PHARMACOLOGY

◉ Sheets ○ Slides

Number: 10

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Subject: Autonomic Nervous System 4

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08/11/2015

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In the previous lecture, an introduction about cholino-receptor activation and inhibition as well as some points about acetylcholine esterase inhibitors for both reversible and irreversible types were explained.

In this lecture, the hydrolysis of acetylcholine (ACh) by acetylcholinesterase (AChE) as well as the action of reversible and irreversible inhibitors of acetylcholinesterase will be explained.

Acetylcholine Hydrolysis

AChE is composed of a macromolecule and this macromolecule is an enzyme (protein) which is composed of many subunits. Each subunit of the enzyme contains many active centers. The active center is composed of 2 components: one is called anionic site bears negative charge and the other site is the esteratic site, it holds amino acid serine with a reactive OH group.

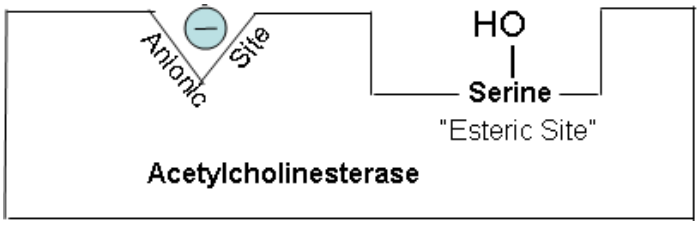


Figure 1 - Active Center of AChE

Acetylcholine molecule (shown in Figure 2) always has a positive charge because of nitrogen. A nitrogen atom usually binds to 3 groups to be stable since it needs 3 electrons to become fully stable. However, since nitrogen binds to 4 groups in acetylcholine, ACh gains a positive charge.

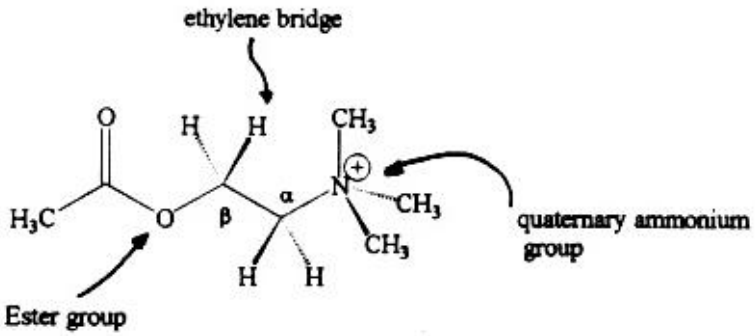


Figure 2 - ACh Molecule

Electrostatic attraction is the attraction between opposite charges. There is an electrostatic attraction between the **positive** nitrogen on the acetylcholine molecule and the **negative** anionic site on the active site of one of the subunits of acetylcholinesterase. This attraction serves to position and align the acetylcholine so that it comes closer to the acetylcholinesterase. This proximity allows the active oxygen, in the amino acid serine, to interact with the acetate carbon atom. The ester linkage, in acetylcholine, is broken. The result is an intermediate which is an acetated enzyme. This enzyme is rapidly hydrolysed to release acetate. The result is acetic acid and the choline. Both are utilized again for synthesis and acetate can be incorporated into the Krebs cycle and so on.

