◉ Sheet ○ Slides

number : 3

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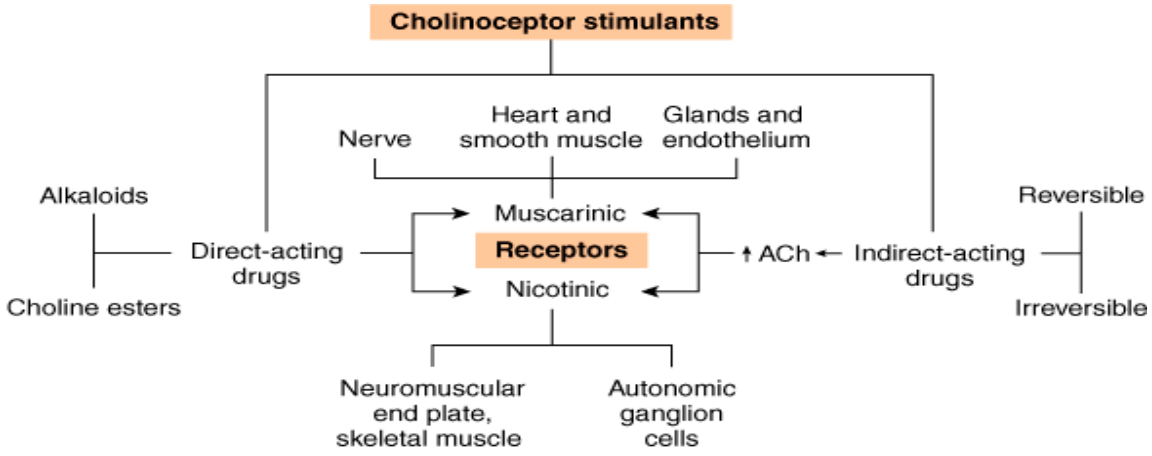
Today we will start a new topic about cholinoceptor so let's talk about it:

Note: this sheet written according to the record and katzung book 13 edition, google ^^ and it's also contain all the slides regard this lecture so you don't need to refer to the slides.



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

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**Cholinoceptor stimulants are classified pharmacologically by their spectrum of action depending on the type of receptor (muscarinic or nicotinic) that is activated.
**Cholinomimetics are also classified by their mechanism of action because some bind directly to cholinoceptor (active) whereas others act indirectly by inhibiting the hydrolysis of endogenous acetylcholine.

Muscarinic receptor: cholinergic receptor of autonomic effectors cell, has an extracellular domain that contain a recognition site for Ach when combine it with Ach physiologic changes occur (slow heart rate, increase glandular secretory) we treat it by stimulated it with Muscarine in mashrooms and parasympathomimetic drugs and antagonize by atropine.

Nicotinic receptor: a class of cholinergic and acetylcholine receptors on skeletal muscle cell that are linked to ion channels in the cell membrane, also it is found on the postganlionic neuron of both (sympathetic and parasympathetic) systems. It responds to nicotine in tobacco .

Cholinomimetic : have an action similar to acetylcholine .

** Direct acting drugs are two classes :

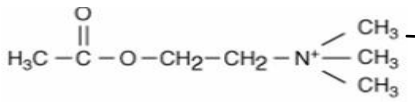
1- natural occurring alkaloid : that found in some mushrooms and plants like muscarine.

2- Choline esters:

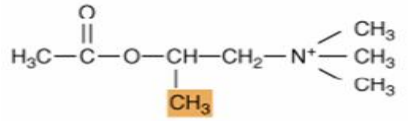
a group of cholinergic drugs that act at sites or organs where acetylcholine is the neurotransmitter. Acetylcholines also consider one of them.

** Indirect acting drugs that inhibit acetyl cholinesterase which catalyze the breaking down of Ach.

"If the Ach not hydrolyze it will stay at the receptor site and this produce extinctive action "



Acetylcholine



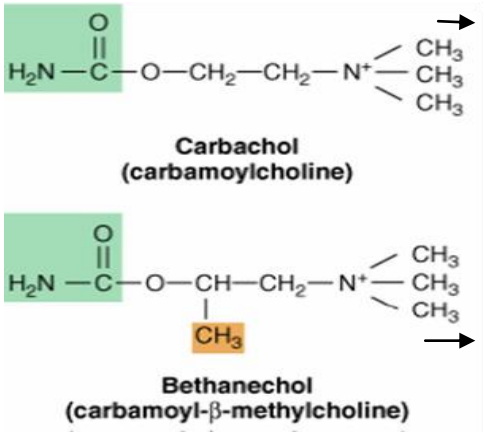
Methacholine
(acetyl-β-methylcholine)

Look at the structure of it, Ach isn't useful as drug critically because its unstable highly metabolize so as soon you inject the patient it will go through the body with no much effectiveness, so to overcome this we can make some changes on the structure.

