◉ Sheet ○ Slides

number : 4

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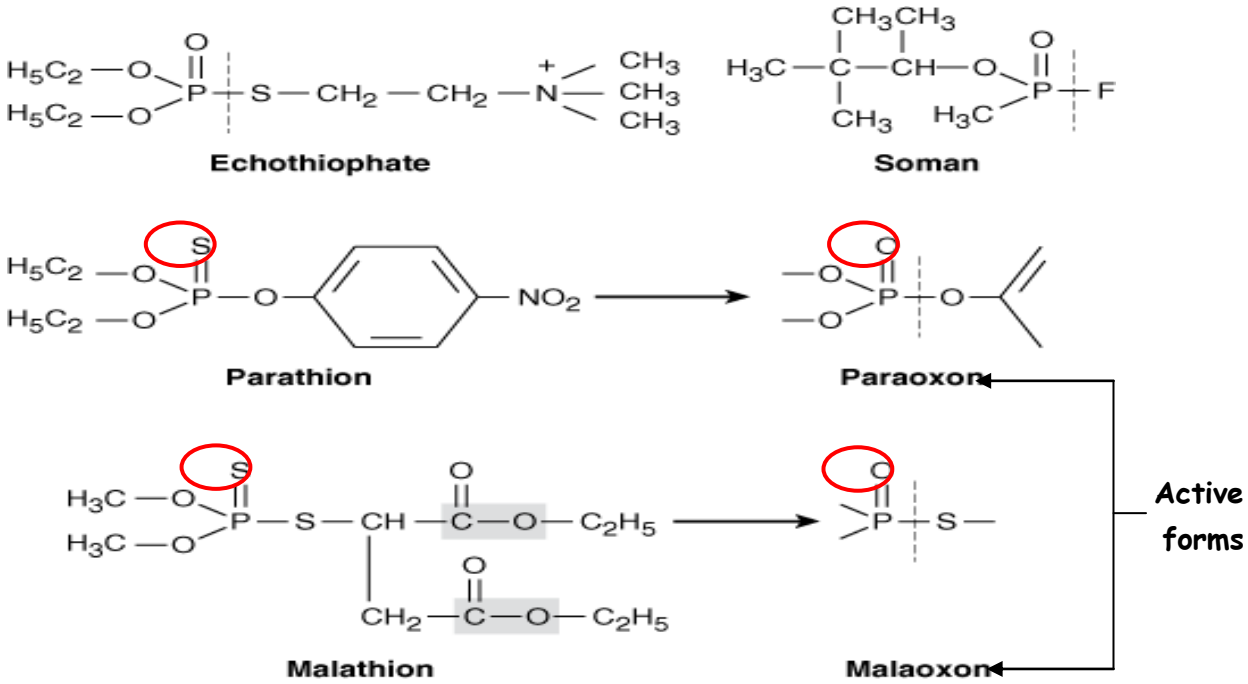
In the previous sheet, we talked about the hydrolysis of acetylcholine (ACh) by acetyl cholinesterase (ACh.E) , as well as the action of ACh.E reversible inhibitors . In this sheet we will talk about irreversible inhibitors of acetyl cholinesterase.