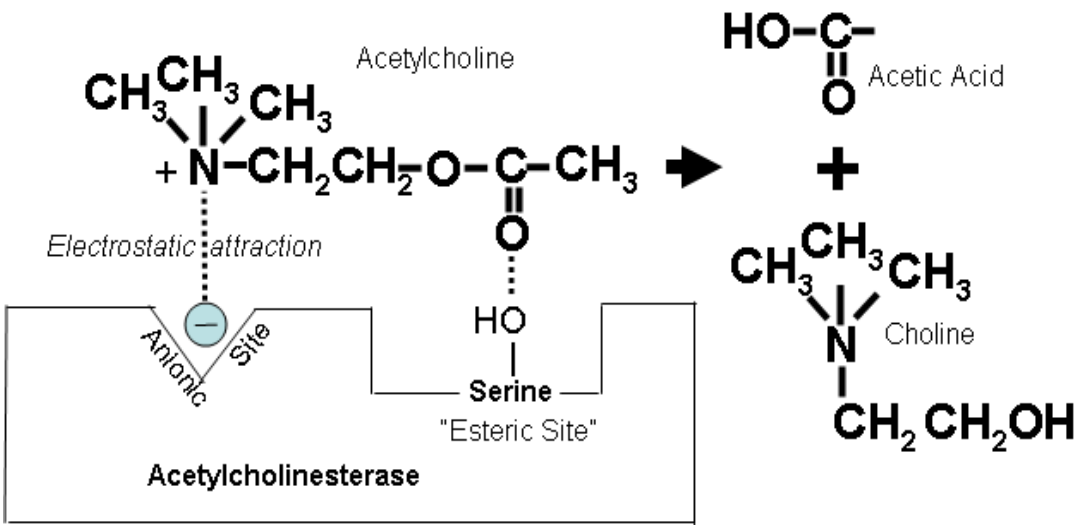


Figure 3 - Acetylcholine Hydrolysis

After understanding how acetylcholine is hydrolyzed, what do anti-cholinesterases do to acetylcholinesterase enzyme?

Reversible Cholinesterase Inhibitors

A **Neostigmine** molecule may look different than acetylcholine but if you look closely it has quaternary nitrogen bearing a positive charge and the **distance** between the nitrogen and the carbon is exactly the same as the distance seen between the nitrogen and the carbon in the acetylcholine molecule.

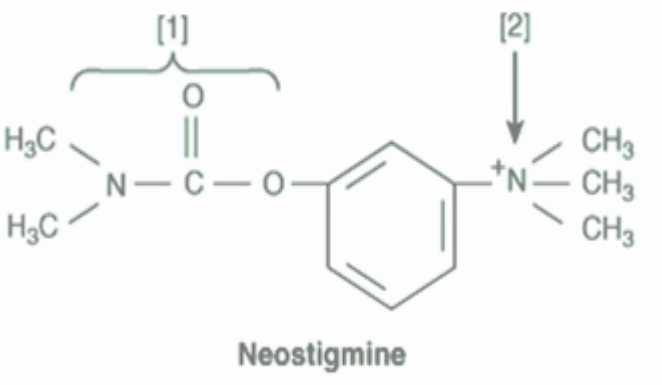


Figure 4 - Neostigmine Molecule

Again, the positive and negative charges allow the Neostigmine molecule to align appropriately with the acetylcholinesterase and this allows a good interaction between the oxygen in the serine molecule and the carbon atom. The result is the breakage of ester linkage and formation of intermediate. This intermediate is hydrolyzed slowly and then the covalent bond is broken and the intermediate will be liberated from acetylcholinesterase. The enzyme during intermediate phase cannot hydrolyze acetylcholine.

This anti-choline esterase is a substrate for the same enzyme but the second phase of the reaction, which is the removal of the intermediate, takes a *longer time* for the regeneration of the enzyme. The enzyme is

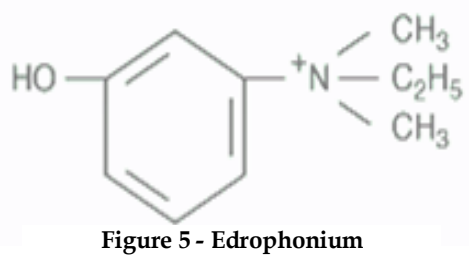


Figure 5 - Edrophonium

incapable of hydrolysing acetylcholine for a period of time ranging from a *few minutes to several hours*.

Edrophonium molecule doesn't have the full structure of Neostigmine and there is no carbon atom to interact with. However, it can also inhibit the enzyme activity for short period of time ranging from 2 to 10 minutes, but why? Again, there is an attraction between positive and negative charge on the esteratic and when edrophonium comes close to the active center then another weak hydrogen bond exists and this straightens the complex resulting of the binding, but it is not strong to last for hours. So this compound is stabilized by an ionic bond at the anionic site and weak hydrogen bonding at the esteratic site.

