Cholinergic Neurotransmitters

Cholinergic system:-

parasympathetic system is also known as cholinergic system.

-> cholinergic system is one where is Acetylcholine is used or released in the body.

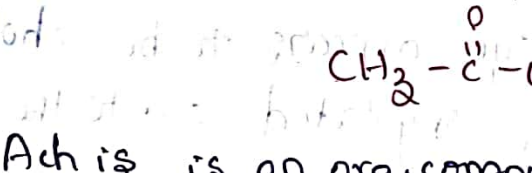
-> Acetyl choline is a neurotransmitter which propagates impulse transmission in the parasympathetic nervous system.

-> Acetylcholine has functions both in the peripheral nervous system and in the central nervous system (CNS).

-> Acetylcholine is major neurohumoral transmitter at autonomic, somatic as well as central sites.

Cholinergic Neurotransmitters:-

* Acetylcholine is the major neurotransmitter at post ganglion Synapses of cholinergic / parasympathetic nerve endings.
* Ach was first reported by Reid hunt and Taveau in 190



* Ach is is an org. compound / chemical which acts in the brain and body of animals & humans as a neurotransmitter.
* It is an ester of acetic acid choline.
* In the brain acts as a neuromodulator and as a neurotransmitter.

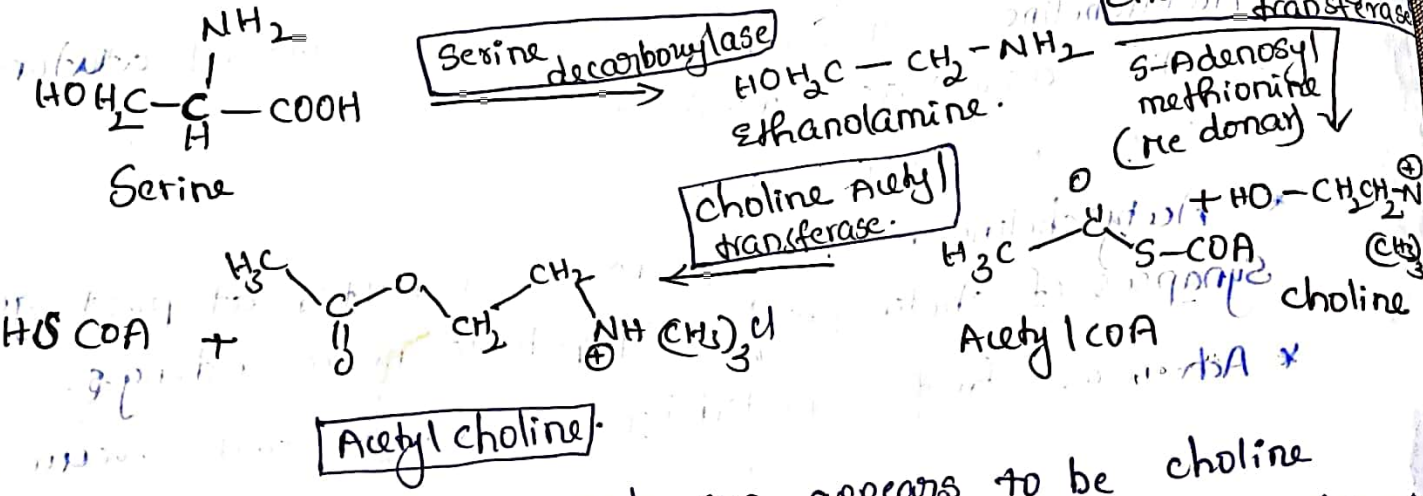
Biosynthesis of Acetylcholine :-

-> Acetylcholine is synthesized within the nerve terminal from choline, most which is taken up into the nerve terminal by special choline transport system.

--> Ach is synthesized, stored & released by cholinergic neurons.

-> Its synthesis takes place in the nerve endings by the transfer of an acyl group from acetyl coenzyme A (CoA) to choline.

-> The reaction is catalyzed by choline acetyl transferase (CAT / ChAT) enzyme synthesized in the neuron.



-> The rate limiting process in Ach syn appears to be choline transport, the activity of which is regulated acc to the rate at which Ach is being released.

-> cholinesterase is present in the presynaptic nerve terminals and Ach is continually being hydrolysed & re synthesized.

Storage of Ach :-

-> The highly polar choline is taken up into the axoplasm by the specific choline transporter.

-> The newly formed Ach is loaded into the storage vesicles by the vasicular Ach transporter (VAchT).

-> Each storage vesicle contains about 1000 to 50,000 molecules of Ach.

-> Large amt of Ach is also present in Extravesicular cytoplasm

-> Ach is transported into the storage vesicle by a carrier which can be inhibited by a chemical agent called vesamicol;

Release :-

-> Ach is stored in vesicles along with other potential co-transmitters (co-T) such as ATP.

