Medicinal Chemistry

Chapter 9

CHOLINERGICS, ANTICHOLINERGICS, AND ANTICHOLINESTERASES

Contents

CHOLINERGIC NERVOUS SYSTEM

1. Nerve transmission

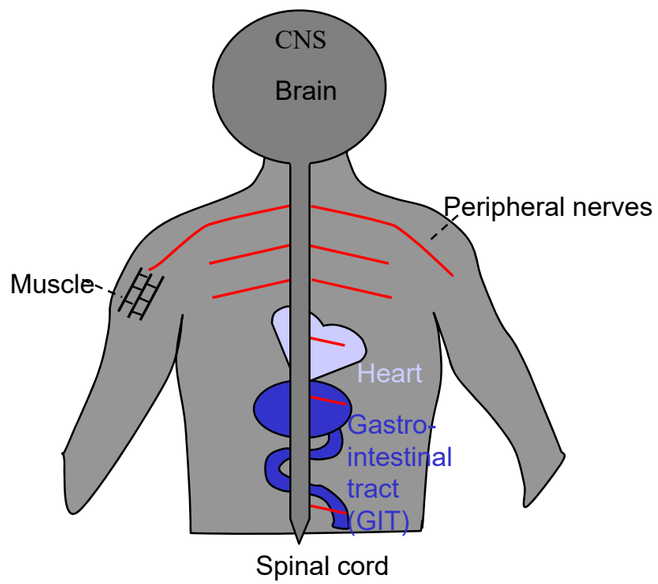
Peripheral nervous system

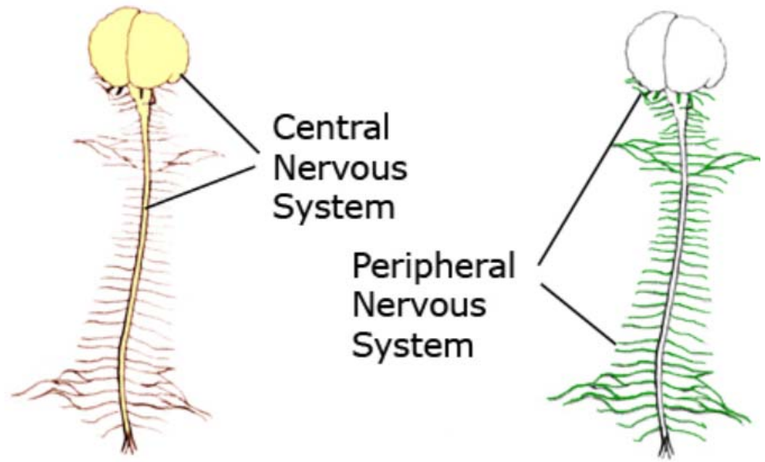
- The peripheral nervous system (PNS) is so called because it is peripheral to the central nervous system (CNS)

➢ PNS can be divided into two divisions:

- sensory nerves “which take messages from the body to the CNS”.
- motor nerves “which carry messages from the CNS to the rest of the body.

➢ Here we are only concerned with the motor nerves. An individual nerve cell is called a **neuron**.