In this structure you will notice it's the same as Ach but here we found also methyl group on beta carbon so that why it called methacholine so this small difference change the affinity of the drug (stronger action on muscrinic receptor than Ach, with no action on nicotinic receptor) and make it little affected by Ach esterase as you see in the table .

Choline Ester	ACE	Muscarinic	Nicotinic
Acetylcholine	++++	+++	+++
Methacholine	+	++++	None
Carbachol	Negligible	++	+++
Bethanechol	Negligible	++	None

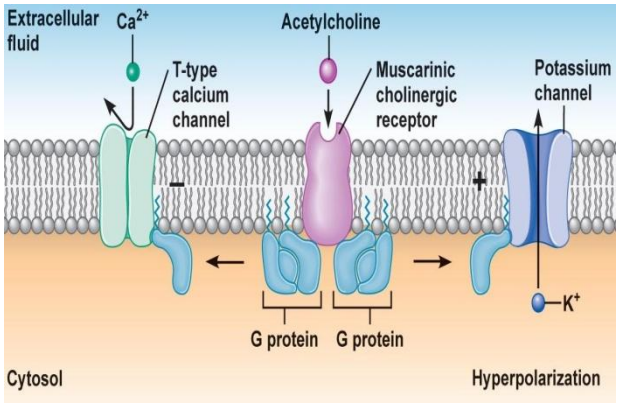
That make methacholine is more potent and long duration than Ach so we don't give it IV because it will cause immediate heart arrest , but it may used in certain situation like atropine poisoning to reduce peripheral and central symptoms (keep in mind this symptoms are similar to acute psychosis , so methacholine used to distinguish between atropine poisoning and acute psychosis by inject the patient with small amount of it, so if there is no meiosis and decrease in heart rate which mean its atropine poisoning)



carbachol notice the difference in structure between it and Ach (they replace methyl group with N) this change make it not affected by Ach esterase modest effect on muscarinic and same effect on nicotinic as Ach, it used on the eye to cause meiosis to treat glaucoma.

bethanechol it has that the two changes that happen to carbachol and methacholine regarding the structure,so it isn't affected by Ach esterase, no effect on nicotinic and modest effect on muscarinic, its muscarinic effect is more selective to GIT and urinary system (so you can name it urecholine) so it has less of effect on the heart and because of that it's the only safe drug among these drugs to be used therapeutically (by parenteral route).

- **Ach** which bind to **M2 receptors**. The receptors open potassium channels through stimulatory G proteins producing hyperpolarization, and close calcium channels through an inhibitory G protein.
- The frequency of action potentials as a result decreases and decreases the heart rate.



**let's start talking about muscarinic transmition in the heart:
the picture above you will notice M2 receptors (because we talk about the heart) these receptor are G protein coupled receptor through it, it will produce action with second messenger.

How the receptor activate:

1-At first Ach bind to the receptor ,then it activate G protein 2-this activation open potassium ion channel (the K go from inside to outside making inside of the membrane more negative) so it make the membrane go from polarize state to hyperpolarize (this mean its need strong stimulant to de hyperpolarize it) .