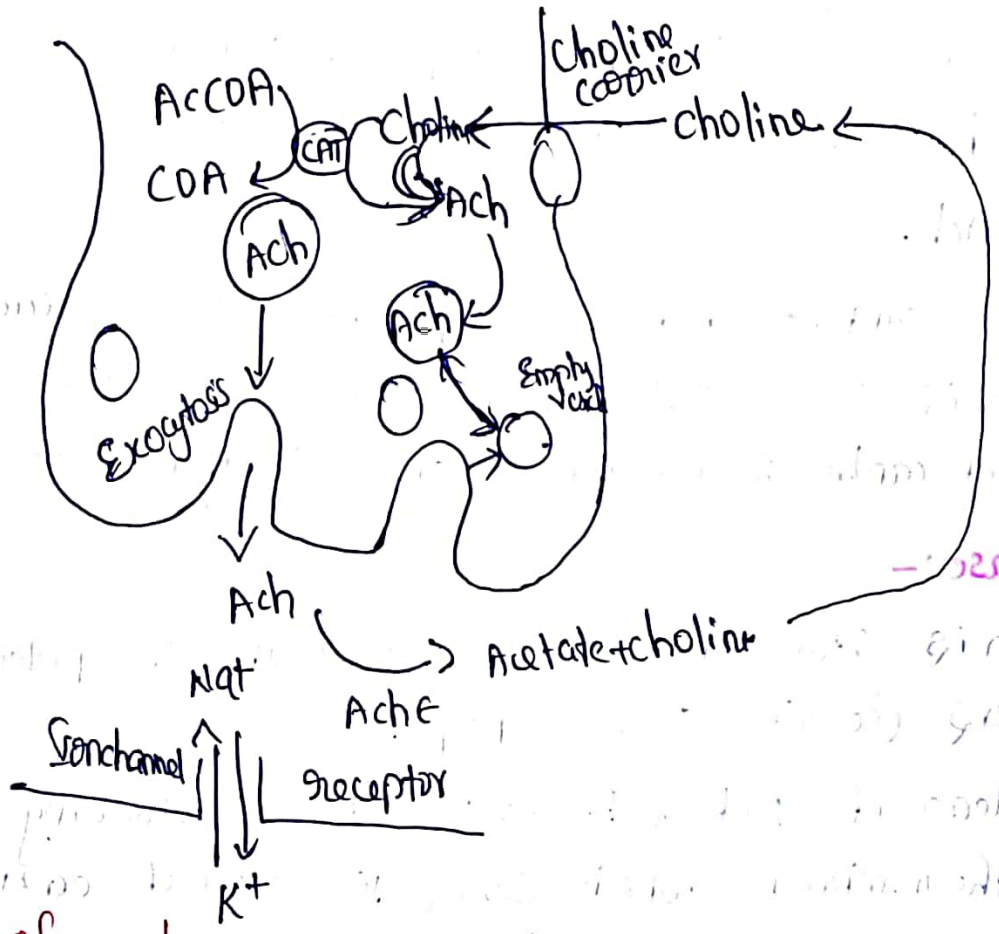
-> Release of Ach & the co-T occurs following depolarization of the membrane, which allows the entry of ca^{2+} through voltage-dependent ca^{+2} channels.

-> During the activation of nerve membrane, ca^{+2} is though to enter the axoplasm through voltage-gated channels and to activate protein kinases that phosphorylate synapsin.

-> As a result, vehicles close to the membrane are detached from their anchoring and allowed to fuse with the presynaptic membrane.

-> During fusion, vesicles discharge their contents into the synaptic gap and simultaneously insert specific-choline transporter (CHT) into the plasma membrane.

→ Ach quickly diffuses, exaytosis through the synaptic gap
→ At the postsynaptic effector cell membrane, Ach reacts with receptors.



Catabolism of Ach:-

→ After the release and action of Ach, the functions and effects of Ach can be terminated by the help of enzymatic hydrolysis.
→ Cholinesterases rapidly hydrolysis Ach into choline and acetic acid.
→ cholinesterases also known as Ach esterases (AchE).
→ It only causes hydrolysis of the Ach which is released from cholinergic nerve terminals.

Acetylcholine $\xrightarrow{\text{Cholinesterases}}$ Choline + Acetic acid.

Cholinergic receptors

Cholinergic receptors are like other transmembrane receptors and are chemical sites at synapses through which acetylcholine exerts its action.

Acetylcholine acts on two different classes of receptors.

1) Muscarinic receptors (M-receptors).
2) Nicotinic receptors (N-receptors)

*These are binds Ach and transmit signal.

1 Muscarinic receptors :-

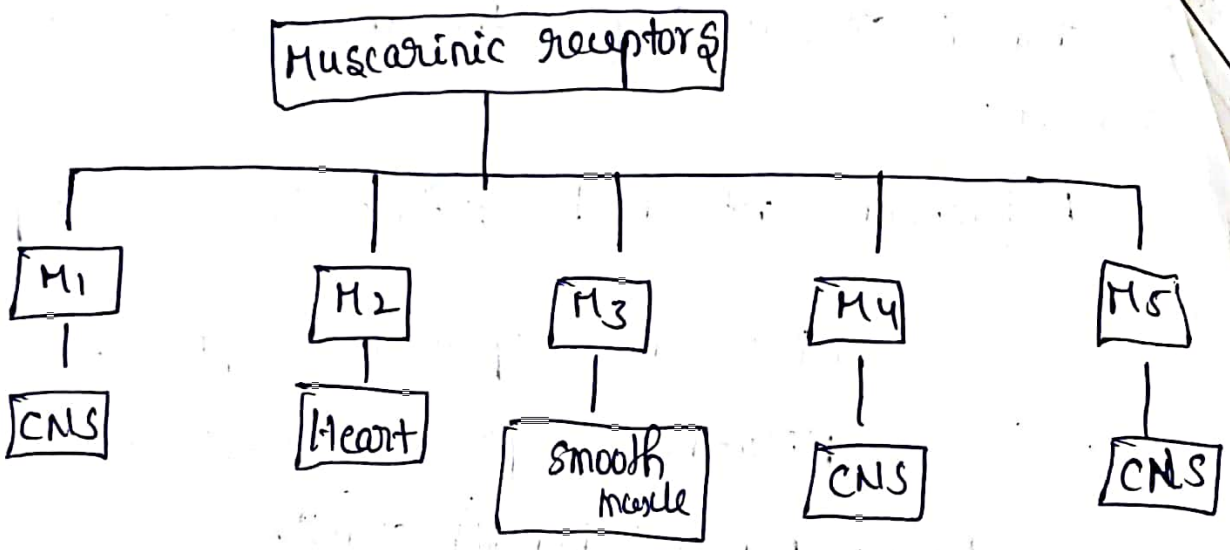
-> The prototype agonist for this receptor is muscarine, so named they specifically stimulated by muscarinic.

-> They belongs to ~~class~~ superfamily of G-protein coupled receptors which activates other ion channels.

-> Muscarinic receptors are involved in a large no. of physiological functions including, Heart rate & force, contraction of smooth muscle and release of neurotransmitters. cholinergic transmission that activates muscarinic receptors occurs mainly at autonomic ganglia

-> Binding of a Ach to a muscarinic Ach R' causes conformational change in the receptor that is responsible for its association with and activation of an intracellular G protein. the later converting GTP to GDP in order to become activated and dissociate from the receptors.

-> Binding studies have identified 5 classes of muscarinic receptors. —> M_1, M_2, M_3, M_4, M_5.



M_1 :- present in autonomic ganglia, gastric gland & in the CNS. It causes depolarization, histamine release, acid secretion, affects learning, memory & motor functions.

M_2 :- present in the heart. It decreases velocity of conduction and also ↓s the strength of contractility.

M_3 :- present in smooth muscles of the blood vessels and of the lungs. It causes contraction of smooth muscles and releases NO to produce vasodilation.

M_4 :- present in the CNS and heart and has no significant clinical effects. It may have direct regulatory action on K^+ and Ca^{+2} ion channels.