Irreversible cholinesterase inhibitors

Organophosphates

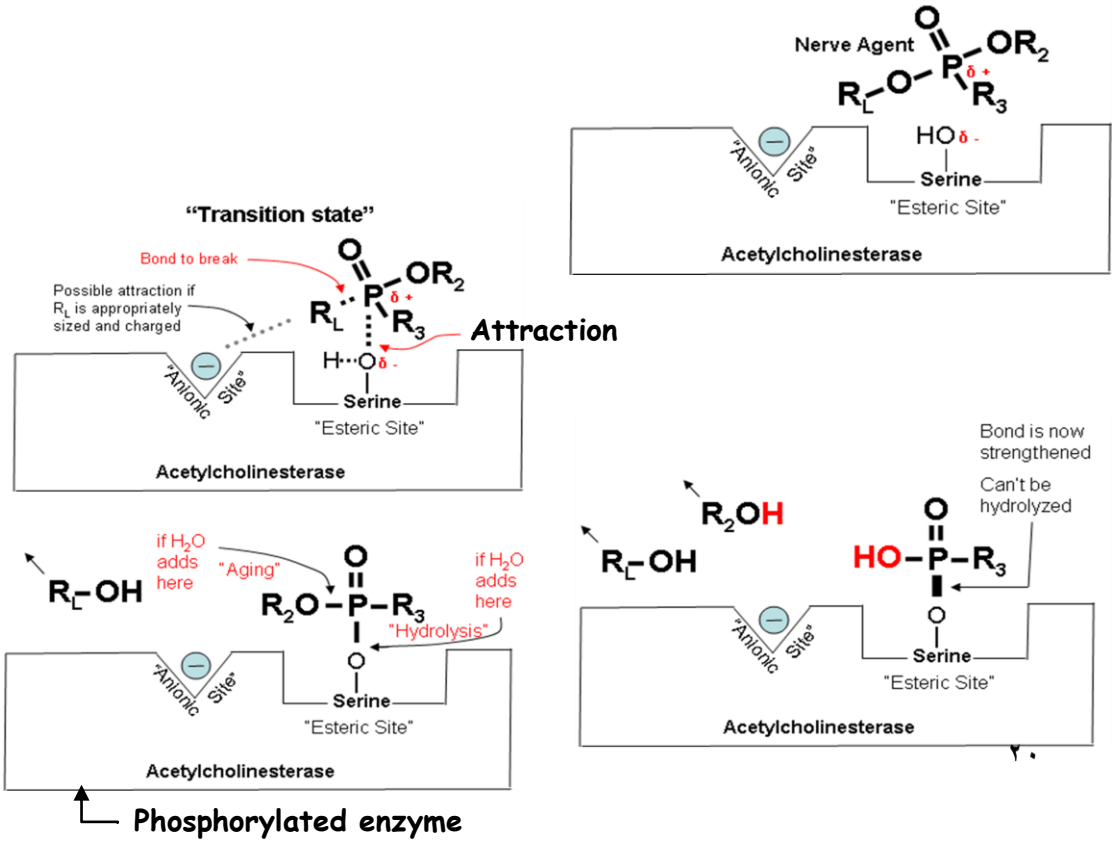


* Organophosphates are very toxic phosphorus containing compounds (pentavalent phosphorus atom) which act as irreversible inhibitors of acetyl cholinesterase.

Examples of irreversible inhibitors:

1) **Echothiophate**
2) **Soman**: a rare and very toxic gas (a nerve gas , used in warfare) .
3) **Parathion**: used as agricultural insecticides to kill insects that invade crops.
4) **Malathion**: used as agricultural insecticides to kill insects that invade crops.

In the case of parathion and malathion, they are *inactive in their native structures*, so they are rapidly activated in the body, HOW? Sulfur in parathion and malathion is displaced by oxygen, this displacement can happen in humans, animals, and insects.



