Pharmacology

Cholinergic drugs

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Ph.D Pharmacology

Lecture 4

Introductions

- Drugs affecting the autonomic nervous system (ANS) are divided into two groups according to the type of neuron involved in the mechanism of action.
- The cholinergic drugs, which are described in this lectures, act on receptors activated by acetylcholine (ACh).
- The adrenergic drugs (described in the next lectures) act on receptors stimulated by norepinephrine or epinephrine.

Cholinergic drugs

- **It is also called parasympathomimetics or cholinomimetics drugs.**
- **Classified into the following**:

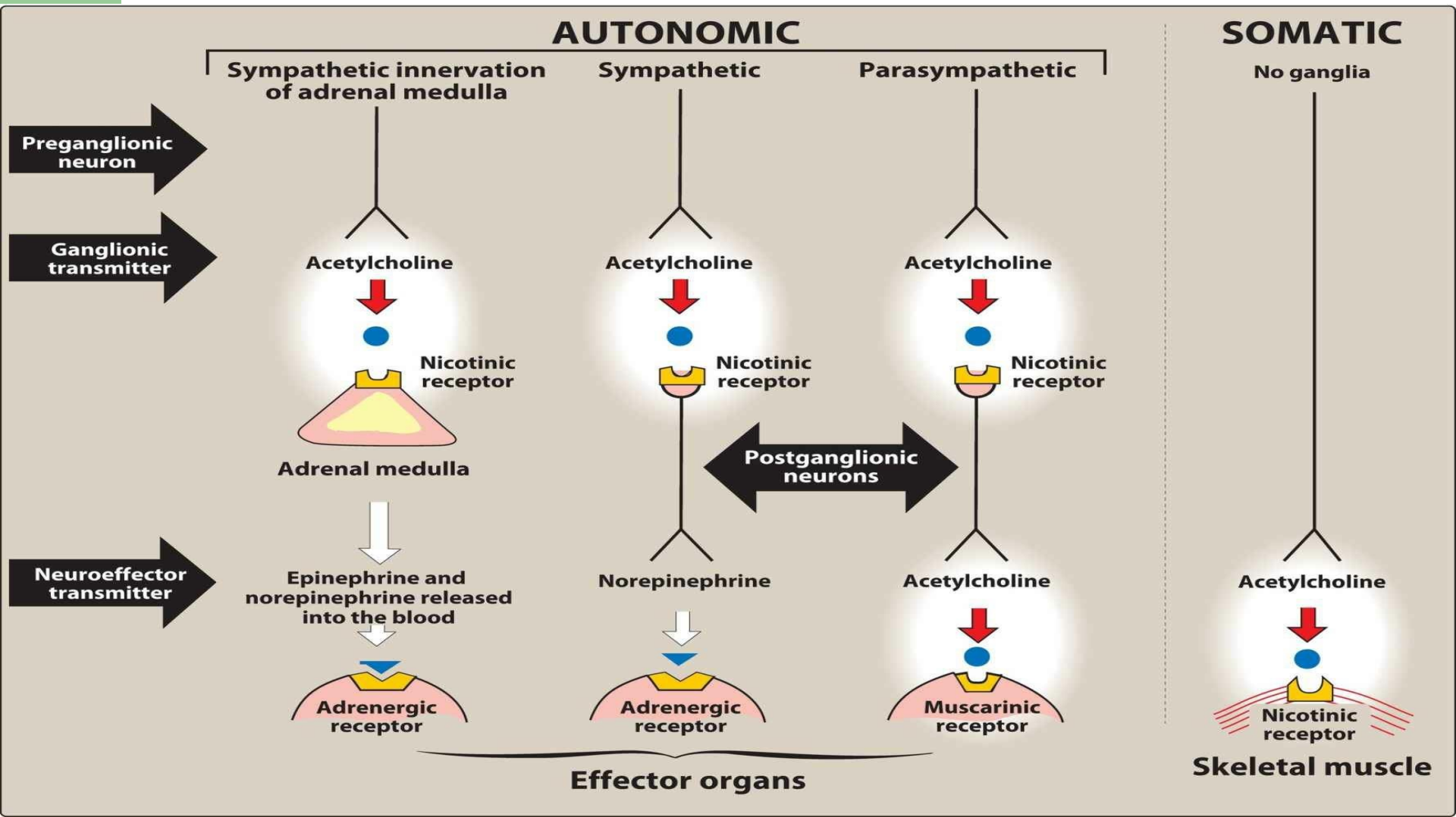
1. Direct acting
2. Indirect acting (reversible)
3. Indirect acting (irreversible)
4. Reactivation of acetylcholineesterase

The Cholinergic Neuron

Any neuron releases Ach from nerve terminal called Cholinergic neuron. They include the following neurons:

1. The preganglionic fibers terminating in the adrenal medulla
2. The autonomic ganglia (both parasympathetic and sympathetic)
3. The postganglionic fibers of the parasympathetic division use ACh as a neurotransmitter.
4. The postganglionic sympathetic division of sweat glands also uses ACh.
5. Cholinergic neurons innervate the muscles of the somatic system and play an important role in the central nervous system (CNS).