M_5 :- present in the CNS and no clinical effects produced by this type of receptor. It may regulate dopamine release at terminals within Striatum.

-> M_1, M_4 & M_5 receptors (CNS):

involved in complex CNS responses such as memory, arousal, attention and analgesia.

-> M_2 receptors (heart): Activation of M_2 receptors lowers conduction velocity at sinoatrial and atrioventricular nodes, thus lowering heart rate.

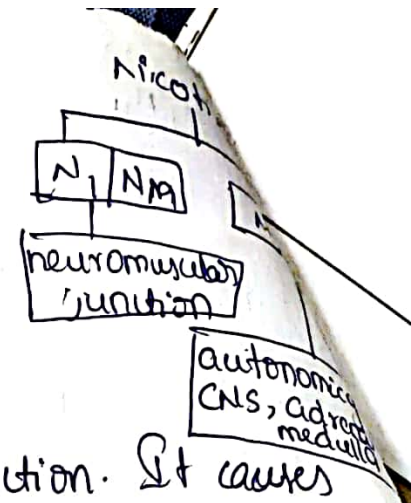
-> M_3 receptors (Smooth muscle): Activation of M_3 receptors at the smooth muscle level produces responses a variety of organs that include bronchial tissue, bladder, exocrine glands...

Nicotinic receptors:-

- Nicotinic receptors derived their name from nicotine, which does not stimulate the nicotinic receptors but selectively binds to the receptors
- It is ionotropic (ligand-gated) receptor which is linked to ion channels
- Nicotinic receptors are selectively activated by nicotine and blocked by tubocurarine or hexamethonium.
- These receptors rosette like pentameric in structure which encloses a ligand gated cation channel and their activation causes opening of the channel and rapid flow of cations result in depolarization and generation of action potential.

Nicotinic receptors are 2 types:-

1) Muscle type nicotinic receptors (Nm)
2) Neuronal type nicotinic receptors (Nn).



Muscle type nicotinic receptors :- Nm

These are located in the skeletal neuromuscular junction. It causes end plate depolarisation ~~and~~ and skeletal muscle contraction.

Neuronal type nicotinic receptors (Nn):

- These are located in adrenal medullary cells, in spinal cord, in ganglionic cells and in certain areas of brain
- It produces excitatory functions like depolarization, firing post ganglion and secretion of catecholamines.

parasympathomimetic agents

-> These agents mimic the action at parasympathetic system.
-> parasympathomimetic agents are the compounds which mimic the actions of Ach, causes nerve stimulation.
-> These are also called cholinomimetic agents / cholinergic agonists
-> cholinomimetic drugs that are resistant to the hydrolytic action of cholinesterase or agents that inhibits cholinesterase.

Classification

parasympathomimetic agents

- Direct acting (agonists)
  - Acetylcholine
  - Carbachol
  - Bethanicol
  - methacholine
  - pilocarpine
  - cevimeline
  - Arecoline
  - Oxotremorine
  - Xanomeline
- Indirect acting (Anticholinesterase)
  - Reversible (Direct acting)
    - physostigmine
    - Neostigmine
    - pyridostigmine
    - Endrophonium Cl
    - Tacrine HCl
    - Galantamine
    - Donepzil
    - Rivastigmine
  - irreversible (indirect acting)
    - parathion
    - malanthion
    - Dichlorofenthion
    - paraoxon
    - chlorpyrifos
- cholinesterase reactivator.
  - pralidoxime chloride.

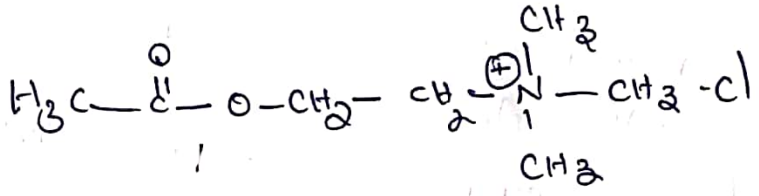
1. Direct acting :— These drugs bind to the nicotinic & or muscarinic receptors and causes excitation of cholinergic system.
2. Indirect acting:— These drugs inhibits the hydrolysis of Ach by acetyl cholinesterases and hence ↑es the life of Ach and causes increased conc. of Ach at the receptor site to produce excitation of cholinergic system.

Both the direct and indirect acting parasympathomimetic are used –

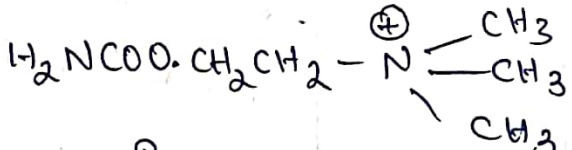
1) In reducing intraocular pressure in glaucoma.
2) In the relief of post operative atony of urinary bladder and gut.
3) It relieves muscular weakness in myasthenia gravis.

Direct acting :–

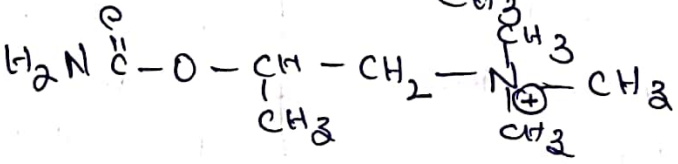
1) Acetylcholine :–



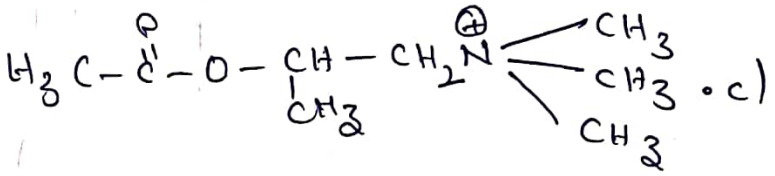
*2) Carbachol :–



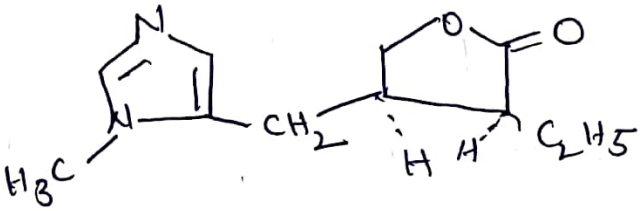
3) Bethanicol :–



4) Methanidoline :



5) Pilocarpine :



Indirect acting agents / cholinesterase inhibitors:

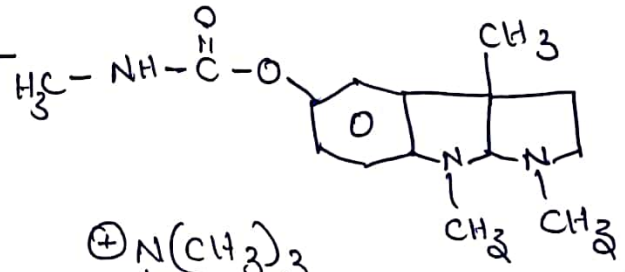
1) Reversible inhibitors - these drugs binds reversibally to the choline subsite. These drugs causes aylation of the hydroxyl group of the serine residue of acetylcholinestera
- These agents form an ester like carbonate or phosphate and covalently binds to the active site of the enzyme

eg: physostigmine, neostigmine.