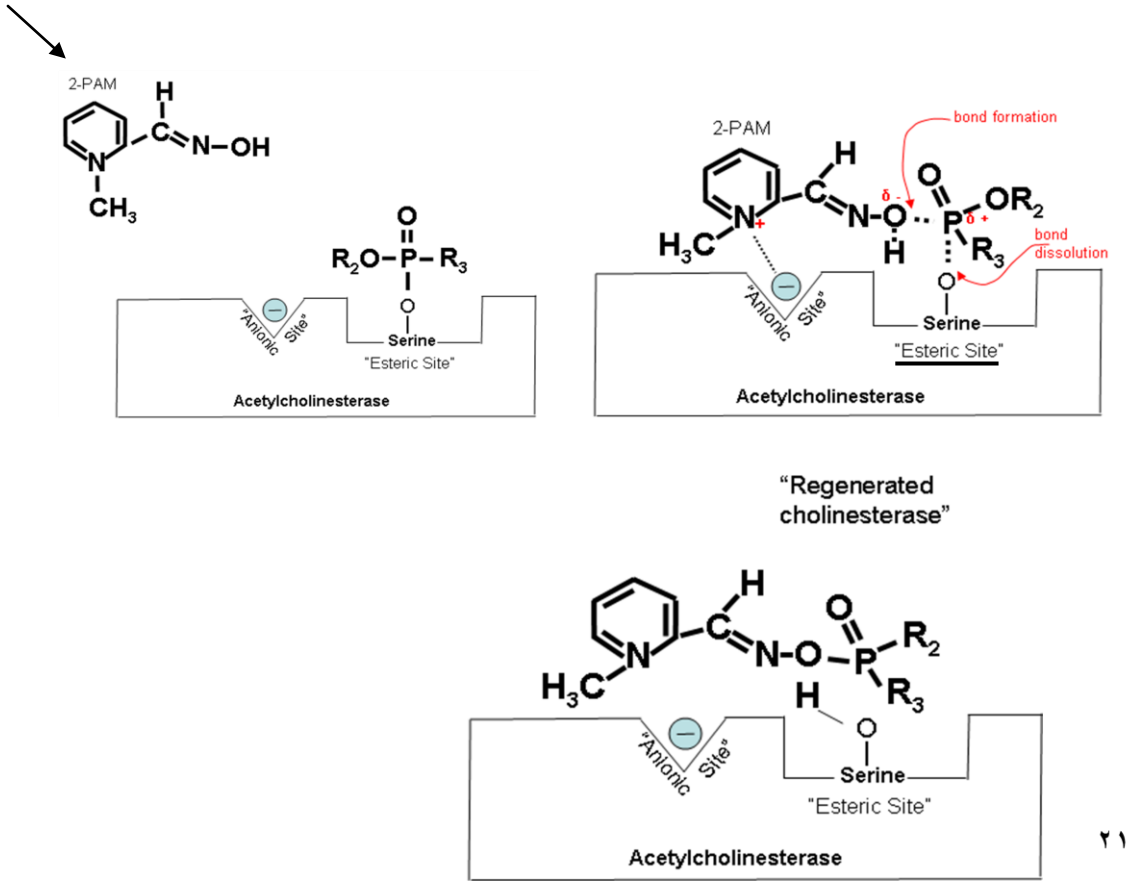
How do these compounds interact with the AChE?

There is a delta positive charge (small positive charge indicated by the symbol **δ**) on the phosphorus and there is an opposite charge on serine. The proximity permits the interaction between the phosphorus and oxygen on serine. There is a possible attraction between the RL and the negative anionic site.

The result is a covalent bond between phosphorus and oxygen. RL – OH ; a free radical, is cleaved and released. The rest of the compound is attached by the covalent bond between phosphorus and oxygen. This is quite a strong bond but it is **strengthened further** by a process which is called Aging, it involves the removal of the 2nd radical.
The enzyme can still be regenerated after initial phosphorylation but when "Aging" occurs after the initial phosphorylation of the enzyme, the inhibitor **is permanently attached** to the enzyme by straightening the phosphorus-oxygen covalent bond which can no longer be hydrolyzed. In

other words, The enzyme can still be regenerated, but if aging happens, the inhibitor is permanently attached to the enzyme by straightening the bond which can no longer be hydrolyzed , this is why their binding is toxic. Therefore, the enzyme is lost and the body has to synthesize new enzymes to compensate for the acetylcholinesterase enzymes it lost by irreversible inhibition.

This aging process takes place after a while. The time elapsed between aging and the first reaction between the organophosphorus compound and the enzyme differs according to each individual. **For nerve gases, aging can occur in minutes**, but for the rest of the toxic compounds, aging can take several hours, during which the victim can still be saved.



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<u>The arrow in the above figure indicates :</u>

<u>2- PAM</u> (Pralidoxime) a drug which comes to the complex between the enzyme and the substrate (irreversible inhibitor) before aging has occurred. 2-PAM holds a positive charge and is attracted to the anionic site which has a negative charge. As a result, it comes closer with additional attraction between the P and oxygen and this allows the creation of a covalent bond between phosphorus and 2-PAM oxygen, and

the covalent bond on the esteric site breaks therefore regenerating the enzyme. This can only occur before aging.