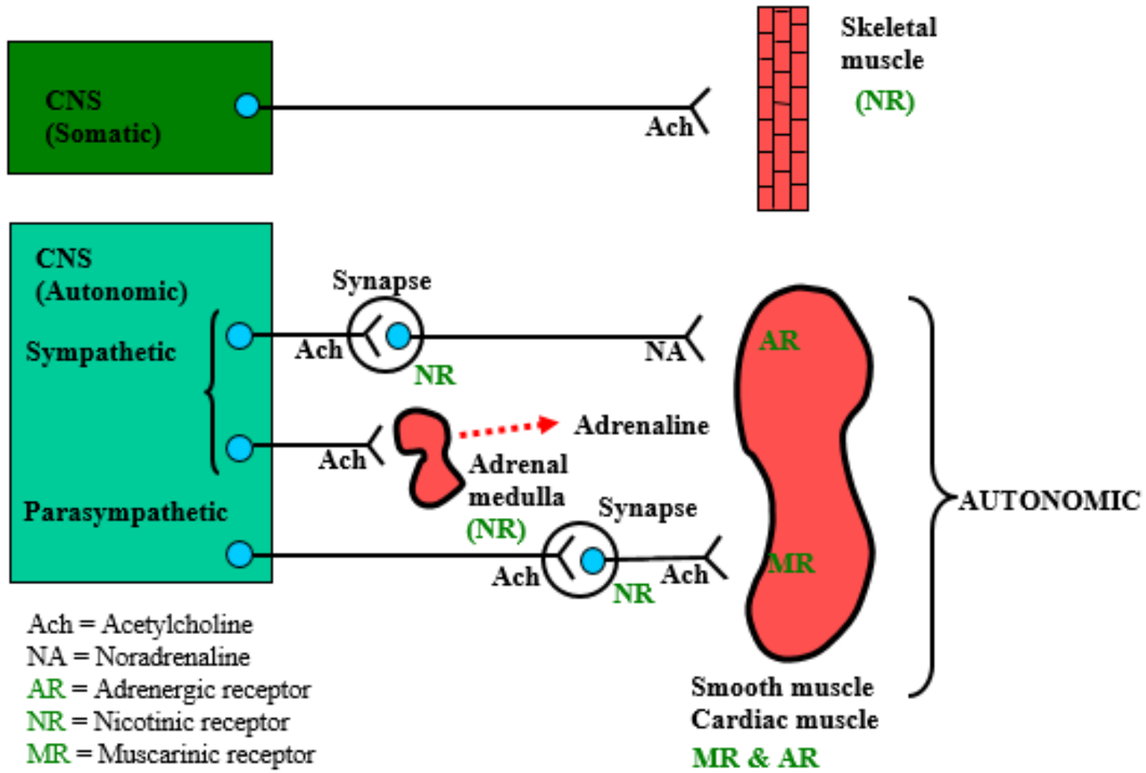




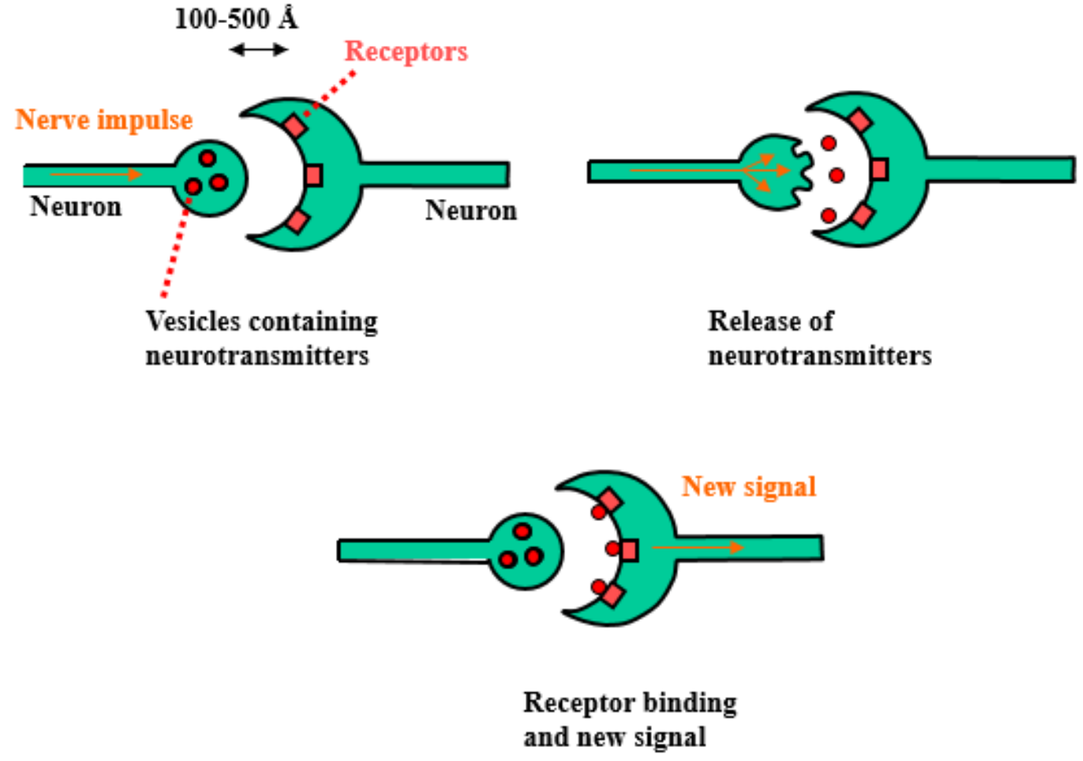
❖ Motor nerves of the PNS

- motor nerves **take** messages from the **CNS** to various parts of the **body** such as skeletal muscle, smooth muscle, cardiac muscle and glands.