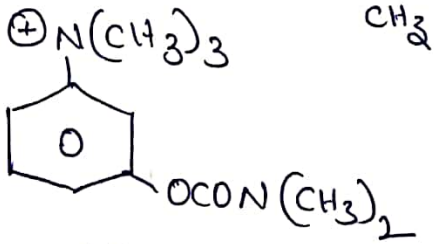
2) Irreversible Inhibitors:- These drugs produces irreversible inactivation of the acetylcholinesterase. This categeory includes various organophosphorous compounds. These are long lasting and binds irreversibly by covalant bonding to the active site of the enzyme.
- The resulting phosphorylated enzyme is very stable and causs inactivation of the acetylcholinesterases enzyme.

eg: parathion & malathion.

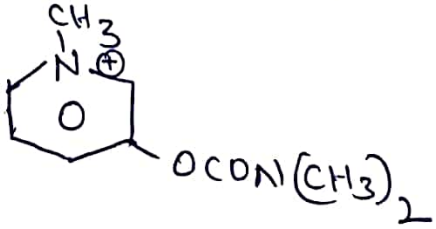
1. physostigmine :-



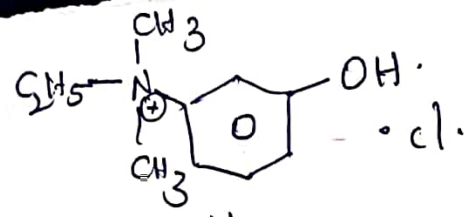
2. Neostigmine :-



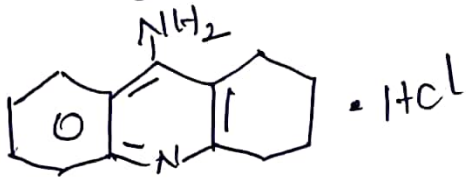
3. pyridostigmine :-



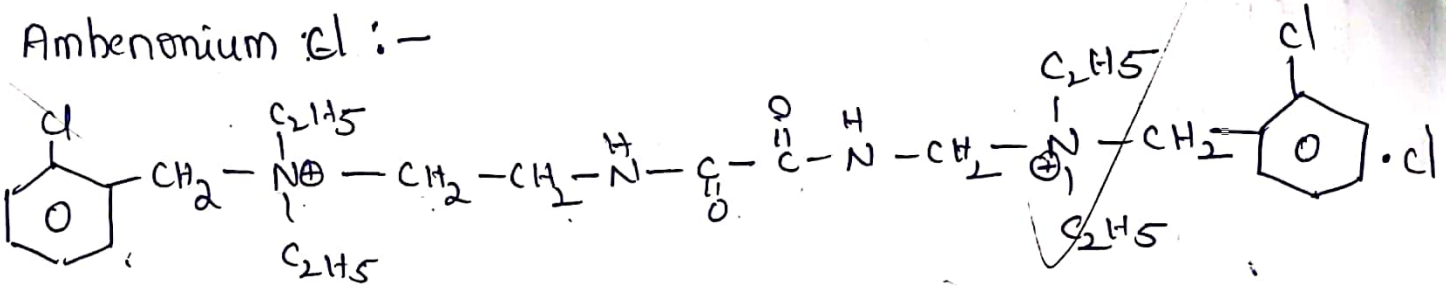
4. Edrophonium cl:-



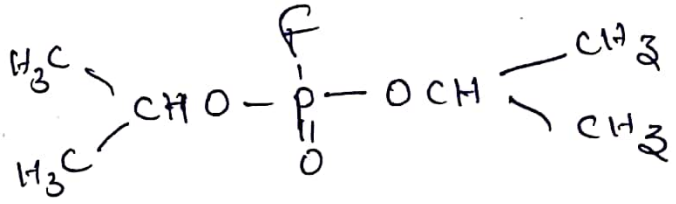
5. Tacrine Hcl:-



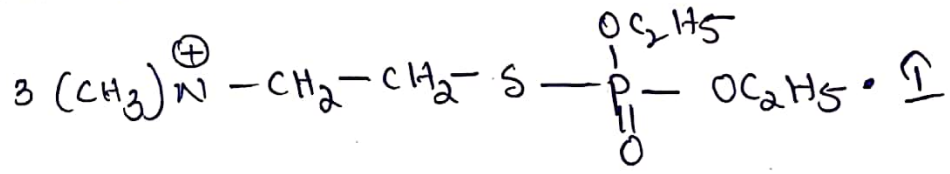
6. Ambenonium Cl:-



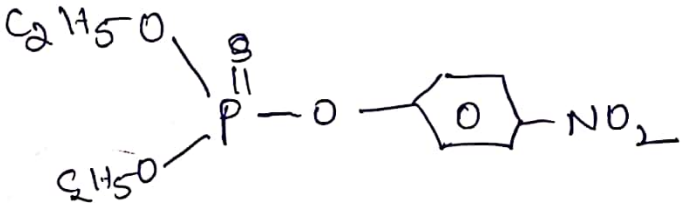
7. Isoflurophate:-



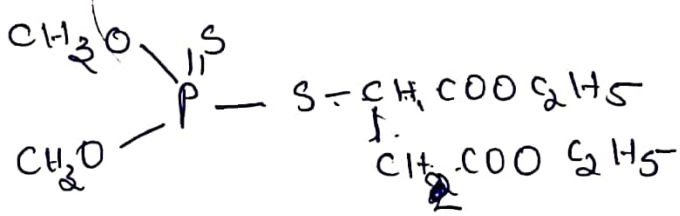
8. Ecothiophate iodide:



9. parathion:



10. Malathion:

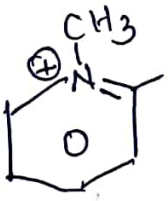


Cholinesterase reactivators :-

These are the drugs which causes the reverse of the inactivation of cholinesterases produced by organophosphates or irrversible agents: i.e these agents causes reactivation of cholinesterases.