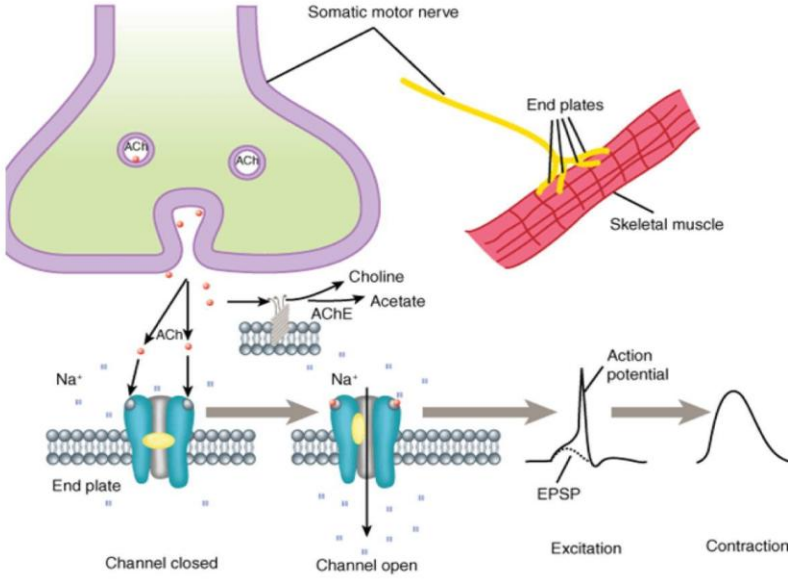
3- at the same time other G protein inhibitory (be aware it's not the same G protein used for K channel it's a stimulant G protein) affected calcium ion channel making it closed (remember that Ca ion needed for muscle contraction) the closure of Ca channel make slow inward of Ca ion. All these actions of M2 receptor cause slowing the peacemaker rate.

Nicotinic transmission at the neuromuscular junction.

Ach interacts with subunits of the nicotinic receptor to open it, allowing Na+ to produce an excitatory postsynaptic potential (EPSP).

The EPSP depolarizes the muscle membrane, generating an action potential, and triggering contraction.

the extracellular Acetylcholinesterase(AChE) hydrolyzes Ach. **5**



**Nicotinic receptor is ion gated receptor (open or close).

Note: AchE hydrolyze Ach to choline+acetate.

We will talk about effects of direct acting cholenceptor stimulants (I organize it in a table to be easy)

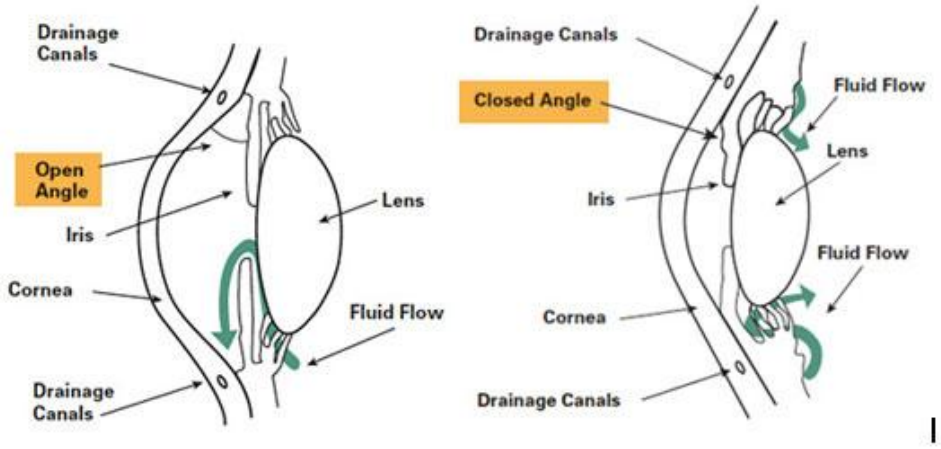
Note : you will see some small numbers in the table they mean there is some explanation about this under the table.

Organ	Response

Eye : 1-sphincter (circular) muscle of iris 2-ciliary muscle : has two function a-control accommodation for viewing object at varying distance. b- facilitation of aqueous humor outflow into the canal of shlemm. (1)	 -Contraction(miosis) - contraction for near vision
Heart: 1-sinoatrial node 2-Atria 3-atrioventicular node (3) 4-ventricles (4)	 -Decrease in rate(negative chronotropy) -decrease in contractile strength (negative inotropy)decrease in refractory period.(2) -decrease conduction velocity (negative dromotropy) increase refractory period. -small decrease in contractile strength.
Blood vessel (5) Artries and veins	-dilation via nitric oxide
Lung 1-Bronchial muscle(bronchoconstriction) 2-bronchial gland	 -contraction -stimulation
Gastrointestinal tract -Motility -sphincters -secretion	 -increase -relaxation -stimulation
Urinary bladder Detrusor (smooth muscle remain relaxed To store urine in the bladder) Trigone and sphincter	 -contraction (that cause urination) -relaxation voiding urination
Glands -sweat, salivary, lacrimal, nasopharyngeal	 -Secretion

(1)- ciliary muscle attach to a ligament that attach to the lens so that how it control it
- aqueous humor is a liquid inside the eye containing low protein concentrations. It is secreted from the ciliary epithelium , a structure supporting the lens, it fills both the anterior and the posterior chambers of the eye, in the drainage angle of the eye (filtration) there is a trabecular meshwork which filtrate this liquid.

