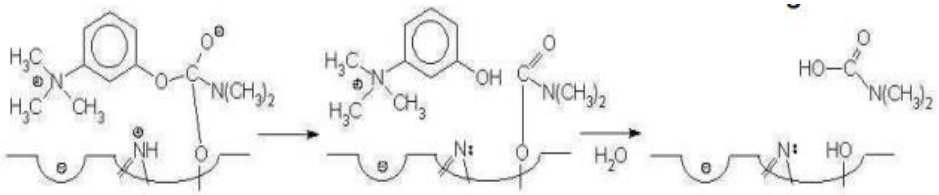
Edrophonium isn't an ester but it can still bind to acetylcholinesterase and inhibit it by forming an ionic bond at the anionic site and a weak hydrogen bond at the esteratic site thus stabilizing it briefly and then it gets detached, this enzyme inhibitor complex doesn't involve a covalent bond and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



Metabolism of Acetylcholine



Inhibition by Edrophonium



Inhibition by Neostigmine

2. Irreversible Cholinesterase Inhibitors:

They are also known as **Organophosphates** (composed of pentavalent phosphorus), here are a few examples:

a. Echothiphate

b. Soman (it is a toxic nerve agent)

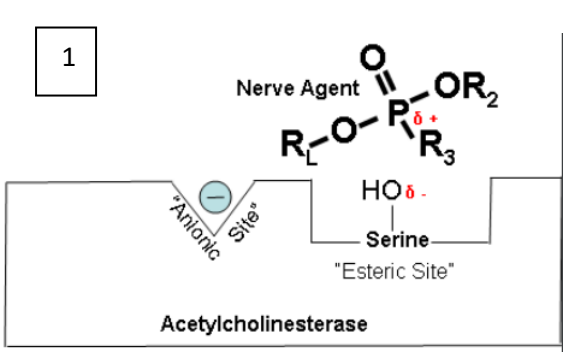
c. Parathion → Paraoxon

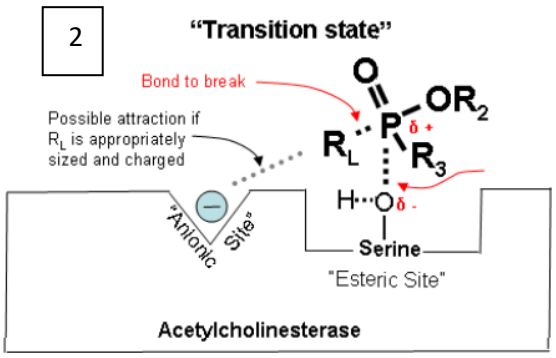
d. Malathion → Malaoxon

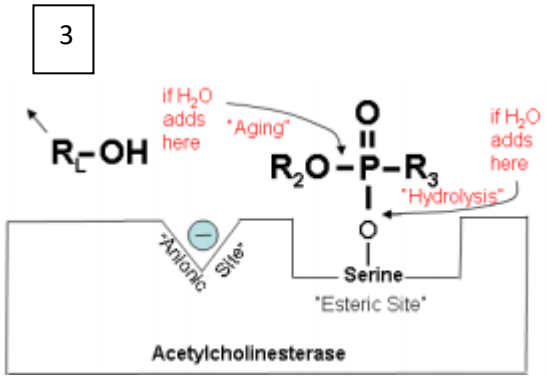
Note: The sulfur in Parathion and Malathion has to be replaced by oxygen in order for the drug to be active and so they are converted to Paraoxon and Malaoxon.

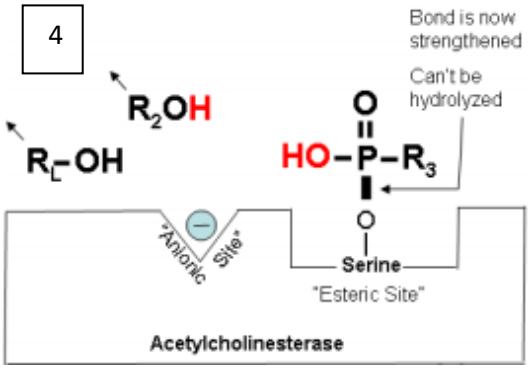
The interaction between these organophosphates and acetylcholinesterase also depends on charges, the phosphorus atom in these organophosphates is partially positive (not as strong as the positive charge of quaternary ammonium) which directs the drug towards the active sites and breaks the bond between 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** (which is a dealkylation reaction [removal of R2O-P bond in this case] of the organophosphate bound to the enzyme) happens, preventing the hydrolysis of the P-O bond on the esteratic site ultimately converting the inhibited enzyme into a non-reactivable form (it further strengthens the phosphorus enzyme bond).