- These drugs are used in treatment of poisioning produced by organo phosphorous compounds.

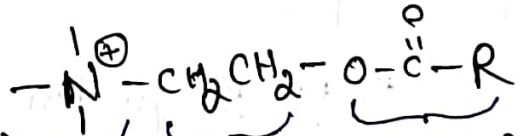
1. pralidoxime chloride :-



SAR of parasympathomimetic agents :-

Structural alteration on molecule may be divided into 3 categories.

1) The onium group modification
2) Modification of ester group
3) Modification of Ethylene bridge.



Onium - Choline Ester function
quaternary ammonium gp. Ethylene bridge acyloxy group.

1) Modification of onium :-

-> The onium group is essential for intrinsic activity.

-> The trimethyl ammonium group is the functional moiety activity through some exceptions are known

eg: pilocarpine, Nicotine & Oxotremorine.

-> The phosphonium, sulfonium, arsenonium isosteres of substituent larger than methyl group on the nitrogen increase the size of onium ion, les the activity.

2). Modification of ester group :-

-> The ester group of Ach contributes to the binding of the compound to the muscarinic receptor.

-> when acyl group is substituted by its higher homologues, less active compound is formed.

-> Carbachol, where the acetyl group is replaced by a carbamyl, has both muscarinic and nicotinic properties.

-> The concept that the ester i.e Carbamic or other group is not essential for activity but may enhances the affinity of the molecule for the receptor.

-> For maximal muscarinic activity the quaternary ammonium group should be followed by a chain of five atoms.

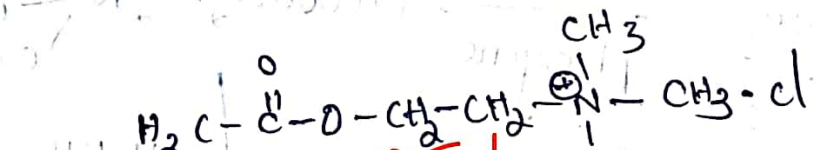
3) Ethylene bridge modification :-

-> Shortening or lengthening the chain of atoms that separates the ester group from the onium moiety reduces muscarinic activity.

- An α- Substitution in choline moiety, decreases both nicotinic and muscarinic activity, but muscarinic activity to greater extent.
- β-Substitution decreases the nicotinic activity to more extent.
- Hydrolysis by acetyl cholinesterase is more affected by β-Substitution than α- carbon.

(1) Direct acting :-

Acetyl choline :-



2-Acetoxy-N,N,N-trimethyl ethonium chloride.

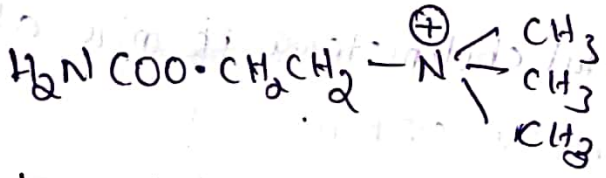
mechanism of action :-

- Ach is directly acting quaternary ammonium cholinergic drug that has the muscarinic effects of Ach.
- Its transit action is due to its destruction by cholinesterase.

Uses:-

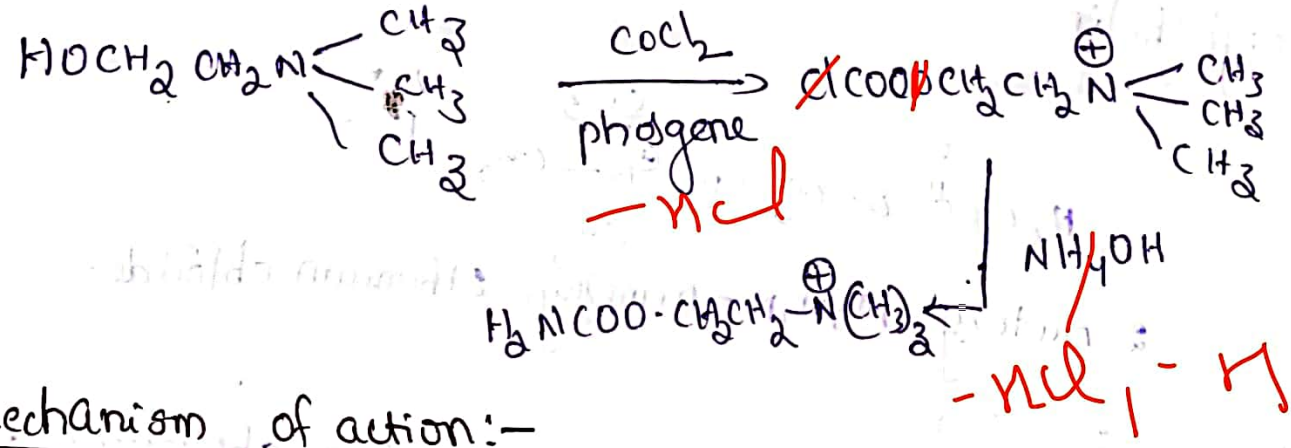
- It is used as miotic to reduce post-operative rises in intra-ocular pressure associated with cataract surgery.
- It also used as a vasodilator and cardiac depressant, a stimulant of vagus and the parasympathetic nervous system.
- It has tonic action on smooth muscle. It also increases lacrymal, salivary and other secretions.

Carbachol:



2 carbamoyloxy -N, N, N- trimethyl ethyl ammonium chloride

Synthesis:-



Mechanism of action:-

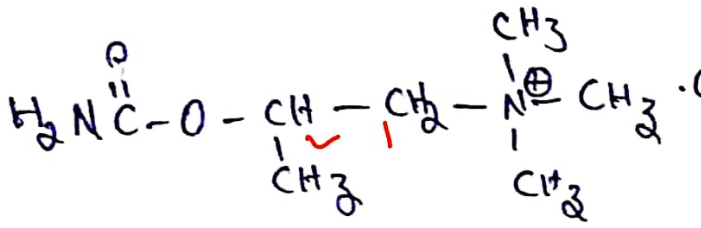
- Carbachol is a quaternary ammonium parasympathomimetic possess both muscarinic and nicotinic action of Ach.