The rate of production of aqueous humor equals the rate of drainage so the pressure inside the eye remains constant .
**some people have some problem in the drainage area (cause red eyes), lead to : aqueous humor drainage stops while the manufacture process continued of aqueous humor this lead to build up the pressure in the eye, when the pressure increased it will push the internal structure of the eye continues process will cause irreversible damage to the retina and optic nerve (irreversible blindness). We treat these people by giving the drugs that cause miosis, so circular muscles go from the periphery to center (contraction), this will lead to open schlemm's canal that drain the fluid.



(2) refractory period: the period where the AV node don't allow any impulses to go through so there will not be straight forward impulses go from atrium to ventricle directly causing contraction of both at the same time when the atrium still not full of blood lead to complication (so the ventricle will have a chance to relax while the atrium contract with this period).

(3) -atrioventicular node (AV node) is the place where the electrical conduction of the heart found (electricity impulses come from SA node and pacemaker of the heart) the electricity end in the ventricle (the only place)
**some people have problem in the heart like foramina in the heart (accessory circle, extra electrical pathway) so the electricity will be lose from other area than AV node causing tachycardia and other complications (named woff- Parkinson-white syndrome)
-decrease conduction velocity: the speed of crossing impulses in the area.

(4) ventricle: the other chamber of the heart the one that pump the blood, blood fill the atrium then the atrium contract in the response of action potential, at the same time ventricle relaxed so the blood go toward the ventricle. The effect on ventricles is small because of the little or no supply of vagal nerve to them.

(5) All the blood vessels innervated by sympathetic only the tongue and penis innervated by parasympathetic. Despite that, they contain muscarinic receptors. when muscarinic receptor on blood vessel stimulated it will release NO and cause vasodilatation.

Organ System Effects

Cardiovascular System: M2

- IV infusions of low doses of **Ach** cause vasodilation, reduction in blood pressure, and a reflex increase in heart rate.
- Larger doses of a **Ach** produce bradycardia and decrease a AV node conduction velocity and hypotension.
- Decrease the contractility of atrial & ventricular cells.
- The direct slowing of sinoatrial rate & atrioventricular conduction is often opposed by reflex sympathetic discharge, elicited by the decrease in blood pressure.

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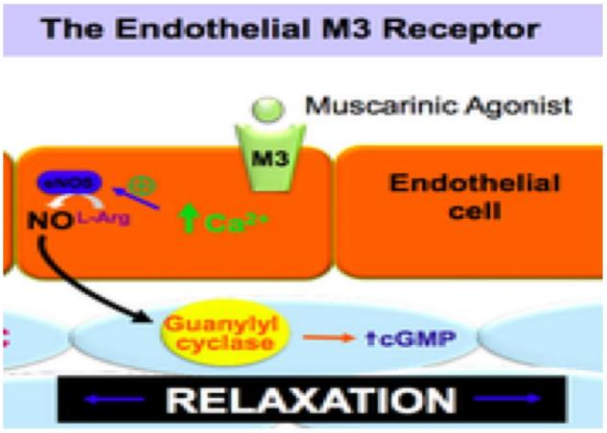
**if we want to see the effect of Ach on the heart we inject an animal with IV infusion Ach so :

1-if we give small doses of infusion Ach will cause vasodilatation because it acts on muscarinic receptor of the blood vessels, by releasing NO which also lead to decrease blood pressure "hypotension" affecting baroreceptors. This causes reflex increase in heart rate.

2- if we give large dose of Ach (mention above in the slide)

- IV injection of muscarinic agonists produces marked vasodilation.
- Muscarinic agonists release nitric oxide (NO), from the endothelial cells.
- The NO diffuses to adjacent vascular smooth muscle, where it activates guanylyl cyclase & increases cGMP, resulting in relaxation.