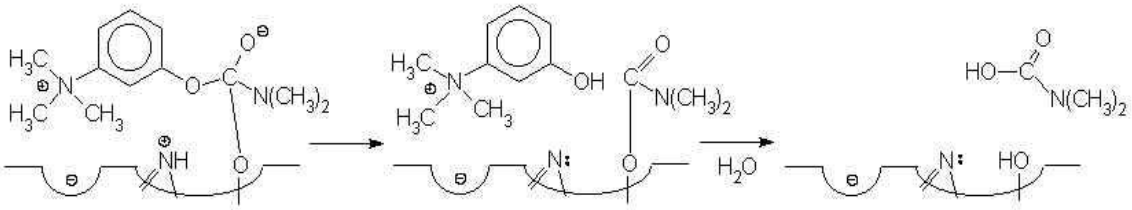


Figure 6 - Reversible Inhibition by Neostigmine

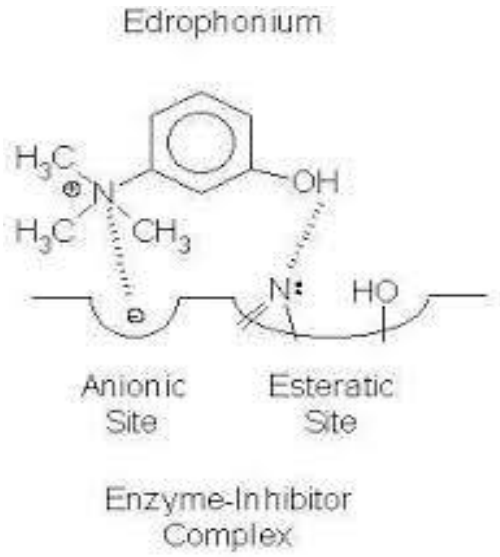
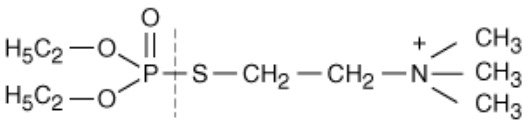


Figure 7 - Reversible Inhibition by Edrophonium

Irreversible Inhibitors

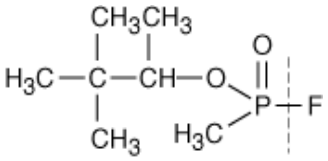


Echothiophate

Organophosphates are very toxic phosphorus containing compounds which act as irreversible inhibitors of acetylcholinesterase.

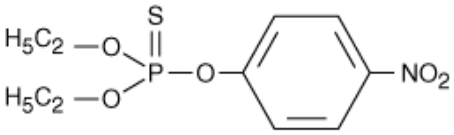
Examples of irreversible inhibitors:

1) **Echothiophate**
2) **Soman**: a rare and very toxic gas.
3) **Parathion**: used as agricultural insecticide to kill insects that invade crops.
4) **Marathion**: used as agricultural insecticide to kill insects that invade crops.



Soman

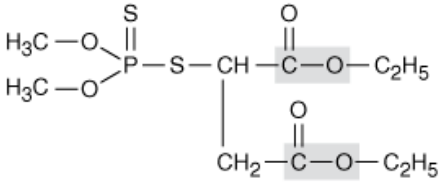
In the case of parathion and marathion, they are *inactive*, but they are rapidly activated in the body. Sulfur in parathion and marathion is displaced by oxygen and this displacement can happen in humans, animals, and insects.



Parathion

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 R_L and the negative anionic site.



Malathion

The results is a covalent bond between phosphorus and oxygen. R_L – 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 that 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 enzymes by straightening the phosphorus-oxygen covalent bond which can no longer be hydrolysed. The enzyme can still be regenerated, but if aging happens, the inhibitor is permanently attached to the enzymes by straightening the bond which can no longer be hydrolysed. 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.

2- PAM (Pralidoxime) 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 oxygen and the covalent bond on the esteratic site breaks therefore regenerating the enzyme. This can only occur before aging.

Absorption, Distribution and Metabolism

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.

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.

Metabolism of the quaternary carbamates occurs by the enzyme they have inhibited and non-specific esterases. Duration of the effect of these reversible types depends on the duration of the inhibition of the enzyme (i.e. duration of the formation of the intermediate complex).

The duration depends on the disability of the enzyme substrate complex.