Sites of actions of cholinergic agonists in the autonomic and somatic nervous systems.



Neurotransmission at cholinergic neurons

Neurotransmission in cholinergic neurons involves six sequential steps:

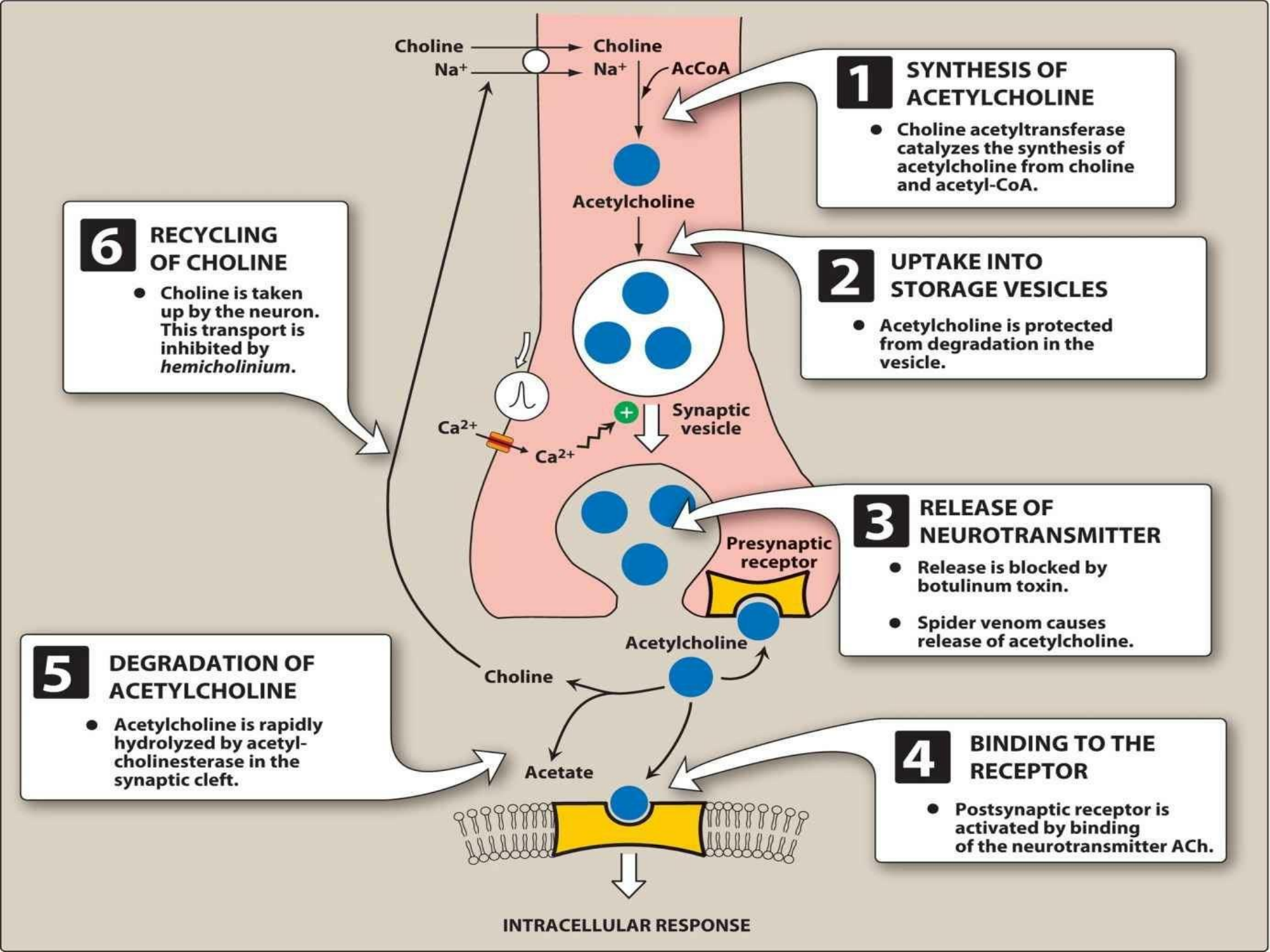
1) synthesis of Ach (can be inhibited by hemicholinium)

2) storage,

3) Release (This release can be blocked by botulinum toxin. In contrast, the toxin in black widow spider venom causes increase the release of ACh into the synaptic gap) .