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- **Pilocarpine (**Natural alkaloid**)** may produce hypertension after a brief initial hypotensive response.
- The longer-lasting hypertensive effect is due to sympathetic ganglionic activation caused by activation of ganglionic **M1** receptors, which elicit slow excitatory (depolarizing) postsynaptic potentials.
- This effect, like the hypotensive effect, can be blocked by atropine, an antimuscarinic drug.

Respiratory System:

- Bronchoconstriction.
- Increases bronchial secretion.

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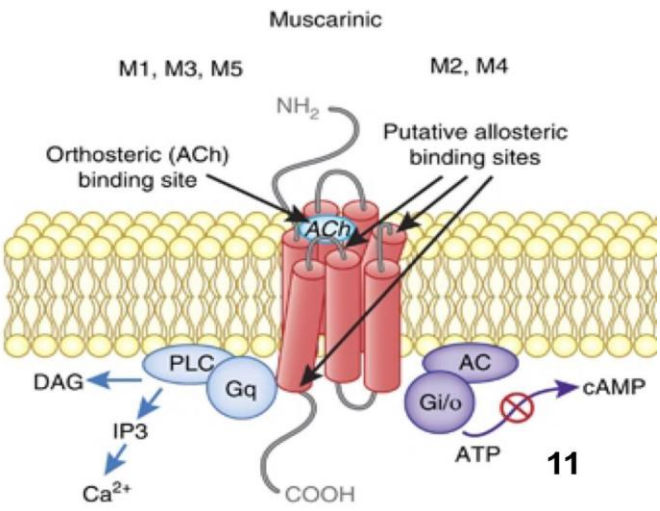
**pilocarpine obtained from silver like leaves of tropical south American shrub from genus pilocarpus (the name of the plant), absorbed very well, not charged (tertiary amine), diffused everywhere in the body, produce briefly hypotension then it produce long lasting hypertension (although its main function is to produce hypotension) how?!

**They found that people with hypertensive have stimulated M1 receptor in the autonomic ganglia (sympathetic nerves, few number of M1 receptor) but most of the ganglia contain nicotinic receptor.
-nicotinic receptor cause fast excitatory postsynaptic potential while M1 slow the excitatory postsynaptic potential ,pilocarpine will stimulate M1, so conduction occur (this mimics stimulating of nicotinic receptor in the sympathetic ganglia at the postsynaptic nerve), so release nor epinephrine which stimulate alpha 1 receptor which produce vasoconstriction that cause hypertension.

**the first useful hypertension drug was ganglia blockers, they block autonomic ganglia so no nor epinephrine release so no hypertension, there problems were severe side effects and rapid tolerance occur so the drug will be no more effective. Nicotinic receptor block and the M1 receptor increase in its number (recall previous lectures) and conduction will be still there so M1 one of the reason for tolerance and side effect.
**if we give atropine which is a muscarinic blocker and after that we give pilocarpine it will not cause vasodilatation (the first rapid effect) nor vasoconstriction (the second prolonged effect of sympathetic ganglia actvation).

Gastrointestinal Tract:

- increases the secretory and motor activity of the gut.
- The salivary and gastric glands are strongly stimulated.
- Peristaltic activity is increased and most sphincters are relaxed.
- The **M3** receptor is required for direct activation of smooth muscle contraction, whereas the **M2** receptor reduces **cAMP** formation & relaxation caused by sympathomimetic drugs.



**we know that cholinergic system stimulate activity and secretion of the GIT and sympathetic system inhibit the activity of GIT.

**sympathetic system inhibits the activity through cAMP which cause relaxation in smooth muscle of GIT.
**cholinergic receptor in GIT: M3 receptor.
Note: M1,M3,M5 work by the same mechanism.
1-Ach bind to its site on the receptor lead to activation G protein and this activate phospholipase C ….> phospholipase C then form IP3 and diacylglycerol which increase the intracellular Ca ion which cause contraction of smooth muscle .
2-At the same time to inhibit the action of sympathetic system, the M2 receptor will be activated ,M2 activate inhibitory proteins that inhibit the formation of cAMP leading to relaxation.

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.
- The human uterus is not sensitive to muscarinic agonists.

Miscellaneous Secretory Glands

- Muscarinic agonists stimulate secretion of sweat, lacrimal, and nasopharyngeal glands

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Central Nervous System:

The CNS contains both muscarinic and nicotinic receptors, the brain is richer in muscarinic sites and the spinal cord contains more nicotinic sites.