- motor nerves have been divided intro 3 subsystems: somatic motor nervous system, the autonomic motor nervous system and the enteric nervous system.



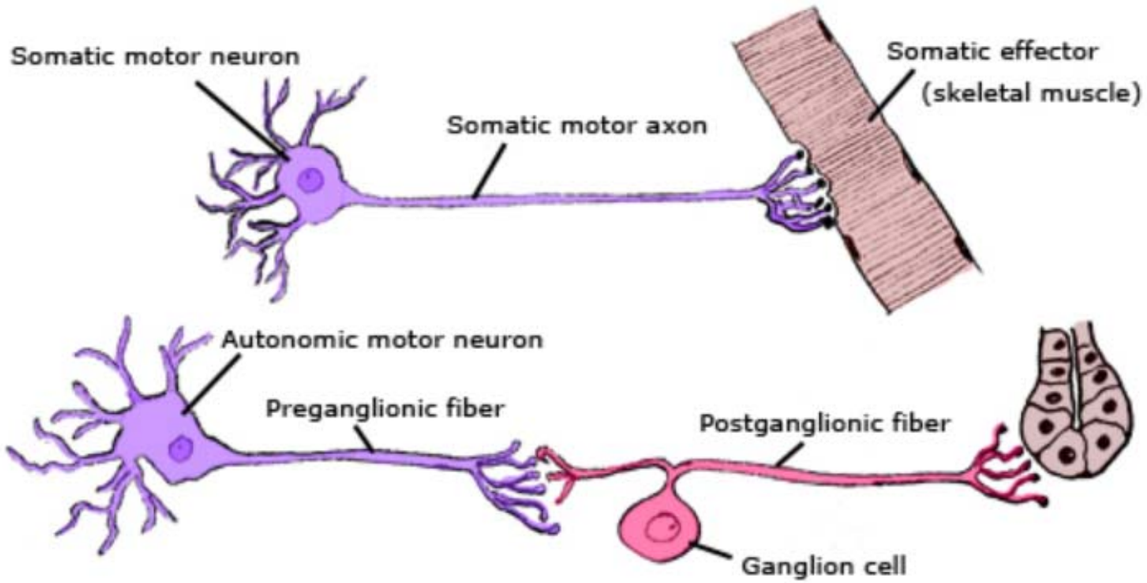
- Neurons must communicate with each other in order to relay messages "not physically connected". but there are gaps which are called **synapses**.
- If a neuron is to communicate its message to another neuron (or a target organ), it can only do so by releasing a chemical that crosses the synaptic gap and binds to receptors on the target cell.
- This interaction between neurotransmitter and receptor can then stimulate other processes, which, in the case of a second neuron, continues the message.
- As these chemicals effectively carry a message from a neuron, they are known as **chemical messengers** or neurotransmitters " **acetylcholine and noradrenaline**"



❑ ***Somatic motor nervous system***

- they carry messages from the CNS to the skeletal muscles. There are **no synaptic** "junctions" en route and the neurotransmitter at the neuromuscular junction is acetylcholine.

•Acetylcholine binds to cholinergic receptors within the cell membranes of muscle cells and the final result is contraction of skeletal muscle.