4) binding of ACh to the receptor (Binding to a receptor leads to a biologic response within the cell, such as the initiation of a nerve impulse in a postganglionic fiber or activation of specific enzymes in effector cells, as mediated by second messenger molecules).

5) degradation of ACh in the synaptic cleft (the space between the nerve endings and adjacent receptors on nerves or effector organs), **6)** recycling of choline


Choline
Na+
Choline
Na+
AcCoA
Acetylcholine
Ca2+
Ca2+
Synaptic vesicle
Presynaptic receptor
Acetylcholine
Choline
Acetate
INTRACELLULAR RESPONSE
1 SYNTHESIS OF ACETYLCHOLINE
Choline acetyltransferase catalyzes the synthesis of acetylcholine from choline and acetyl-CoA.
2 UPTAKE INTO STORAGE VESICLES
Acetylcholine is protected from degradation in the vesicle.
3 RELEASE OF NEUROTRANSMITTER
Release is blocked by botulinum toxin.
Spider venom causes release of acetylcholine.
4 BINDING TO THE RECEPTOR
Postsynaptic receptor is activated by binding of the neurotransmitter ACh.
5 DEGRADATION OF ACETYLCHOLINE
Acetylcholine is rapidly hydrolyzed by acetyl-cholinesterase in the synaptic cleft.
6 RECYCLING OF CHOLINE
Choline is taken up by the neuron. This transport is inhibited by hemicholinium.

Cholinergic Receptors (Cholinoceptors)

Two families of cholinoceptors:

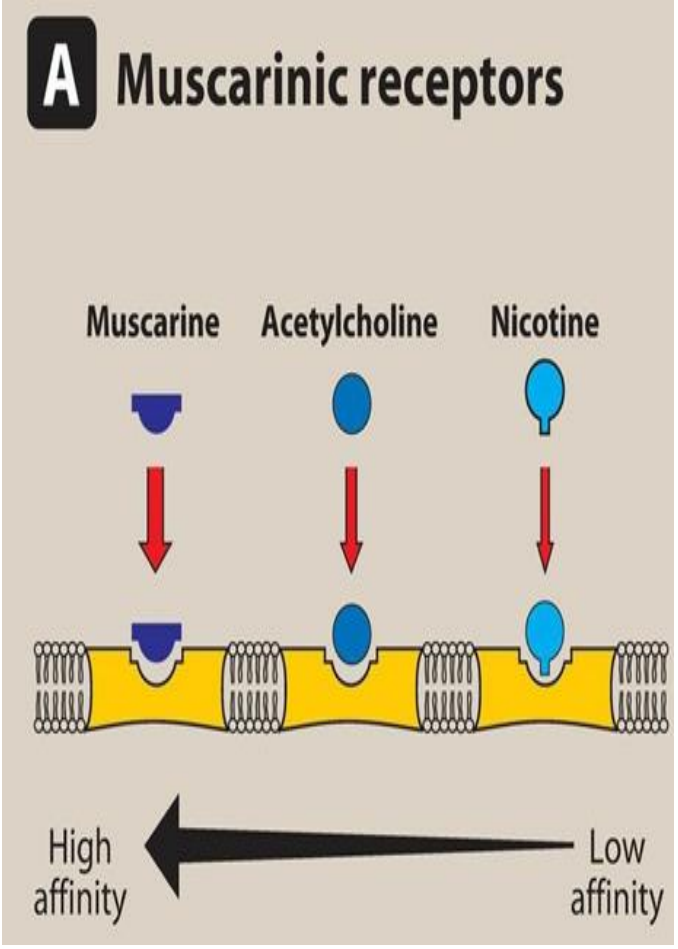
1. Muscarinic
2. Nicotinic receptors,

It can be distinguished from each other on the basis of their different affinities for agents that mimic the action of ACh (cholinomimetic agents).

1-Muscarinic receptors

Muscarinic receptors belong to the class of G-protein–coupled receptors. These receptors, in addition to binding ACh, also recognize muscarine, an alkaloid in certain poisonous mushrooms. By contrast, the muscarinic receptors show only a weak affinity for nicotine, an alkaloid found in tobacco and other plants . There are five subclasses of muscarinic receptors; however, only M1 , M2 , and M3 receptors have been functionally characterized.

Example on Muscarinic agonists is Pilocarpine which is a nonselective muscarinic agonist used to treat xerostomia and glaucoma.