Absorption, Distribution, and Metabolism

- Absorption of the **quaternary carbamates** from the conjunctiva, skin, and lungs is **poor**, since their permanent charge renders them relatively insoluble in lipids.
- Thus, much larger doses are required for oral administration than for parenteral injection.

- Distribution into the CNS is negligible. **Physostigmine**, in contrast, is well absorbed from all sites and can be used topically in the eye.
- It is distributed into the CNS and is more toxic than the more polar quaternary carbamates.

Absorption depends on lipid solubility.

➡ Organophosphorus compounds are highly lipid soluble. They are absorbed from all routes: from skin, orally, etc…

➡ The quaternary carbamates like edrophonium are not easily absorbed. To give them orally, they have to be given in high doses (much higher than the parentral dose.) They are not lipid soluble due to their high polarity , thus do not enter the CNS and cannot cross blood brain barrier.

➡ Physostigmine, on the other hand, is a tertiary amine that is a lipid soluble compound. Absorption is complete after oral intake and distributed everywhere including the CNS and it inhibits the acetylcholinesterase enzyme both centrally and peripherally. That's why this drug is considered **very toxic**. Physostigmine is used parenterally only for atropine poisoning. Atropine poisoning causes symptoms related to central actions and peripheral actions.

➡ The other quaternary anti-cholinesterases are useless in this case because they do not cross the blood brain barrier. Physostigmine is the only one that can agonize the central actions.

- 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 , not by metabolism or excretion.

- The organophosphates (except for echothiophate) are **well absorbed** from the skin, lung, gut, and conjunctiva—thereby making them dangerous to humans and highly effective as insecticides.
- Parathion, malathion, must be activated in the body by conversion to the oxygen analogs

★ Metabolism of the quaternary carbamates occurs by the enzyme they have inhibited (which they're its susbstrates) , and non-specific esterases in plasma .

Sample Case:

A worker used to work in farms spraying insecticides. While he was working some of the insecticide was spilled on his clothes. His clothes absorbed these toxic compounds. He was rushed to the hospital nearly dead. After 3 weeks, he recovered and went back to work wearing the same clothes. The moment he wore the same clothes the insecticide was spilled on, he was poisoned again and rushed again to the hospital.

Conclusion:
The organophosphates remained on the worker's clothes and the skin absorbed the insecticide because organophosphates are well absorbed from any route, and the skin is just one example of these routes.

Therapeutic Uses and Durations of Action of Cholinesterase Inhibitors

	Uses	Approximate Duration of Action
Alcohols		
• Edrophonium	Myasthenia gravis, ileus,	5–15 minutes ← **Short**
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 **(In the eye)**	100 hours **(8 – 9 days)**

Terminology:

Myasthenia Gravis: a condition causing abnormal weakness of certain muscles.

Glaucoma: a group of eye diseases which result in damage to the optic nerve and vision loss. A major risk factor is increased pressure in the eye.

Ileus: a painful obstruction of the ileum or other part of the intestine.

- A Note related to the table :

Edorphonium is a small compound , it doesn't have a full structure like Neostigmine , so it cannot form a bond with serine oxygen , and therefore stabilized by 2 forces : by an electrostatic interaction , and by formation of a hydrogen bond .

Mechanism of Action

- **increase** the concentration of endogenous **acetylcholine** at cholinoceptors.
- **Edrophonium** is a quaternary alcohols, bind **electrostatically and by hydrogen bonds** to the active site, thus preventing access of acetylcholine.
- The enzyme-inhibitor complex does not involve a covalent bond and is short-lived (on the order of 2–10 minutes).

❖ The inhibitors inhibit acetylcholinesterase so acetylcholine will be released from the neurons and will not be destroyed; therefore, it accumulates so this produces *cholinergic effect*.

In the next 3 slides , the doctor mostly just read the info written :D

- Carbamate esters, e.g., **neostigmine** and **physostigmine**. undergo a two-step hydrolysis sequence analogous to acetylcholine.