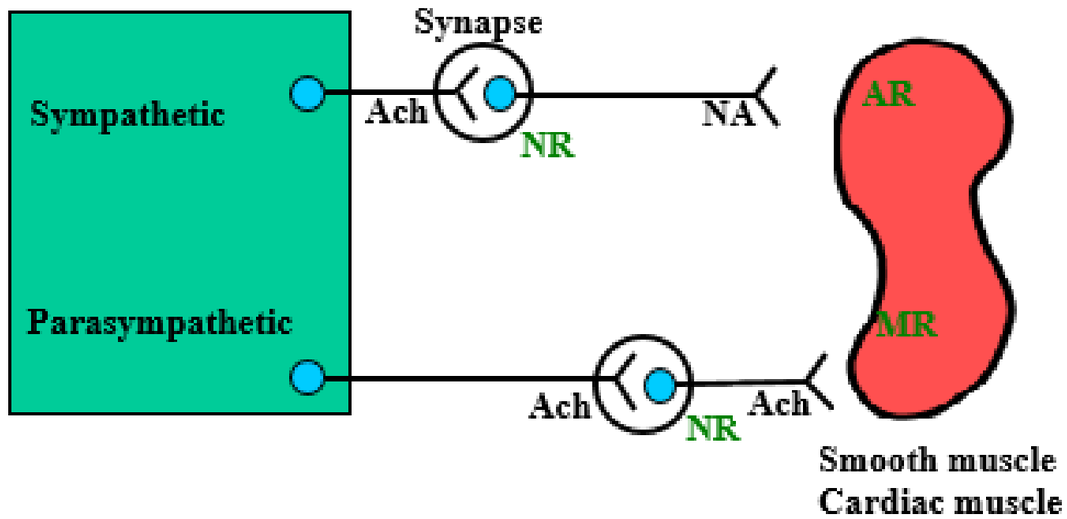
❑ ***Autonomic motor nervous system***

•they carry messages from the CNS to smooth muscle, cardiac muscle & the adrenal medulla. This system can be divided into two subgroups: Parasympathetic nerves & Sympathetic nerves

❑ ***Enteric system:*** it is located in the walls of the intestine. It receives messages from sympathetic & parasympathetic nerves, but it also responds to local effects to provide local reflex pathways which are important in the control of GIT function.

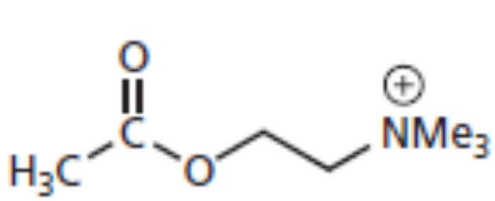
Parasympathetic nerves: leave the CNS, travel some distance, then each neuron synapses with a second neuron, which then proceeds to the final synapse with smooth muscle. The neurotransmitter at both synapses is acetylcholine.

Sympathetic nerves: leave the CNS, but almost immediately each neuron synapses with a second neuron "neurotransmitter here is acetylcholine", which then proceeds to the same target organs as the parasympathetic nerves.

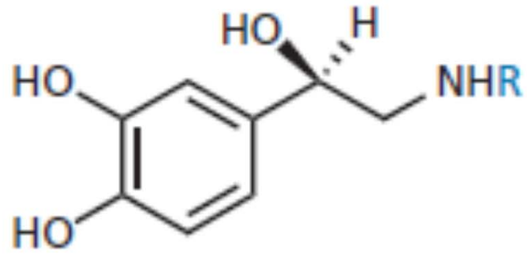


2. Neurotransmitters

In the PNS we need to consider only two neurotransmitters- **acetylcholine** "crucial in cholinergic system" & **noradrenaline** "is essential in adrenergic system".