Muscarinic receptors

Muscarinic receptors are located in most internal organs. This includes the cardiovascular, respiratory, gastrointestinal, and genitourinary. Stimulation of the muscarinic receptors may result in either excitation or inhibition, depending on the organ involved.

Example on muscarinic agonists

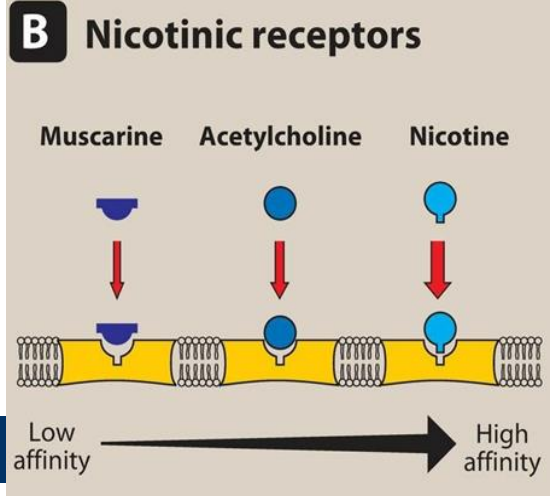
Pilocarpine is a nonselective muscarinic agonist used to treat xerostomia and glaucoma. Attempts are currently underway to develop muscarinic agents that are directed against specific receptor subtypes.

Receptor	Typical locations	Parasympathetic effect
Muscarinic		
M1	Ganglia, CNS, some of presynaptic nerve mainly in the cortex and hippocampus	Play a major role in the mediating gastric acid secretion, learning , memory and motor function
M1	Prostate gland	Contraction
M2	Heart	Decrease the heart rate and contractility
M2	In presynaptic nerve in the CNS	Discontinuation the release of neurotransmitter
M3	Gastro intestinal tract smooth muscle Wall Sphincters Secretion	Contraction Relaxation Increase secretion of gland
M3	Gento-urinary smooth muscles Bladder wall Sphinictors Uterus	Contraction Contraction Relaxation Contraction
M3	Lung (Bronchial smoth muscle)	Contraction
M3	Eye (Ciliary muscle)	Contraction
M	Penis, seminal vesicles	Errection
Nicotinic		
NN	Ganglia, adrenal medula	Stimulation the release of neurotransmitter
NM	Neuromuscular junction	Contraction the skeletal muscle

Mechanism of acetylcholine signal transduction

A number of different molecular mechanisms transmit the signal generated by ACh occupation of the receptor. For example, when M_1 or M_3 receptors are activated, the receptor undergoes a conformational change and interacts with a G-protein that activates phospholipase C. This ultimately leads to production of second messengers inositol-1,4,5trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 causes an increase in intracellular Ca^{2+}. Calcium can then interact to stimulate or inhibit enzymes or to cause hyperpolarization, secretion, or contraction. DAG activates protein kinase C, an enzyme that phosphorylates numerous proteins within the cell. In contrast, activation of the M_2 subtype on the cardiac muscle stimulates a G-protein that inhibits adenylyl cyclase and increases K^+ conductance. The heart responds with a decrease in rate and force of contraction.

2-Nicotnic receptors



These receptors, in addition to binding ACh, also recognize nicotine but show only a weak affinity for muscarine . The nicotinic receptor functions as a ligand-gated ion channel (ionotropic receptor). Binding of two ACh molecules elicits a conformational change that allows the entry of sodium ions, resulting in the depolarization of the effector cells. Nicotine at low concentration stimulates the receptor, whereas nicotine at high concentration blocks the receptor. Nicotinic receptors are located in the CNS, the adrenal medulla, autonomic ganglia, and the neuromuscular junction (NMJ) in skeletal muscles. Those at the NMJ are sometimes designated Nm , and the others, Nn . The nicotinic receptors of autonomic ganglia differ from those of the Nm at NMJ. For example, ganglionic receptors are selectively blocked by mecamylamine, whereas NMJ receptors are specifically blocked by atracurium.

Direct-Acting Cholinergic Agonists

Cholinergic agonists mimic the effects of ACh by binding directly to cholinoceptors (muscarinic or nicotinic). These agents may be broadly classified into two groups:

1) choline esters, which include endogenous ACh and synthetic esters of choline, such as *carbachol* and *bethanechol*, and
2) naturally occurring alkaloids, such as *nicotine* and *pilocarpine*.

All direct-acting cholinergic drugs have a longer duration of action than ACh. The more therapeutically useful drugs (*pilocarpine* and *bethanechol*) preferentially bind to muscarinic receptors and are sometimes referred to as muscarinic agents. However, as a group, the direct-acting agonists show little specificity in their actions, which limits clinical usefulness.