- However, the covalent bond of the *carbamoylated* enzyme is more resistant to the second (hydration) process, and this step is correspondingly prolonged (**30 minutes to 6 hours**).

- 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.

Aging occurs within 10 minutes with the chemical warfare agent, soman, and in 48 hours with the agent, VX.

- **Pralidoxime** If given before aging has occurred, is able to break the phosphorus-enzyme bond and can be used as "cholinesterase regenerator" drugs for organophosphate insecticide poisoning.

Organ System Effects

Central Nervous System

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

Eye, Respiratory Tract, GIT, Urinary Tract

The effects are qualitatively similar to the effects of the direct-acting cholinomimetics**(Parasympathatic reactions) .**

Central Nervous System

❖ High concentration of acetylcholine produces an arousal alerting response.

❖ For the very potent warfare agents, the only symptom is death! For less toxic agents, there are many parasympathetic and cholinergic effects.

Eye, Respiratory Tract, Gastrointestinal Tract, Urinary Tract

They cause increase in the concentration of acetylcholine thus increase alertness.

Cardiovascular System

Mimic the effects of vagal nerve activation on the heart.

Negative **chronotropic, dromotropic**, and **inotropic** effects and cardiac output falls.

The fall in cardiac output is attributable to bradycardia, decreased atrial contractility, and some reduction in ventricular contractility.

The latter effect occurs as a result of prejunctional inhibition of NE release.

Cardiovascular System :

❖ Mimic the effects of vagal nerve on the heart so the effect is similar to the parasympathetic activation of the heart.

❖ Negative chronotropic (decreased heart rate)
Negative dromotropic (decreased rate of conduction through AV node)

❖ The reduction in ventricular contractility occurs as a result of prejunctional inhibition of norepinephrine release. The ventricles are not affected by parasympathetic nerves. Increased Acetylcholine concentration can stimulate hetero receptors on the sympathetic nerves innervating the ventricles (inhibitory release of norepinephrine).

Minimal effects by direct action on vascular smooth muscle because most vascular beds lack cholinergic innervation (NO parasympathetic innervation).

The *net* cardiovascular effects of moderate doses of cholinesterase inhibitors consist of: modest bradycardia

a fall in cardiac output

an increased vascular resistance (**sympathetic ganglion stimulation**) that result in a rise in blood pressure.

increased vascular resistance (sympathetic ganglion stimulation increases norepinephrine release in post ganglionic nerves leading to vasoconstriction) that result in a rise in blood pressure.

Neuromuscular Junction

- Low concentrations prolong and intensify the actions of Ach.

  This increases the strength of contraction, especially in muscles weakened by curare-like neuromuscular blockers or by myasthenia gravis.
- At higher concentrations fibrillation of muscle fibers. **Antidromic firing** (nerve impulses in a direction opposite to normal) of the motor neuron may also occur, resulting in fasciculations that involve an entire motor unit.

Neuromuscular Junction :

Normal people don't have that much increase, but this is seen in patients with muscles weakened by neuromuscular blocking agents. An example is Curare. Curare is a competitive neuromuscular blocker. It competitively

blocks nicotinic receptors in the neuromuscular junction so it prevents acetylcholine from stimulating the muscles.

 Originally it was poison endogenous to South America where they used to cover the tip of arrows with curare. When the arrow hits a living organism, curare is distributed in the organism and caused paralysis started from the lips to the lungs.

This increases the strength of contraction, especially in muscles weakened by curare-like neuromuscular blocking agents or by myasthenia gravis.

At higher concentrations, the accumulation of acetylcholine may result in fibrillation of muscle fibers. Antidromic firing (nerve impulses in a direction opposite to normal) of the motor neuron may also occur, resulting in fasciculation (a muscle twitch) that involves an entire motor unit , so each fiber will contract on its own with no coordination .

- With marked inhibition of acetylcholinesterase, **depolarizing neuromuscular blockade** occurs followed by a phase of **nondepolarizing blockade** as seen with succinylcholine.

- 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.