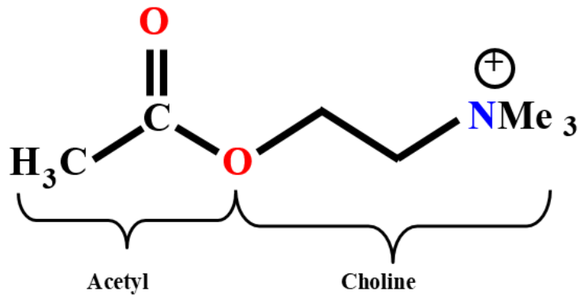


Acetylcholine



R = H Noradrenaline
R = Me Adrenaline

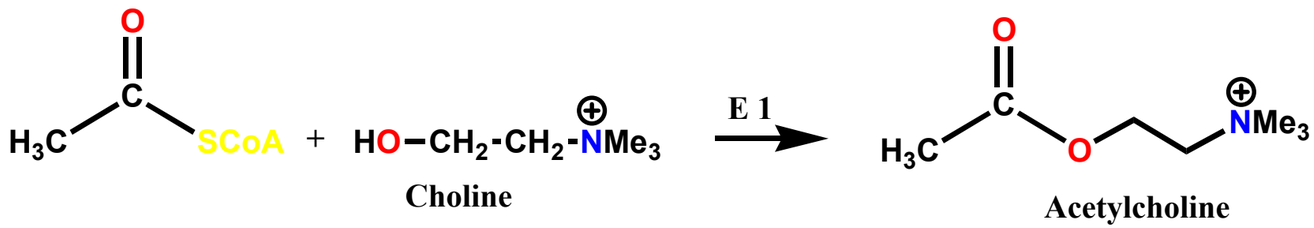
3. Cholinergic system

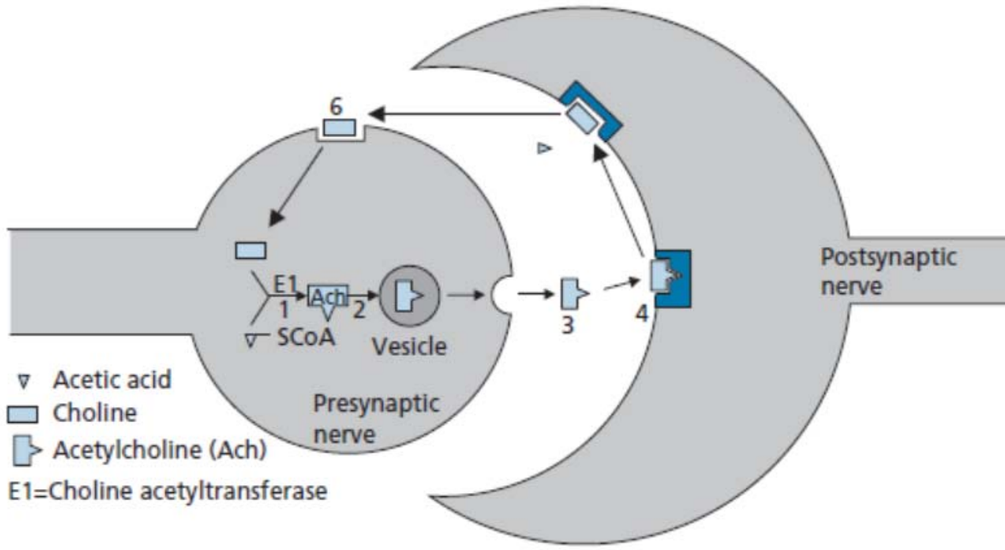


What happens in the synapse between two neurons.

1.the first stage involves the biosynthesis of acetylcholine. It is synthesized from **choline** & **acetyl coenzyme A** at the ending of the pre-synaptic neuron. The rxt is catalyzed by the enzyme **choline acetyltransferase**.