Use:- It is used as an alternative to pilocarpine in the management of glaucoma.

- Carbachol is given intra-ocularly to produce miosis in ocular surgery

reduce post operative rises in intraocular pressure.

3. Bethanicol :-



2 carbamoyloxy-1-(N,N,N, trimethyl) propyl ammonium chloride.

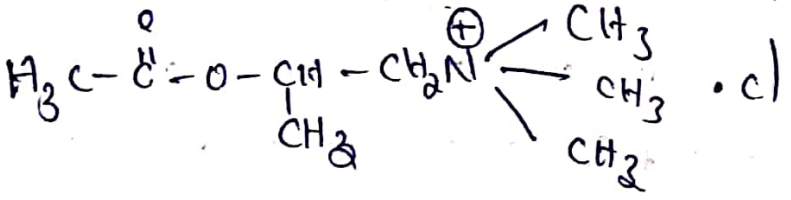
mechanism of action :-

- Bethanicol is choline ester mainly exhibits the muscarinic action of Ach.
- It is not inactivated by cholinesterases.

Uses :-

- It is usually used in stimulation of G.I tract and urinary bladder to relieve postoperative atony. It has prolong effect than Ach.

4. Methacoline :-



2 (Acetoxy)-N,N,N- trimethyl propan-1- ammonium chloride.

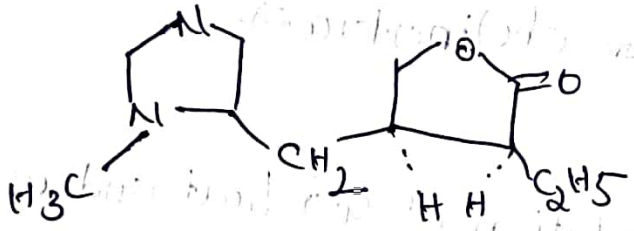
MOA :-

- methacholine is quaternary ammonium parasympathomimetic coith the muscarinic actions of Ach.

Uses:-

1. It is used in treatment of reynaud's syndrome & glaucoma.
2. It is used in eye drops as a miotic for diagnostic purposes.
3. used to diagnose bronchial hyper reactivity.
4. It is also used for peripheral vascular disease.

5. pilocarpine:-



3-ethyl-4-(1-methyl-5-imidazolyl methyl) tetrahydrofuran-2-one.

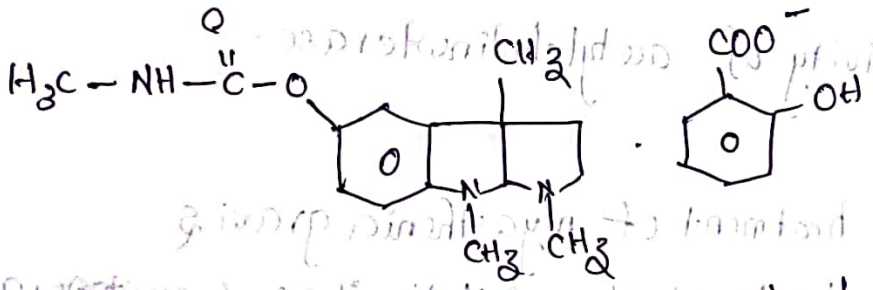
MOA:-

- Direct acting tertiary ~~amine~~ amine cholinergic that has muscarinic effects of Ach.
- pilocarpine may be used for surgery as part of the emergency treatment of acute attacks of angle-closure glaucoma.
- Also used in diaphoretic in diagnostic tests for cystic fibrosis & leprosy.

1. It is mainly used in the treatment of glaucoma and in treatment of dry eye or dry mouth.
2. It is used in the treatment of open angle glaucoma.
3. Pilocarpine may be used before surgery as part of the emergency treatment of acute attacks of angle-closure glaucoma.
4. It has also been used as a diaphoretic in diagnostic tests for cystic fibrosis and uptocy.

Indirect acting / Cholinesterase inhibitors.

1. Physostigmine Salicylate



1, 3 (a) - trimethyl - 2, 3, 3 (a), 8 (a), - tetrahydropyrrolo [2, 3-b] - indole 5-yl - N-methyl carbamate.

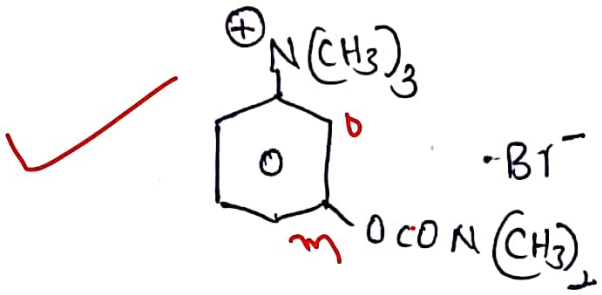
MOA :—

→ physostigmine is a reversible tertiary amine inhibitor of cholinesterase activity with actions similar to those Neostigmine.

Uses :—

1. physostigmine has been used alone or more usually with other miotics such as pilocarpine, to decrease intraocular pressure in glaucoma.
2. It is more potent miotic than pilocarpine but is rarely tolerated for prolonged periods.
3. It can also be used parenterally for reversal of effects caused by anticholinergic & tricyclic depressants.

2. Neostigmine bromide



N,N,N - trimethyl - meta - dimethyl - carbamoyloxy - benzenaminium bromide.

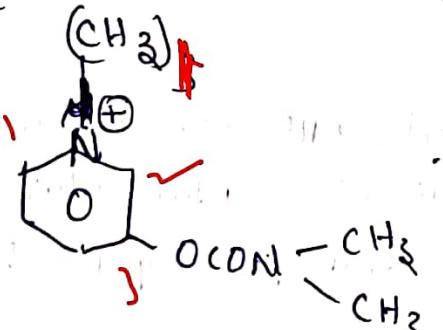
MOA :-

- Indirectly stimulates both muscarinic and nicotinic receptors.
- It binds to the anionic and esteric site of cholinesterase and block the activity of acetylcholinesterase.

Uses :-

1. It is used in the treatment of myasthenia gravis
2. It is used in the treatment of paralytic ileus & postoperative urinary retention.
3. It has also been used to lower intra-ocular pressure in the management of glaucoma.

3. pyridostigmine.