Acetylcholine

- Acetylcholine [ah-see-teel-KOE-leen] is a quaternary ammonium compound that cannot penetrate membranes.
- Although it is the neurotransmitter of parasympathetic and somatic nerves as well as autonomic ganglia, it lacks therapeutic importance because of its multiplicity of actions (leading to diffuse effects) and its rapid inactivation by the cholinesterases.
- ACh has both muscarinic and nicotinic activity.

Actions of Acetylcholine

1. **Decrease in heart rate and cardiac output**

Injection of Ach produces a brief decrease in cardiac rate (bradycardia) and cardiac output, mainly because of a reduction in the rate of firing at the sinoatrial (SA) node.

Actions of Acetylcholine

2- Lowering the blood pressure. How??

Injection of ACh causes vasodilation and lowering of blood pressure by an indirect mechanism of action. Ach activates M receptors found on endothelial cells lining the smooth muscles of blood vessels. This results in the production of nitric oxide from arginine. Nitric oxide then diffuses to vascular smooth muscle cells to stimulate protein kinase G production, leading to hyperpolarization and smooth muscle relaxation via phosphodiesterase-3 inhibition. In the absence of administered cholinergic agents, the vascular cholinergic receptors have no known function, because ACh is never released into the blood in significant quantities. Atropine blocks these muscarinic receptors and prevents ACh from producing vasodilation.

Other actions of Acetylcholine

3. Increased tone and contractility in GI smooth muscle, relaxation of sphincters, increased salivary gland and GI secretions.
4. Increased tone and contractility of detrosal smooth muscle in urinary bladder and relaxation of the sphincter.
5. Increased tone and contractility of bronchial smooth muscle and increase the bronchial secretion (Methacholine, used to assist in the diagnosis of asthma) .
6. stimulation of ciliary muscle contraction for near vision and in the constriction of the pupillae sphincter muscle, causing miosis

Mechanisms of Action—Direct Acting Cholinergic drugs

- Non-selective cholinergic drugs or (cholinergic agonist) or direct acting cholinergics drugs stimulate both muscarinic receptors (M1, M2, M3, M4 and M5) and also nicotinic receptors (example carbachol).
- Selective cholinergic drugs are:
- Muscarinic agonist (Bethanicol and pilocarpine)
- Nicotinic agonist such as Nicotine and Varenicline.

Bethanecol (used orally)

- **Mechanism of action:** Directly stimulates muscarinic receptors (M3), causing increased intestinal motility and tone. It also stimulates the detrusor muscle of the bladder, whereas the trigone and sphincter muscles are relaxed. These effects stimulate urination.
- **Used** to treat nonobstructive urinary retention due to bladder atony and for postoperative abdominal distention due to paralytic ileus.
- **Side effects** include sweating, salivation, flushing, decreased blood pressure (with reflex tachycardia), nausea, abdominal pain, diarrhea, and bronchospasm. Atropine may be administered to overcome severe cardiovascular or bronchoconstrictor responses to this agent.

Carbachol (carbamylcholine) (non-selective)

Carbachol has profound effects on both the cardiovascular and GI systems because of its ganglion-stimulating activity, and it may first stimulate and then depress these systems.

It can cause release of epinephrine from the adrenal medulla by its nicotinic action.

Locally instilled into the eye, it mimics the effects of ACh, causing miosis and a spasm of accommodation (constriction of the ciliary muscle. The vision becomes fixed at some particular distance, making it impossible to focus.[Note the opposing effects of *atropine]*

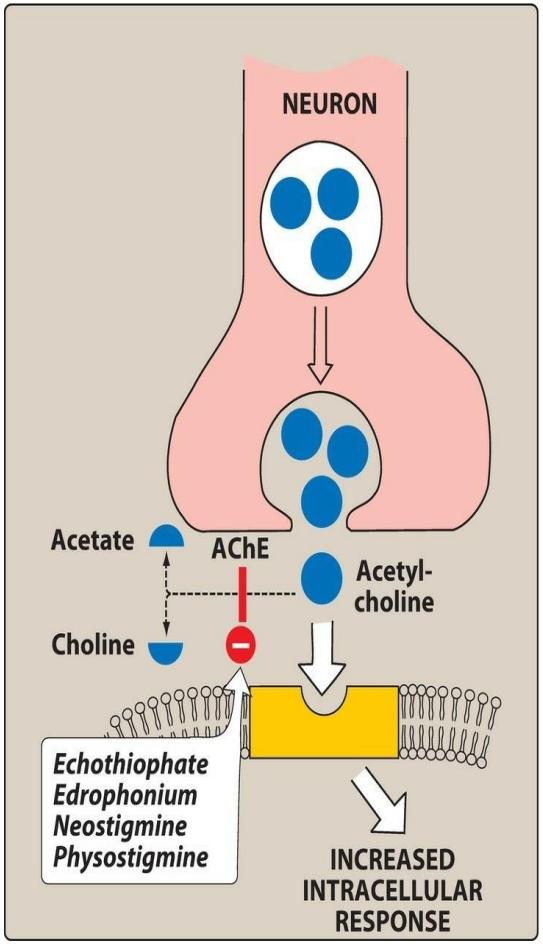
Uses: carbachol is rarely used. Intraocular use provides miosis for eye surgery and lowers intraocular pressure in the treatment of glaucoma.