Succinylcholine is composed of 2 molecules of acetylcholine joined back to back and is used for electrocompulsive therapy for psychological therapy like in treatment of mental depression .

Clinical Uses

The Eye

- **Glaucoma** was treated with pilocarpine, methacholine, carbachol) or ChEIs physostigmine, demecarium, echothiophate, isoflurophate).
- These drugs have been replaced by **topical -β-blockers** and **prostaglandin** derivatives.
- **Acute angle-closure glaucoma** is a medical emergency that usually requires **surgery.**
- Initial therapy consists of a combination of a direct muscarinic agonist and a cholinesterase inhibitor (e.g., pilocarpine plus physostigmine).

The Eye:

❖ There are 2 effects of cholinergic drugs on the eye: miosis and contractions of ciliary muscles. Both enhance flow of the aqueous humor in the eye.

❖ If a person has glaucoma, there is increased pressure in the eye because the drainage of aqueous humor is impaired.

Drugs typically used to treat it are effective but they impair vision (the patient has to stay in the light in order to be able to see).

❖ Topical -β-blockers and prostaglandin derivatives are as effective as the previously mentioned drugs, however, they do not cause any major side effects and do not impair vision.

❖ Acute angle-closure glaucoma (a type of glaucoma that is a sudden rise in the pressure in the eye and patients do not have any history of glaucoma)

GI and Urinary Tracts

- **Postoperative ileus** (atony or paralysis of the stomach or bowel following surgical manipulation) and **congenital megacolon**.

- **Urinary retention** postoperatively or postpartum or secondary to spinal cord injury or disease (neurogenic bladder).

- **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.

GI and Urinary Tracts:

❖ congenital megacolon (medical condition that causes the movement of the colon, which is called peristalsis, to stop or to be very slow).

❖ **Bethanechol** (Remember that is a choline ester that has moderate muscarinic effect, not nicotinic, and not affected by anti-cholinesterase) has a selective effect on the GI tract and bladder so it can be given for systematic effect. Also, Neostigmine is used

- **Pilocarpine**
  has long been used to increase salivary secretion.
- **Cevimeline**
  A new direct-acting muscarinic agonist used for the treatment of dry mouth associated with Sjögren's syndrome.
  (shoh-grinz, a systemic autoimmune disease in which immune cells attack and destroy the exocrine glands2 that produce tears and saliva.) and that caused by radiation damage of the salivary glands.

Cevimeline is a new synthetic drug used for treatment of dry mouth and it's a derivative of acetylcholine.

Neuromuscular Junction

Myasthenia gravis is an autoimmune disease affecting skeletal muscle neuromuscular junctions. **Antibodies** are detected in 85% of myasthenic patients.

The antibodies **reduce nicotinic receptor function**.

Frequent findings are ptosis, diplopia, difficulty in speaking & swallowing, and extremity weakness.

Severe disease may affect all the muscles, including those necessary for respiration. ٣٨

(Autoimmune disease is what happens when your immune system starts attacking healthy cells .)

antibodies *reduce the nicotinic receptor function* by several means (like distortion of the structure of the motor end-plate; Cross-linking receptors & lysis of the postsynaptic membrane & binding to the receptor and inhibit its function .) . THE NUMBER OF RECEPTORS REMAINIG IS LOW›››LOW TRANSMISSION›››WEAK MUSCLES.

- in symptoms we have to discuss :

1) **Ptosis** : dropping of the eyelid due to paralysis or a disease .

2) **Diplopia** : the double vision of subjects .

- The disease resembles the neuromuscular paralysis produced by *d*-tubocurarine.
- Patients with myasthenia are very sensitive to the action of neuromuscular blockers and other drugs that interfere with neuromuscular transmission, eg, aminoglycoside antibiotics.
- Patients with **ocular myasthenia** may be treated with cholinesterase inhibitors alone.
- Patients having more widespread muscle weakness are also treated with **immunosuppressant** drugs (steroids, cyclosporine, and azathioprine).
- In some patients, the **thymus** gland is removed.