The organophosphates, except for echothiophate, are well absorbed from any route.

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 min
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
Demacarium	Glaucoma	4-6 hours
Organophosphates		
Echothiophate	Glaucoma	100 hours

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.

Mechanism of Action

- 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*.
- **Edrophonium** is a quaternary alcohol which binds electrostatically and by hydrogen bonds to the active site, thus preventing access of acetylcholine. Its effect is short-lived (2–10 minutes).
- Carbamate esters such as **Neostigmine** and **Physostigmine** undergo a two-step hydrolysis sequence same as acetylcholine. The covalent bond of the *carbamyolated* enzyme is considerably more resistant to the second (hydration) process. This step is 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.** (Note: When aging happens, the phosphorus-oxygen covalent bond straightens and cannot be hydrolyzed therefore the enzyme is lost permanently) Again, aging time differs from one chemical agent to another. For example, aging occurs within 10 minutes with the chemical warfare agent, **Soman,** and in 48 hours with the agent, **VX**.
- **Pralidoxime (2-PAM)** if given before aging has occurred is able to break the phosphorus-enzyme bond. It can be used as "cholinesterase regenerator" for organophosphate insecticide poisoning.

Organ System Effects

Central Nervous System

- High concentration of acetylcholine produces an arousal alerting response.
- In low concentrations, the lipid-soluble cholinesterase inhibitors cause diffuse activation on the electroencephalogram (when we measure electricity in the heart during EKG, electroencephalogram measures electricity in the brain) and a subjective alerting response.
- In higher concentrations, they cause generalized convulsions, which may be followed by coma and respiratory arrest and death.
- 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

- The effects are qualitatively similar to the effects of the direct-acting cholinomimetics. They cause increase in the concentration of acetylcholine thus increase alertness.

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, dromotropic, and inotropic effects and cardiac output falls. The fall in cardiac output is due to: bradycardia, decreased atrial contractility, and some reduction.
- 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 heteroreceptors 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, and an 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 physiologically released acetylcholine. 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.
- With marked inhibition of acetylcholinesterase, depolarizing neuromuscular blockade occurs and that may be followed by a phase of nondepolarizing blockade as seen with succinylcholine (it is composed of 2 molecules of acetylcholine joined back to back and is used for electrocompulsive therapy for psychological therapy).
- Some quaternary carbamate cholinesterase inhibitors, e.g., neostigmine, have an additional direct nicotinic agonist effect at the neuromuscular junction. They are very good in the treatment of Myasthenia gravis.

Clinical Uses

The Eye

- As mentioned in previous lecture, 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.
- Glaucoma was treated with pilocarpine, methacholine, carbachol or cholinesterases physostigmine, demecarium, echothiophate, isoflurophate). <u>They are effective but they impair vision (the patient has to stay in the light in order to be able to see).</u>
- These drugs are replaced by topical -β-blockers and prostaglandin derivatives. <u>They are as effective as the previously mentioned drugs, however, they do not cause any major side effects and do not impair vision.</u>
- 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) is a medical emergency that usually requires surgery.
- Initial therapy is a combination of a direct muscarinic agonist and a cholinesterase inhibitor such as pilocarpine and physostigmine.

GI and Urinary Tracts

- Postoperative ileus (Remember that ileus is the atony or paralysis of the stomach or bowel following surgical manipulation) and congenital megacolon (medical condition that causes the movement of the colon, which is called peristalsis, to stop or to be very slow).
- Urinary retention postoperatively or postpartum or may be secondary to spinal cord injury or disease (neurogenic bladder).

- **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. These two are the most widely used but *it must be certain that there is no mechanical obstruction to outflow before using the cholinomimetic.*
- **Pilocarpine** has long been used to increase salivary secretion. It has been used for a very long time.

Cevimeline

- New synthetic drug used for treatment of dry mouth.
- A derivative of acetylcholine,
- It is considered a new direct-acting muscarinic agonist used for the treatment of dry mouth associated with Sjögren's syndrome (shoh-grinz,). It is a systemic autoimmune disease in which immune cells attack and destroy the exocrine glands that produce tears and saliva. Also treat dry mouth caused by radiation damage of the salivary glands.

GOOD LUCK!