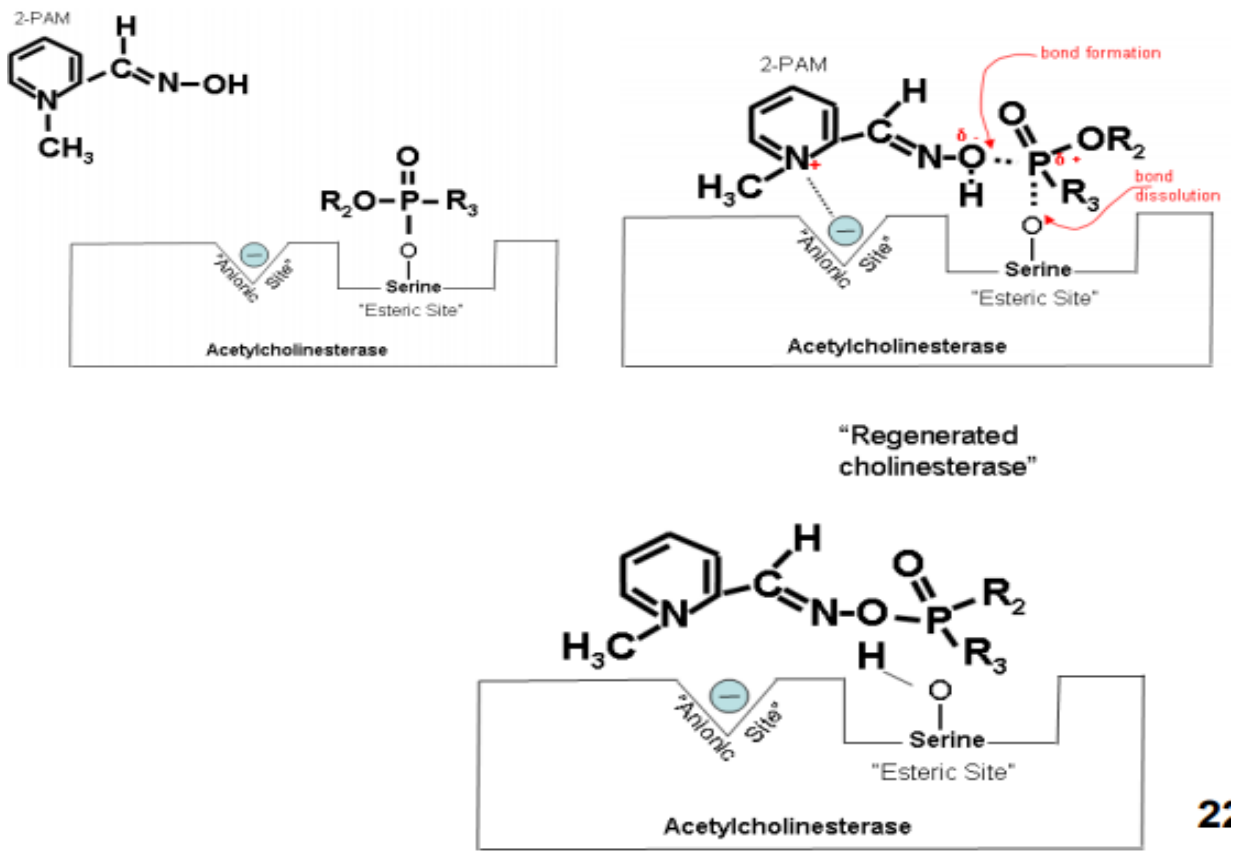








Pralidoxime (2-PAM) can be given before aging occurs, it is able to break the phosphorus-enzyme bond making it a "cholinesterase regenerator" drug to counter organophosphate insecticide poisoning (aging occurs within 10 minutes with the chemical warfare agent, soman, and in 48 hours with the nerve agent VX which is also an organophosphate). The figure in the next page shows how it happens, we are basically saving the enzyme.



- **In summary**: **(what is written in the slides)**
  -The organophosphates undergo initial binding and hydrolysis by the enzyme, resulting in a phosphorylated active site.

  -The covalent phosphorus-enzyme bond is extremely stable and hydrolyzes in water at a very slow rate (hundreds of hours).