E 1 = Choline acetyltransferase



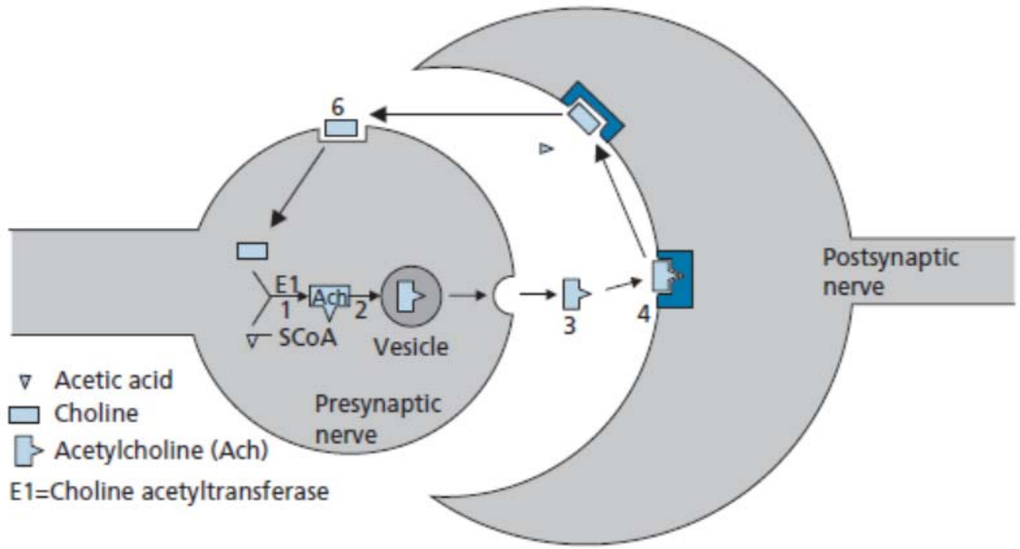


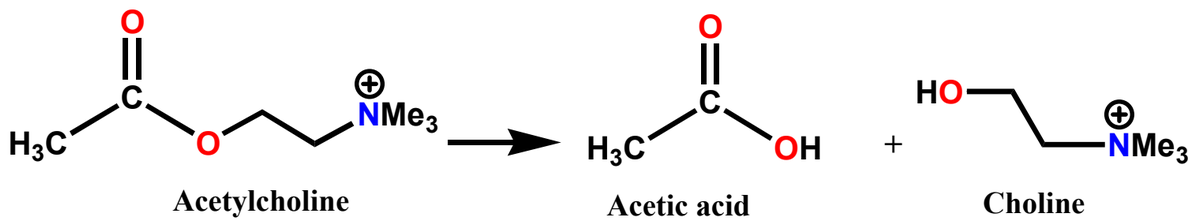
2. Ach is incorporated into membrane-bound vesicles by means of a specific transport protein.

3. The arrival of a nerve signal leads to an opening of calcium ion channels & an increase in intracellular calcium concentration. This induces the vesicles to fuse with the cell membrane & release the transmitter into the synaptic gap.

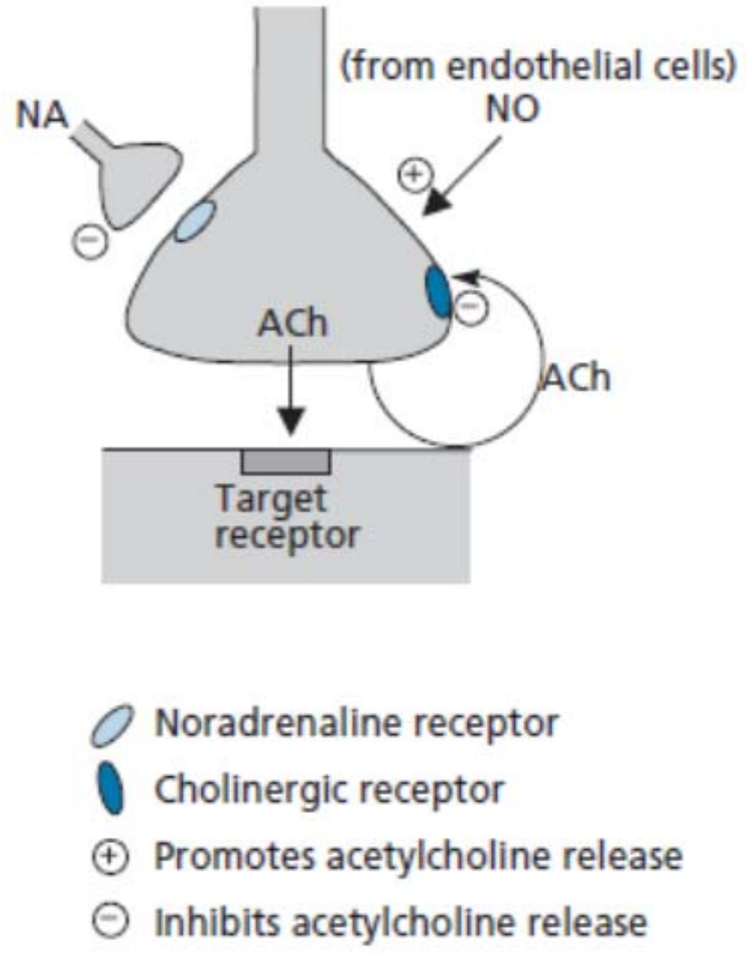
4. Ach crosses the synaptic gap & binds to the cholinergic receptor leading to stimulation of the second neuron.

5. Achmoves to an enzyme called **acetylcholinesterase**, which is situated on the postsynaptic neuron, which catalyses the hydrolysis of acetylcholine to produce choline & acetic acid





➢Cholinergic receptors (called autoreceptors) are present at the terminus of the presynaptic neuron. “The purpose of these receptors is to provide a means of local control over nerve transmission. When acetylcholine is released from the neuron, some of it will find its way to these autoreceptors and switch them on. This has the effect of inhibiting further release of acetylcholine.”