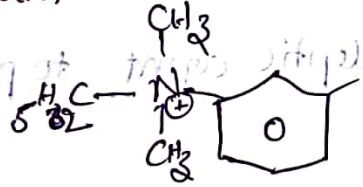
3-Dimethylamino carbonyl]oxy]-1-methyl pyridinium bromide.

MOA:— pyridostigmine block acetyl cholinesterase enzyme and inhibit the destruction of released acetyl choline.

uses:—

1. mainly used in the treatment of myasthenia gravis.
2. It has also been used as prophylaxis against the neuromuscular blockade produced by effects of nerve gas poisioning.
3. It has been used in management of postoperate urinary.
4. pyridostigmine is used to reverse the neuromuscular blockade produced by competetive neuromuscular blockers but it is generally considered less satisfactiry than neostigmine.

4. Edrophonium chloride:—



Ethyl-(3-hydroxy phenyl) dimethyl-ammonium chloride.

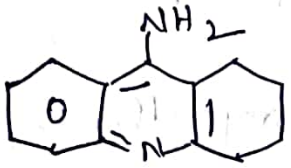
MOA:—

It is a quaternary ammonium compound that is reversible inhibitor of cholinesterase activity. It has a shorter duration of action than neostigmine and pyridostigmine.

uses:—

1. It is mainly useful for the treatment of myasthenia gravis but due to its short duration of action it is not suitable for routine treatment of myasthenia gravis.
2. Edrophonium is also used in the treatment of snake bite.

5. Tacrine Hydrochloride :-



1,2,3,4 - tetrahydroacridin-9- amine.

MOA :-

- Tacrine is centrally acting anticholinesterase and indirect cholinergic agonist.

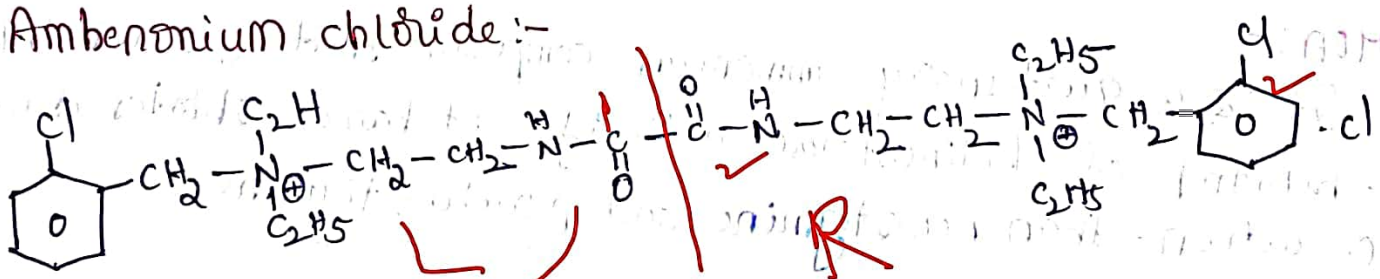
Uses :-

1- It is used in the treatment of mild to moderate severe dementia in Alzheimer's disease.

2- It has also used as an an analeptic agent to promote mental alertness.

3. Tacrine has been used intravenously to antagonize competetive neuromuscular blockers and as a ~~postoperative respiratory stimulant~~

6. Ambenonium chloride :-

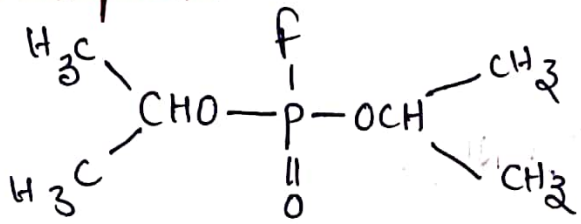


2,2-[(1,2-Dioxoethane-1,2-diyl)diimino]bis(N-(2-chlorobenzyl)-N,N-diethylethanaminium] chloride.

MOA :- It competetively reversibly inhibit the acetylcholinesterase enzyme responsible for hydrolysis of Ach.

Uses :- It is used the management of myasthenia gravis.

7. Isoflurophate



bis (propan-2-yl) fluorophosphonate.

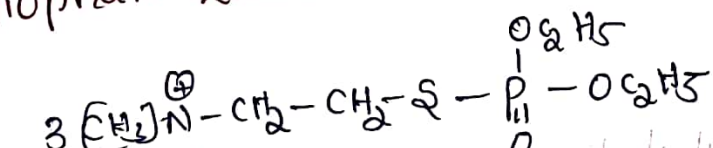
MOA :-

- It produces virtually irreversible inactivation of the acetyl-cholinesterase.

Uses :-

1. It is used as a miotic agent in treatment of glaucoma.
2. It is used in civilian laboratories to mimic lethal nerve gas exposure or organo phosphate toxicities.
3. It also inhibit some proteases enzymes. So it is useful for additive for protein or cell isolation procedure.

8. Ecothiophate Iodide :-

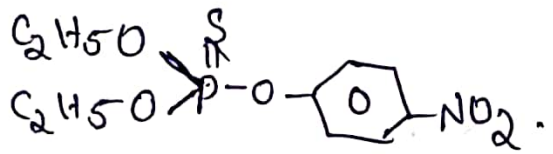


2-(Diethoxyl phosphoryl sulfanyl) ethyl-N,N,N-trimethyl azonium iodide.

MOA :- It is an irreversible acetyl cholinesterase inhibitor. It covalently binds to cholinesterase and permanently inactive the enzyme.

Uses :- It is used as an ocular antihypertensive in the treatment of chronic glaucoma.

9. parathion:-



O,O-Diethyl-O-4-Nitrophenyl Phosphorothioate.

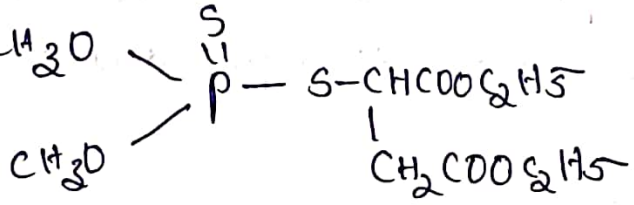
MOA:-

It indirectly acts on the acetyl cholinesterase enzyme.

Uses:-

It is used as insecticide in agriculture. it is often applied by spraying to cotton, rice & fruit trees.