Pilocarpine

- **Mechanism of action**: Pilocarpine stimulate the M3 receptor caused reduction in the intraocular pressure by causing contraction of the ciliary body so as to facilitate outflow of aqueous humor and perhaps also by diminishing the rate of its secretion. It is also stimulate the salivary secretion by stimulating the M3 in salivary gland.
- **Uses:** It is the drug of choice for emergency lowering of intraocular pressure of both open-angle and angle-closure glaucoma. Pilocarpine caused an immediate drop in intraocular pressure because of the increased drainage of aqueous humor. This action occurs within a few minutes, lasts 4 to 8 hours, and can be repeated.
- It promoting salivation in patients with xerostomia resulting from irradiation of the head and neck. Sjögren syndrome, which is characterized by dry mouth and lack of tears, is treated with oral pilocarpine tablets.
- **Side effects:** Blurred vision, night blindness, and eyebrow ache (ألم الحاجب).

Indirect-Acting Cholinergic Drugs (Reversible)

- Inhibitors of AChE (anticholinesterase agents or cholinesterase inhibitors) indirectly provide cholinergic action by preventing the degradation of ACh. This results in an accumulation of ACh in the synaptic space.
- Accumulation of acetylcholine then occurs which enhances the activation of the nicotinic and muscarinic receptors of the ANS, as well as at the NMJ and in the brain.
- The reversible AChE inhibitors can be broadly classified as short-acting or intermediate-acting agents.



Edrophonium (IV injection)

- *Mechanism of action: Edrophonium* binds reversibly to the active center of AChE, preventing hydrolysis of ACh. Act on peripheral nervous system.
- It has a short duration of action of 10 to 20 minutes due to rapid renal elimination.
- It is used in the diagnosis of myasthenia gravis
- may also be used to assess cholinesterase inhibitor therapy, for differentiating cholinergic and myasthenic crises.
- reversing the effects of nondepolarizing neuromuscular blockers (NMBs) after surgery
- Due to the availability of other agents, *edrophonium* use has become limited.

What is Myasthenia gravis?

Myasthenia gravis autoantibodies presumably destroy nicotinic receptors; thus, acetylcholine less able to stimulate muscle contraction, results in severe muscle weakness.

Intravenous injection of edrophonium leads to a rapid increase in muscle strength in patients with myasthenia gravis. Care must be taken, because excess drug may provoke a cholinergic crisis (atropine is the antidote). Edrophonium may also be used to assess cholinesterase inhibitor therapy, for differentiating cholinergic and myasthenic crises, and for reversing the effects of nondepolarizing neuromuscular blockers (NMBs) after surgery.

Neostigmine

Mechanism of action: Act by inhabiting Ach esterase enzyme, it is more polar, is absorbed poorly from the GI tract, and does not enter the CNS. Its effect on skeletal muscle is greater than physostigmine, and it can stimulate contractility before it paralyzes.

Uses: stimulate the bladder and GI tract and as an antidote for competitive neuromuscular-blocking agents and management of myasthenia gravis.

Side effects: salivation, flushing, decreased blood pressure, nausea, abdominal pain, diarrhea, and bronchospasm.

Note: Neostigmine does not cause CNS side effects and is not used to overcome toxicity of central-acting antimuscarinic agents such as atropine.

Contraindicated: Intestinal and urinary bladder obestruction

Pyridostigmine

Pyridostigmine is another cholinesterase inhibitor used in the chronic management of myasthenia gravis. Its duration of action is intermediate (3 to 6 hours) but longer than that of neostigmine.