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d-tubocurarine is a (competitive blocker of the nicotinic receptors in the muscles, it prevents the action of acetylcholine and nicotinic receptors and it's given during anesthesia to produce muscle relaxation)

Ocular myasthenia (related to eyes)

immunosuppressant drugs (Drugs that can lower the body's ability to resist; which in our case lowering the production of the antibodies) .

Thymus gland is removed sometimes (because it produces T-lymphocytes that are attacking the receptors)

Edrophonium is used as a **diagnostic test** for myasthenia.

- A 2 mg dose is injected IV. If the patient has myasthenia gravis, an improvement in muscle strength that lasts 5 minutes can be observed.
- Edrophonium 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**) must be distinguished from excessive drug therapy (**cholinergic crisis**).

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• it's a short-acting drug, because it only binds electro statically (doesn't form covalent bonds) , so it last's 2-10 minutes only.

So why are we using edrophonium and not any other drug?

Because if the patient doesn't have myasthenia gravis and has another disease and we give him a long-acting drug, he might suffer from side effects for hours.

• a patient could come to you with muscle weakness though he's been taking neostigmine or pyridostigmine as assigned ; then he should have one of two conditions :

• Either the dose is **very small** ; so he still suffer from muscle weakness.

• Or he is taking a **large dose** which is related to **cholinergic crisis** because – as you know – excessive acetylcholine can cause a neuromuscular blocking which causes muscle weakness. In order to distinguish between **myasthenic crisis** – due to low dose – and **cholinergic crisis** – due to excessive drug therapy - , we also inject edrophonium . If the patient improves then is taking a low dose so we must increase it (myasthenic crisis) ! But if his condition doesn't improve or gets worse, then he has a high dose so just reduce it(cholinergic crisis) !

- Long-term therapy is usually accomplished with **pyridostigmine**; **neostigmine** or **ambenonium**.
- Muscarinic effects is controlled by **atropine**. Tolerance to the muscarinic effects develops, so atropine treatment is not required.
- Neuromuscular blockade is frequently produced as an adjunct to **surgical anesthesia**. After surgery, **neostigmine** and **edrophonium** are the drugs of choice used to **reverse this pharmacologic paralysis promptly**.

- The use of its drugs like **pyridotigmine** ,**neostigmine** &**ambenonium** cause muscarinic effects (like ;salivation, Diarrhea , bronchospasm) . So we need atropine to control these effects . However , the muscarinic effect after a while, could exhibit tolerance so that atropine wouldn't be needed .

surgical anesthesia .

When a surgeon wants to operate on the patient, he needs the muscles to be at complete relaxation ,which he gets be reducing the concentration of anesthetic agent and complement it with neuromuscular blocker so it's safe .

Muscle relaxation is produced by general anesthesia , which has **4 stages:**

The 4th is irreversible ,reaching it means the death of the patient.

The 3rd stage is called the stage of surgical anesthesia, the danger usually in surgeries is mostly due to anesthesia ,high concentrations of the anesthetic agent can be lethal ,so to make a safe anesthesia ,the physician tries to give the minimal concentration of the anesthetic agent ,this concentration will make the patient unconscious but he/she might still feel pain with incomplete muscle relaxation .so the physician combines the anesthesia with drugs by giving the patient neuromuscular blockers so that he has full muscle relaxation .

Clinic. Uses cont.

Central Nervous System

- **Tacrine** is an anticholinesterase used for the treatment of mild to moderate **Alzheimer's disease**.

  Tacrine's efficacy is modest, and hepatic toxicity is significant.
- **Donepezil**, is newer, more selective used in treatment of cognitive dysfunction in **Alzheimer's** patients.
- Given once daily because of its long half-life, and it lacks the hepatotoxic effect of tacrine.

Alzheimer disease (it is the condition where certain cholinergic nerves in the brain go through atrophy or dry or die , thus the amount of acetylcholine is decreased in these areas which explains its observed signs like forgetting the place and people by the patient) .

" Someday you'll forget the info, forget the doctor, forget the classrooms, but you'll never forget an act of kindness ^.^ "

Best of luck amazing people ♥