10. Malathion:-



MOA:-

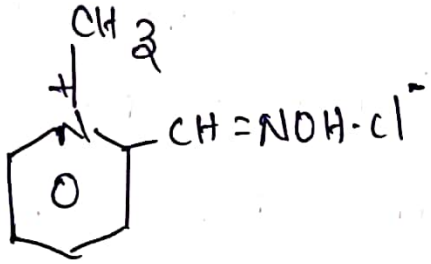
It inhibits the acetyl cholinesterase activity by binding serine residue on the cholicsterase enzyme and irreversibly deactivates the enzyme.

Uses:- Malathion low doeses(0.5%) is used in treatment of head lice and body lice.

- It is also used for treatment of scabies
- It is also used as insecticide.

Cholinesterase Reactivators:

1. pralidoxime chloride:-



MOA:-

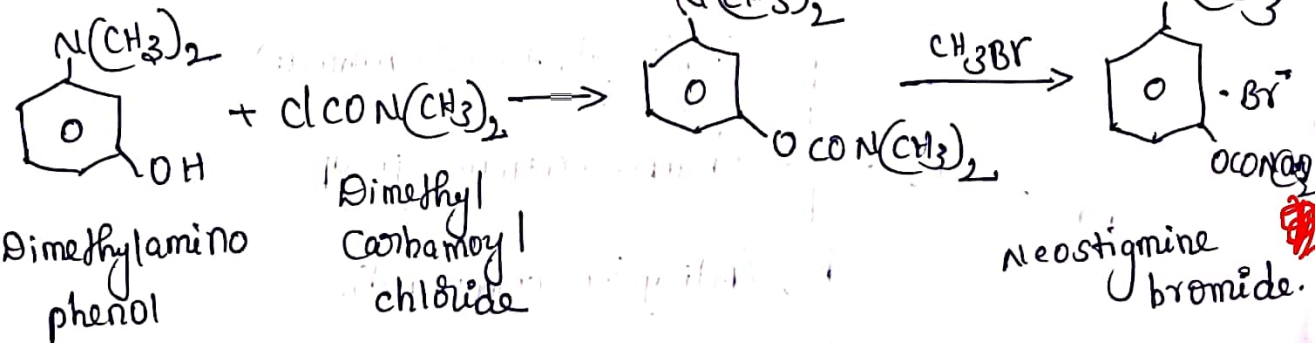
It reactivate the acetyl cholinesterase enzyme rapidly by binding to the anionic site of enzyme and displaces the phosphate from the serine residue.

Uses:-

1- It is mainly used for the treatment of poisioning by organo phosphorous compounds.

2. It is also used for the treatment of overdose by anti-cholinesterase drugs, including those used to treat myasthenia gravis such as neostigmine.

Synthesis of Neostigmine bromide:-



Cholinergic Blocking agents / Cholinolytics

-> These are also called Antispasmodics or parasympatholytics.

-> Anticholinergic drugs inhibit the effect of Ach released from post ganglionic parasympathetic nerve endings.

-> They block muscarinic action of Ach, including smooth muscle contraction and exocrine gland secretion.

-> Because of the ability to relax smooth muscle, they are also referred as antispasmodics.

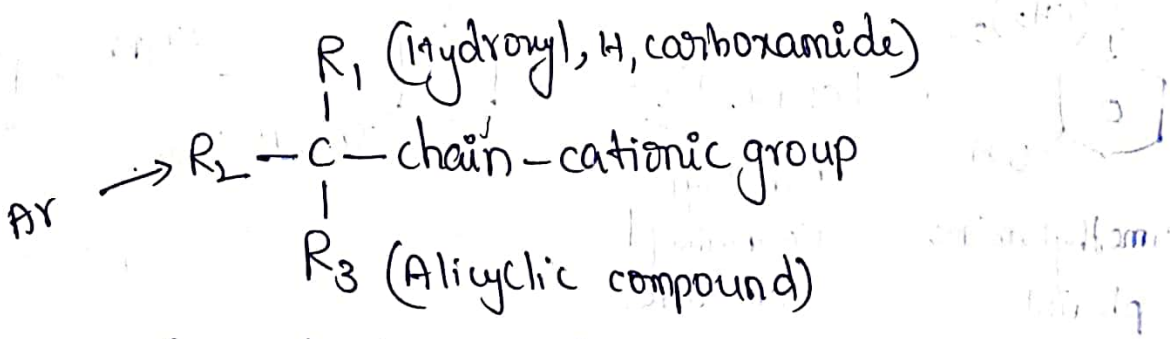
-> The cholinergic blocking agents can be grouped according to their location & type of cholinergic receptors into:-

i) parasympathetic postganglionic blocking agent or anti-muscarinic agents. Eg: Atropine, scopalamine.

ii) Ganglionic blocking agents eg: Hexamethonium, Curare alkaloid pancuronium bromide.

iii) Neuromuscular blocking agents: Eg: Tubocurarine, metocurine.

SAR of Cholinolytics:-



General structure of anticholinergic agent.

1. Anticholinergic agents may contain a cationic group, quaternary ammonium salt or a tertiary amine that is protonated at physiological P_H.

2. steric hindrance that diffuses the charge of anion, decreases the parasympathomimetic activity.

*3. The primary part of attachment to cholinergic sites is through the positively charged nitrogen (cationic head). In case of tertiary amines, they are quaternized by protonation at physiological pH. This property is responsible for their lack of CNS activity because of poor penetration in the brain.

*4. for hydrophobic bond formation and vander-waals interaction an aryl group (phenyl, thienyl) and a cyclo aliphatic (cyclo-hexyl) is a common feature in all anticholinergic molecules.

*5. An ester group is present in Ach, therefore, ester group containing compounds are potent anticholinergics.

*6. The presence of a free hydroxyl or carbamide is also imp. for hydrogen bonding with the receptor.

Classification of Cholinolytics.

Parasympatholytics / Anticholinergic drugs

1. Solanaceous alkaloids & its analogues

Eg: Atropine Sulphate
Scopalamine
Homatropine
Ipratropium bromide

II. Synthetic cholinergic Blockers.

- Aminoalcohol esters
  - clidinium bromide
  - cyclopentolate HCl
  - * Dicycloamine HCl
  - glycopyrolate
  - Methantheline Br
  - propantheline Br.
- Aminoalcohol ethers
  - orphenadrine citrate
  - Benztropine mesylate
- Amino alcohols
  - Biperidine HCl
  - * procyclinidine HCl
  - Tridihexethyl chloride
- Amino amides Eg: Ethopropazine HCl, isopropamide iodide, Tropicamide
- miscellaneous