➢the presynaptic neuron also contains receptors for noradrenaline & inhibit its release. “the sympathetic nervous system is active, noradrenaline is released and binds to these receptors”. The chemical messenger nitric oxide “NO” promotes the release of acetylcholine.

4. Agonists at the cholinergic receptor

- If there is a lack of acetylcholine acting at a certain part of the body, why not just administer more acetylcholine?

❑ **<u>There are three reasons why this is not feasible.</u>**

- Acetylcholine is easily hydrolysed in the stomach by acid catalysis and cannot be given orally.
- Acetylcholine is easily hydrolysed in the blood by esterase enzymes (esterases).
- There is no selectivity of action. Additional acetylcholine will switch on all cholinergic receptors in the body.

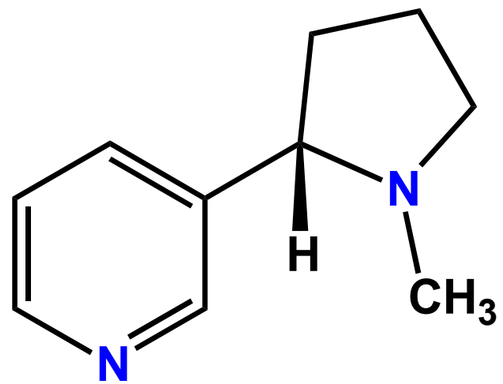
- Therefore, we need **analogues** of **acetylcholine** that are more **stable to hydrolysis** **and** **more selective** with respect to where they act in the body.

- As we know, there are different types of Ach receptors and subtypes from this we can design a specific drug which can bind only at one subtype receptor.

- there are one type of cholinergic receptor on skeletal muscles and at nerve synapses called nicotinic receptor, which has ten subtypes of it "α1-α10".

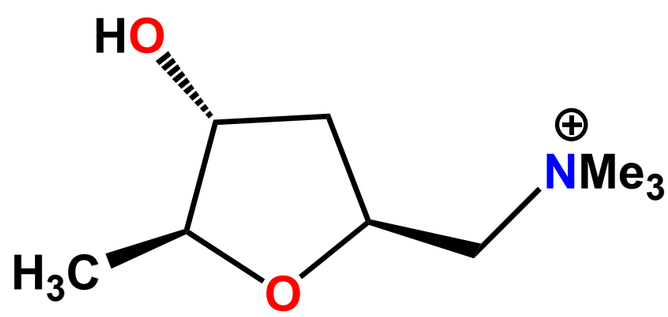
- and a different type of cholinergic receptor on smooth muscle and cardiac muscle called muscarinic receptor, which has five subtypes of it "M1-M5".