-After the initial binding-hydrolysis step, the phosphorylated enzyme complex may undergo a process called aging.

-Aging involves the breaking of one of the oxygen-phosphorus bonds of the inhibitor and further strengthens the phosphorus-enzyme bond.

-Pralidoxime, if given before aging has occurred, is able to break the phosphorus-enzyme bond and can be used as a ‘’cholinesterase regenerator" drug for organophosphate insecticide poisoning.

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). 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 **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

	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

Notes about the figure above:

-Myasthenia gravis is a long-term neuromuscular autoimmune disease that leads to varying degrees of skeletal muscle weakness. It is caused by antibodies attacking nicotinic receptors in motor end plates as well as increasing the space between the folds of it, It can result in double vision, drooping eyelids, trouble talking, and trouble walking.

-Glaucoma is a group of eye conditions that damage the optic nerve caused by an abnormally high pressure in your eye and irregular aqueous humor filtration.

-Ileus is a term for the lack of movement somewhere in the intestines that leads to a buildup and potential blockage of food material. An ileus can lead to an intestinal obstruction. This means no food material, gas, or liquids can get through. It can occur as a side effect after surgery.

❖ Organ System Effects:

1. Central Nervous System:

Drugs that can reach the CNS like Organophosphates and Physostigmine (the lipid-soluble cholinesterase inhibitors):

- In **low concentrations**, they cause a subjective alerting response
- In **higher concentrations**, they cause generalized **convulsions**, which may be followed by coma and respiratory arrest

2. Eye, Respiratory Tract, GIT and Urinary Tract:

The effects are qualitatively similar to the effects of the direct-acting cholinomimetics such as GI increased function, increased secretions, diarrhea, cramps and urinating.

3. Cardiovascular System:

Mimic the effects of vagal nerve activation (parasympathetic) on the heart, negative **chronotropic**, **dromotropic** and **inotropic effects** and cardiac output falls

The fall in cardiac output is due to bradycardia (chronotropic), decreased atrial contractility (dromotropic) and some reduction in ventricular contractility (inotropic).

The reduction in ventricular contractility occurs as a result of prejunctional inhibition of NE release (by heteroreceptors such as M2 receptors).

They have minimal effects by direct actions on vascular smooth muscles because most vascular beds lack cholinergic innervations (they are not innervated by parasympathetic nerves thus there is no acetylcholine).

The net cardiovascular effects of moderate doses of cholinesterase inhibitors consist of:

- A modest bradycardia
- A fall in cardiac output
- An increased vascular resistance (sympathetic ganglion stimulation because of acetylcholine receptors on the post synaptic nerves) that result in a rise in blood pressure.

4. Neuromuscular Junction:

- In **low concentrations**, the action of ACh is intensified and prolonged, this increases the strength of contraction, especially in muscles weakened by curare-like neuromuscular blockers or by myasthenia gravis.

Neuromuscular blockers block nicotinic receptors on muscles thus preventing the action of ACh, and we have 2 types: depolarizing neuromuscular blockers and non-depolarizing neuromuscular blockers.

Succinylcholine is an example of a depolarizing neuromuscular blocker and it causes a sustained depolarization period because it doesn't breakdown as easily as ACh leading to muscle contraction then muscle paralysis (it functions as ACh receptor agonist)

Curare is an example of a non-depolarizing neuromuscular blocker and it competes with ACh for the binding with nicotinic receptors to prevent it's function leading to muscle relaxation and paralysis (it functions as competitive antagonists to ACh and it can be overcome by increasing ACh concentrations)

- In **higher concentrations**, fibrillation of muscle fibers is the result, **Antidromic firing** (nerve impulses in a direction opposite to normal) of the motor neuron may also occur, resulting in fasciculations (brief spontaneous contractions) that involve an entire motor unit.

  (Some **quaternary carbamate cholinesterase inhibitors, e.g., neostigmine**, have an additional direct nicotinic agonist effect at the neuromuscular junction. This may contribute to the effectiveness of these agents as therapy for myasthenia [helps muscles regain strength]).

❖ Clinical Uses of AChEIs:

1. The Eye:

Glaucoma (which is an increase in the intraocular pressure of the eye as a result of the amount of aqueous humor exceeding the amount of drainage) was treated with pilocarpine which was the standard drug used for it's treatment as well as drugs like methacholine, carbachol or ChEIs; physostigmine, demecarium, echothiophate, isoflurophate.