Pilocarpine is used to induce chronic epilepsy in rats, to examine different treatments (M1 effect).

oxotremorine produces tremor, hypothermia, and antinociception (increased tolerance for pain) M2.

Presynaptic nicotinic receptors regulate the release of several neurotransmitters.

In high concentrations, **nicotine** induces tremor, emesis, and stimulation of the respiratory center. At still higher levels, nicotine causes convulsions & fatal coma.

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**Ach is important in central nervous system, for example patient with Parkinson disease .the ganglion have two neurotransmitters: inhibitory (dopaminergic transmition) and excitatory (Ach),,so it will cause destruction in dopamine neurons .

In Alzheimer disease because of the damage in cholinergic receptor in the brain (cerebral cortex).

Autonomic ganglia:

- In the CVS, the effects of nicotine are chiefly **sympathomimetic**.
- Nicotine causes hypertension, tachycardia which may alternate with a bradycardia mediated by vagal discharge.

GIT and urinary tracts:

- The effects are **parasympathomimetic**: nausea, vomiting, diarrhea, and voiding of urine are commonly observed.
- Prolonged exposure may result in depolarizing blockade of the ganglia.

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**depolarizing blockade: the membrane is polarized ,when action potential happen it will be depolarized ,when action potential leave the membrane come back to polarization. If the membrane still depolarized there is no further stimulation and this what we mean by " depolarizing blockade" .

Neuromuscular Junction:

- Nicotinic applied directly causes contractile response varies from disorganized **fasciculations** to a strong contraction of the entire muscle.
- Nicotine also causes rapid development of **depolarization blockade**; transmission blockade persists even when the membrane has repolarized.
- This latter phase of block is manifested as flaccid paralysis of skeletal muscle.

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**After this blockade the depolarizing start to leave slowly. However, desensitization to the receptor occurs (the receptor no longer respond).

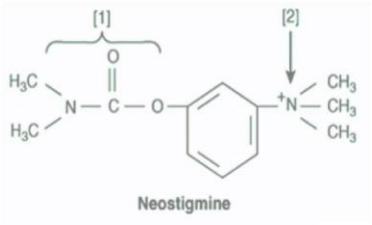
**High nicotine concentrations cause muscle contractions, then in the second stage the muscle will relax at all and it will not respond to any stimulus.

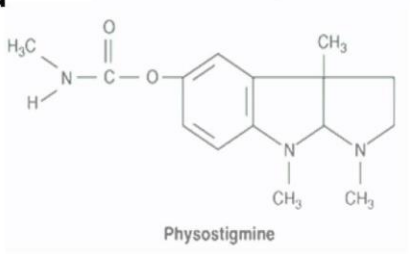
Indirect-Acting Cholinomimetics

Reversible Cholinesterase inhibitors.

Neostigmine

an ester composed of carbamic acid ([1]) and a phenol bearing a quaternary ammonium group([2]).

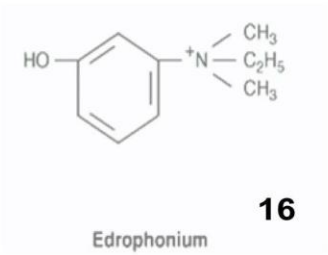




Physostigmine

A naturally occurring carbamate, is a tertiary amine.

Edrophonium is not an ester but binds to the active site of the enzyme.