Physostigmine

- Physostigmine stimulates not only the muscarinic and nicotinic sites of the ANS, but also the nicotinic receptors of the NMJ. Muscarinic stimulation can cause contraction of GI smooth muscles, miosis, bradycardia, and hypotension. Nicotinic stimulation can cause skeletal muscle twitches, fasciculations, and skeletal muscle paralysis (at higher doses). Physostigmine can enter and stimulate the cholinergic sites in the CNS.
- Uses: used in the treatment of overdoses of drugs with anticholinergic actions, such as Atropine and antihistamine. And to reverse the effects of nondepolarizing neuromuscular blockers (NMBs) after surgery.
- Side effects: Convulsions, bradycardia, decrease cardiac output and paralysis of skeletal muscle.

Alzheimer's disease (AD)

- Acetylcholine important neurotransmitter affecting cognitive functioning, memory storage and retrieval
- In *Alzheimer's disease (AD), there is* abnormalities of the cholinergic, serotonergic, noradrenergic, and glutaminergic neurotransmission systems
- In cholinergic system, patient with AD found to have loss of neurons that secrete acetylcholine

Indirect Acting Agents used to treat Alzheimer's disease.

- Donepezil said to delay progression of the disease by up to 55 weeks. Does not cause liver toxicity.
- Galantamine
- Rivastigmine long acting. Twice a day dosing.
- Tacrine: It is hepatoxic. Elevated liver enzymes.

Indirect-Acting Cholinergic Agonists: Anticholinesterase Agents (Irreversible)

A number of synthetic organophosphate compounds have the ability to bind covalently to AChE. The result is a long-lasting increase in ACh at all sites where it is released. Many of these drugs are extremely toxic and were developed by the military as nerve agents (Sarin). Related compounds, such as parathion and malathion, are used as insecticides.

Example on irreversible anticholinestrase is Echothiophate

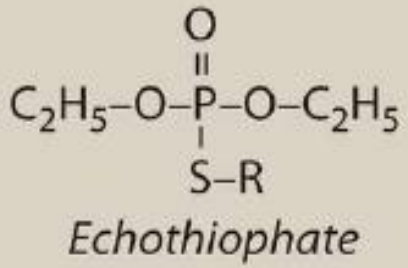
Echothiophate

Mechanism of action: Echothiophate is an organophosphate that covalently binds via its phosphate group at the active site of AChE. Once this occurs, the enzyme is permanently inactivated, and restoration of AChE activity requires the synthesis of new enzyme molecules. Following covalent modification of AChE, the phosphorylated enzyme slowly releases one of its ethyl groups. The loss of an alkyl group, which is called aging, makes it impossible for chemical reactivators, such as pralidoxime, to break the bond between the remaining drug and the enzyme.

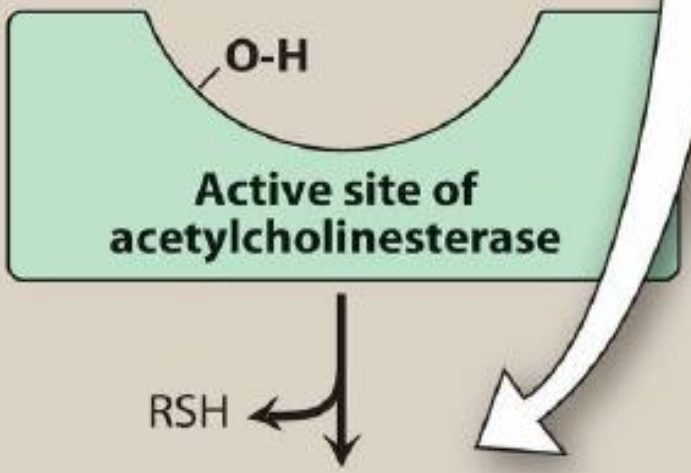
Echothiophate eye drops is used for the treatment of open-angle glaucoma. However, echothiophate is rarely used due to its side effect profile, which includes the risk of cataracts