Even though these drugs were effective, they had a negative effect on vision causing **miosis** leading to patients unable to see in well lit places, as well as a **spasm of accommodation** for near objects (ciliary muscle of the eye remains in constant state of contraction).

These drugs were soon replaced with **topical β-blockers** and **prostaglandin derivatives**, which had the same effectivity without interfering with vision.

There are two types of glaucoma: **Acute angle-closure glaucoma and Chronic open-angle glaucoma,** an acute angle-closure glaucoma could happen due to trauma to the eye due to an accident for example, this causes the blockage of filtration in the eye and prevents the aqueous humor to move from iris to canal of Schlemm or towards cornea causing a buildup of pressure.

Acute angle-closure glaucoma is a medical emergency that usually requires **surgery,** but before surgery, initial therapy consists of a combination of a direct muscarinic agonist and a cholinesterase inhibitor such as Pilocarpine plus Physostigmine.

2. GI and Urinary Tract:

For people who suffer from **postoperative ileus** (atony or paralysis of the stomach or bowel movement following surgical manipulation) and **congenital megacolon**, these drugs can be given to stimulate stomach and intestine activity.

Some people also suffer from **urinary retention** postoperatively or postpartum or secondary to spinal cord injury or disease (neurogenic bladder) these drugs can be given to stimulate the parasympathetic nerves which are responsible for bladder contraction and urination.

Bethanechol and **Neostigmine** are the most widely used, but it must be certain that there is no mechanical obstruction to outflow before using the cholinomimetic agents or this for example might cause a rupture in a case of kidney stones in urethra due to contractions and severe pushing of the stone.

Pilocarpine has been long used to increase salivary secretions in cases of dry mouth, as well as **Cevimeline (a direct-acting muscarinic agonist)**, in cases of treatment of dry mouth associated with Sjögren's syndrome which is a systemic autoimmune disease that causes your immune system to damage salivary glands.

3. Neuromuscular Junctions:

Myasthenia gravis (which we already discussed before) is an autoimmune disease affecting skeletal muscle neuromuscular junctions. Antibodies are detected in 85% of myasthenic patients (they attack the nicotinic receptors and increase the gap between the muscle end plate and the neuron leading to loss of ACh).

The antibodies reduce nicotinic receptor function and severe disease may affect all the muscles, including those necessary for respiration.

The first early symptoms (frequent findings) are:

- Ptosis (drooping of upper eyelid)
- Difficulty in speaking and swallowing
- Diplopia (double vision)
- Extremity weakness

The disease resembles the neuromuscular paralysis produced by d-tubocurarine

Patients with myasthenia gravis are very sensitive to the action of neuromuscular blockers and other drugs that interfere with neuromuscular transmission which is very dangerous, e.g., aminoglycoside antibiotics

Patients with ocular myasthenia may be treated with cholinesterase inhibitors alone, but patients having more widespread muscle weakness can be treated with immunosuppressant drugs that stop the production of antiboides (steroids, cyclosporine, and azathioprine). In some patients, the thymus gland is removed.

Edrophonium is not used for treatment, but it is used as a diagnostic test for myasthenia gravis in which a 2 mg dose of it is injected IV, If the patient has myasthenia gravis, an improvement in muscle strength that lasts 5 minutes can be observed (short active drug), if not then weakness in muscle is caused by another disease.

It is also used to assess the adequacy of treatment with the longer-acting cholinesterase inhibitors in patients with myasthenia gravis. Clinical situations in which severe myasthenia **(myasthenic crisis),** caused by lower doses that must be increased for the therapy to effective, must be distinguished from excessive drug therapy caused by higher doses **(cholinergic crisis).**

To sum up, what you should understand from this lecture:

1- there are two types of indirect AChEIs:

- Reversible: Edrophonium, Physostigmine, Neostigmine
- Irreversible (organophosphates): Ecothiophate, Soman, Malathion, Parathion

2- Aging converts AChE from an inhibited form to non-reactivable form but aging can be stopped by using Pralidoxime (2-PAM)

3- Depending on their polarity and charges these drugs can be distributed to the CNS and peripherally or peripherally only

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

5- AChEIs have multiple effects on organs and their functions depending on their distribution

6- AChEIs have multiple clinical uses according to their mechanism of work and the nature of the disease

THIS SHEET WAS WRITTEN ACCORDING TO LECTURE 12 FOR SECTION 3

GOOD LUCK