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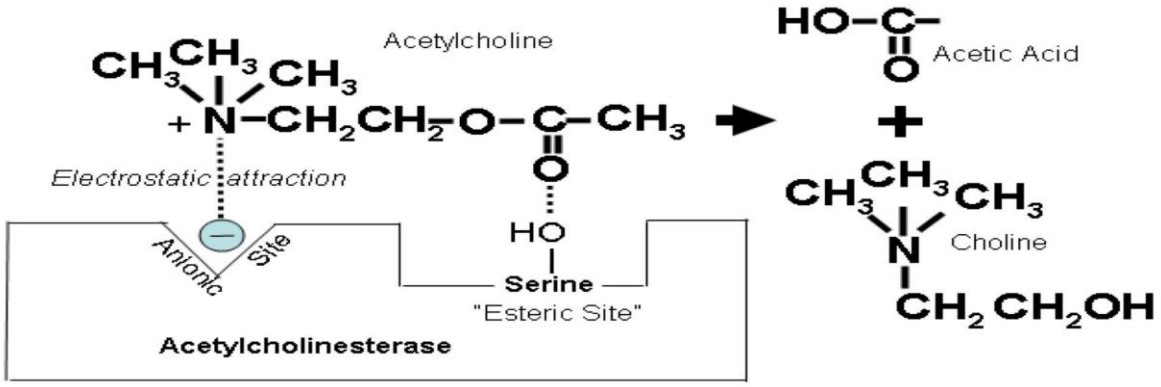
**physostigimine (tertiary amine) is naturally occurring alkaloid. The first one to discover found in Africa,, the doctor tell us a story that this drug found in Africa in a plant look like beans (calabar beans) they use it to distinguish between sorceress and the none one, they made her eat it if she dead then she's deserve to die, if not she is normal person and she continue her life, so American colonist heard about this story they take this plant and extract alkaloid from, then they discover the drug. French scientist discover similar drug from the same plant and they named it eserine.

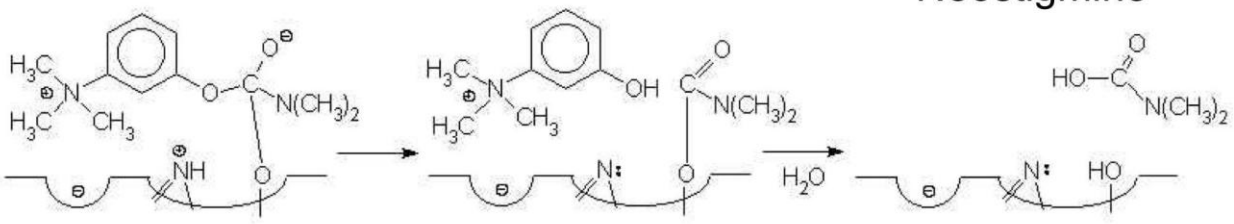
**Neostigmine is a synthetic drug.

Metabolism of Acetylcholine



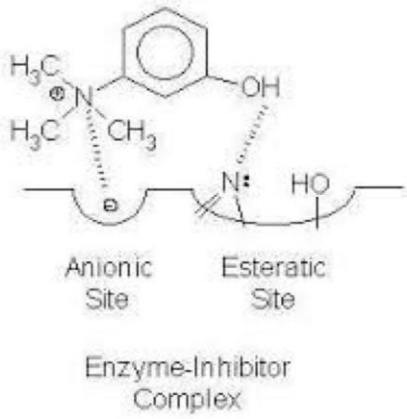
The positively charged nitrogen in the acetylcholine molecule is attracted to the ionic site on acetylcholinesterase, and hydrolysis is catalyzed at the esteric site to form choline and acetic acid. 17

Neostigmine



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

Edrophonium



Stabilized by an ionic bond at the anionic site and through weak hydrogen bonding at the esteratic site.

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**Ach esterase is a macromolecular protein and compose of many subunits, each subunit has very active center, each active center has two sites, one site called anionic site

because it has negative charge, the other side contain serine that has OH and other two amino acids (histidine, glutamate) named esteric site.

**Ach at physiologic ph has always positive charge, because of the electrostatic between charges it will pull the positive Ach to the negative binding site on Ach esterase, after this bond serine attach at the other site of Ach called esteric site which bind to carbonyl by a "covalent bond" catalyze the hydrolysis of ester bond in Ach to give choline and acetic acid. (The covalent bond is broken very fast to release acetate).

**if we look at cholinesterase inhibitors for example let's take neostigmine which contains positive N and carbonyl. The distance between these two groups is equal to the distance between them in Ach. This drug cause the action which is written above in the slide, this is reversible inhibition ,this drug as you see is substrate to the enzyme like Ach and same action occur in it so what the difference!!

The difference found due to last step of the action which becomes slow when the drug binds comparing to Ach which happen fast. The hydrolysis of this bond needs time (minutes-few hours).

** Edrophonium is a very short (5 minutes) acting Ach esterase inhibitor drug because it is not ester it only bind to the active site (anionic site) make ionic bond and weak H bond at the esteric site. (No covalent bond formation)

**Note: Neostigmine and Edrophonium act as substrates for AchE.