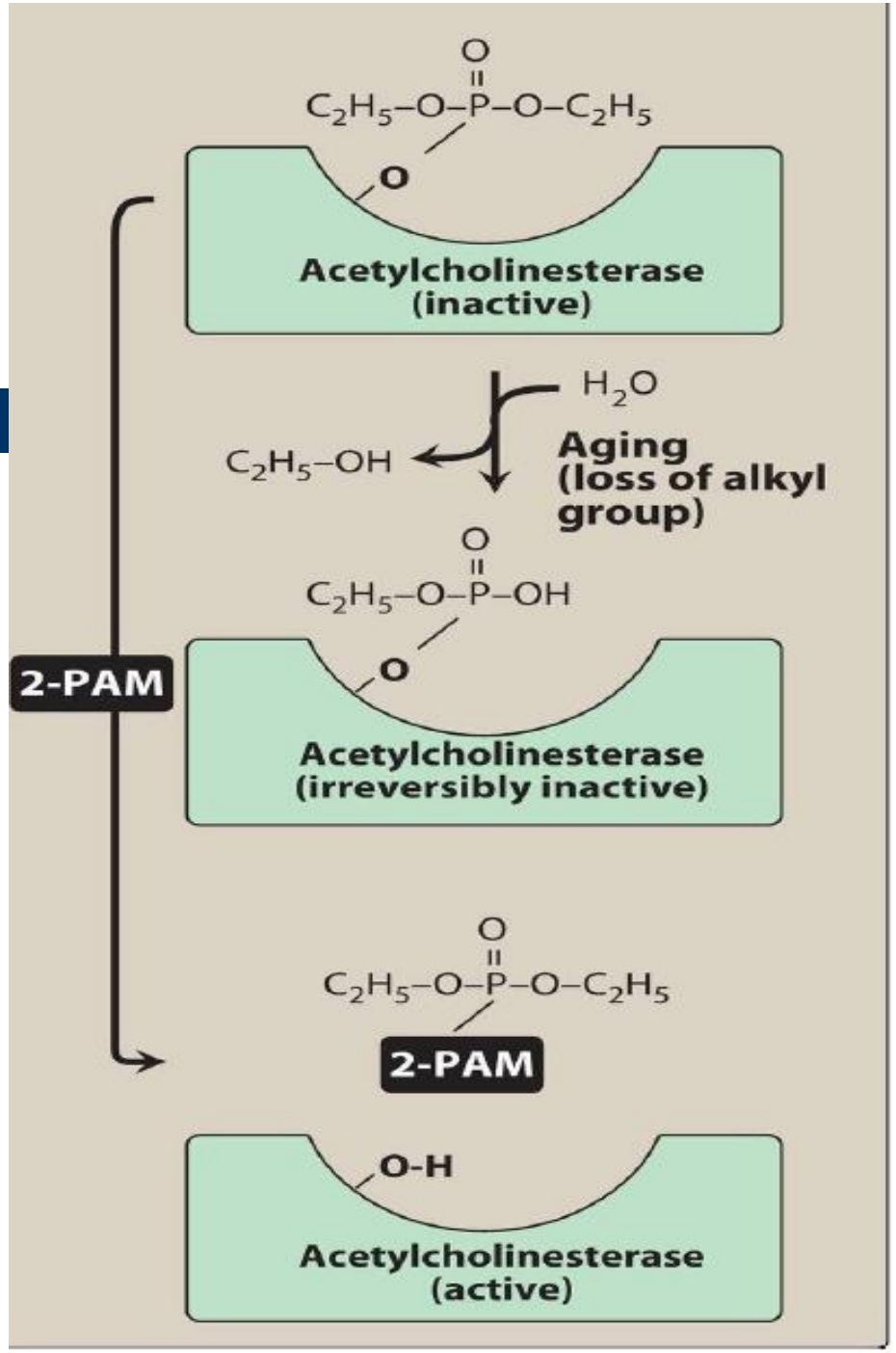
PHOSPHORYLATION OF ENZYME

- Enzyme inactivated
- *Pralidoxime* (2-PAM) can remove the inhibitor



Echothiophate





Toxicity of irreversible Anticholinesterase Agents

Irreversible AChE inhibitors (mostly organophosphate compounds) are commonly used as agricultural insecticides, which has led to numerous cases of accidental poisoning with these agents. In addition, they are frequently used for suicidal and homicidal purposes. Organophosphate nerve gases such as sarin are used as agents of warfare and chemical terrorism. Toxicity with these agents is manifested as nicotinic and muscarinic signs and symptoms (cholinergic crisis). Depending on the agent, the effects can be peripheral or can affect the whole body.

How can treat Toxicity of irreversible Anticholinesterase Agents ??

- By using reactivation of acetylcholinesterase (Pralidoxime) (2-PAM) However, it is unable to penetrate into the CNS and therefore is not useful in treating the CNS effects of organophosphates. The presence of a charged group allows it to approach an anionic site on the enzyme, where it essentially displaces the phosphate group of the organophosphate and regenerates the enzyme. If given before aging of the alkylated enzyme occurs, it can reverse both muscarinic and nicotinic peripheral effects of organophosphates, but not the CNS effects. With the newer nerve agents that produce aging of the enzyme complex within seconds, pralidoxime is less effective.
- In addition, it cannot overcome toxicity of reversible AChE inhibitors (for example,physostigmine).
- Atropine can also used to reverse the muscarinic effect of anticholinestrase agents.


THANKYOU