5. Cholinergic receptor



Nicotine

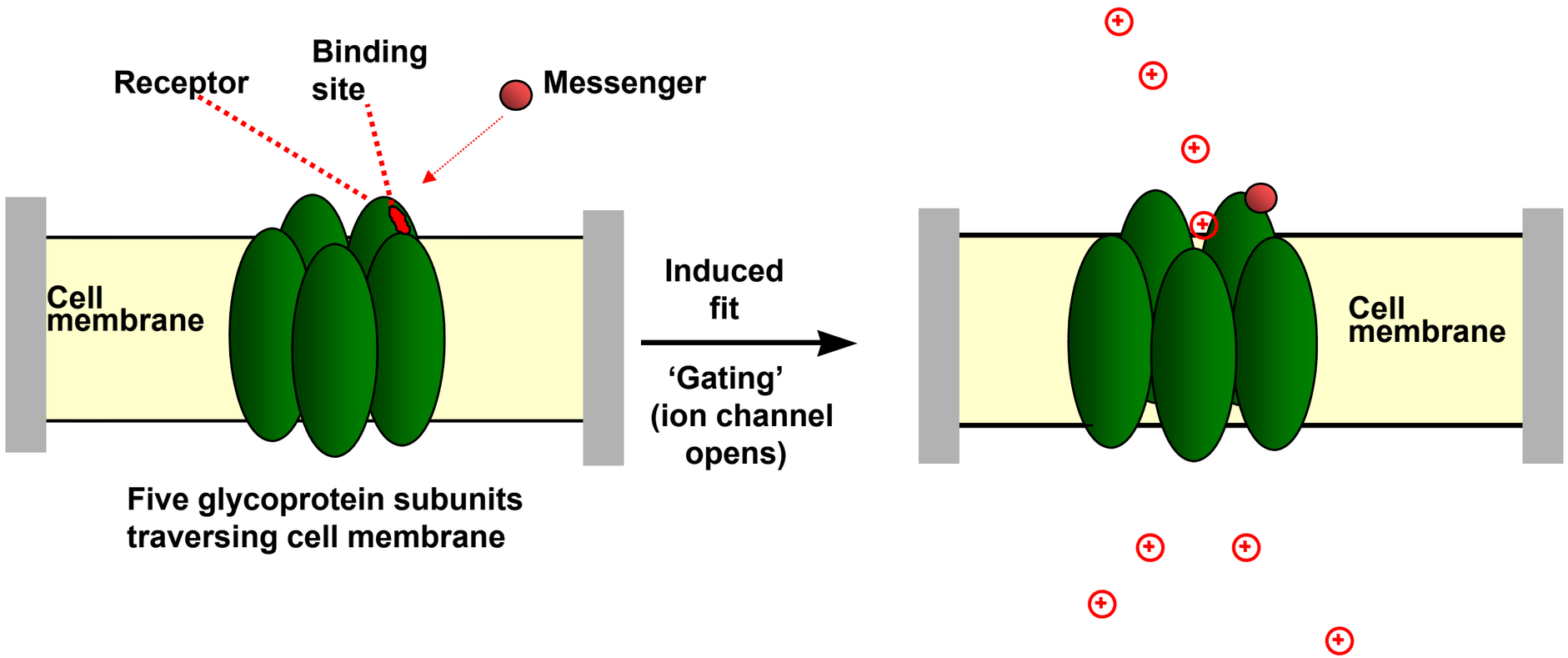
- The first indications that different types of cholinergic receptor existed came from the action of natural compounds.
- It was discovered that the compounds nicotine (present in tobacco) and muscarine (the active principle of a poisonous mushroom) were both cholinergic agonists, but that they had different physiological effects.
- Nicotine showed selectivity for cholinergic receptors present on skeletal muscle or at the synapses between different neurons
- Muscarine showed selectivity for cholinergic receptors present on smooth muscle and cardiac muscle.



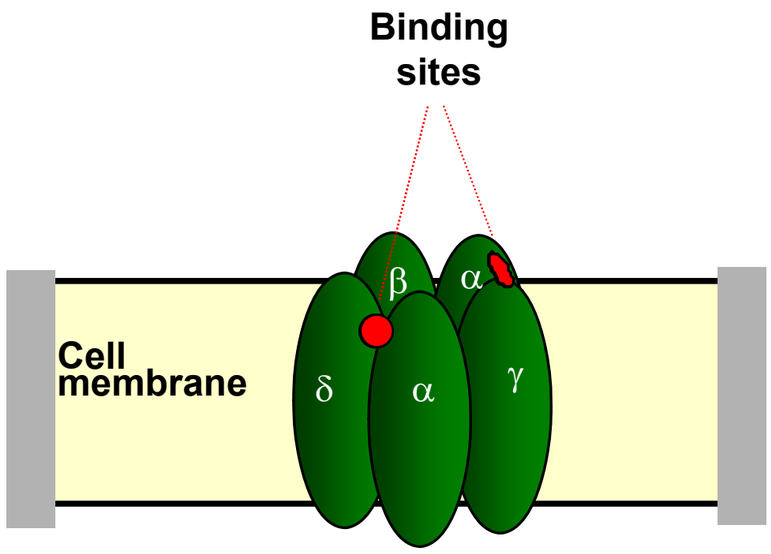
L-(+)-Muscarine

5.1 Nicotinic receptor

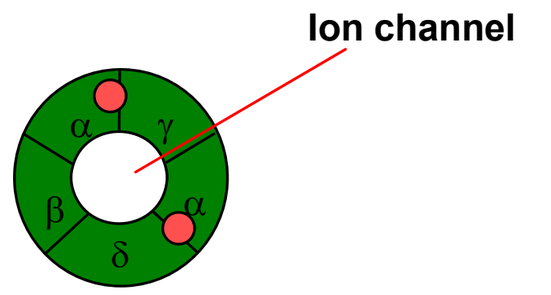
Control of cationic ion channel



The binding sites



$2\alpha, \beta, \gamma, \delta$ **subunits**



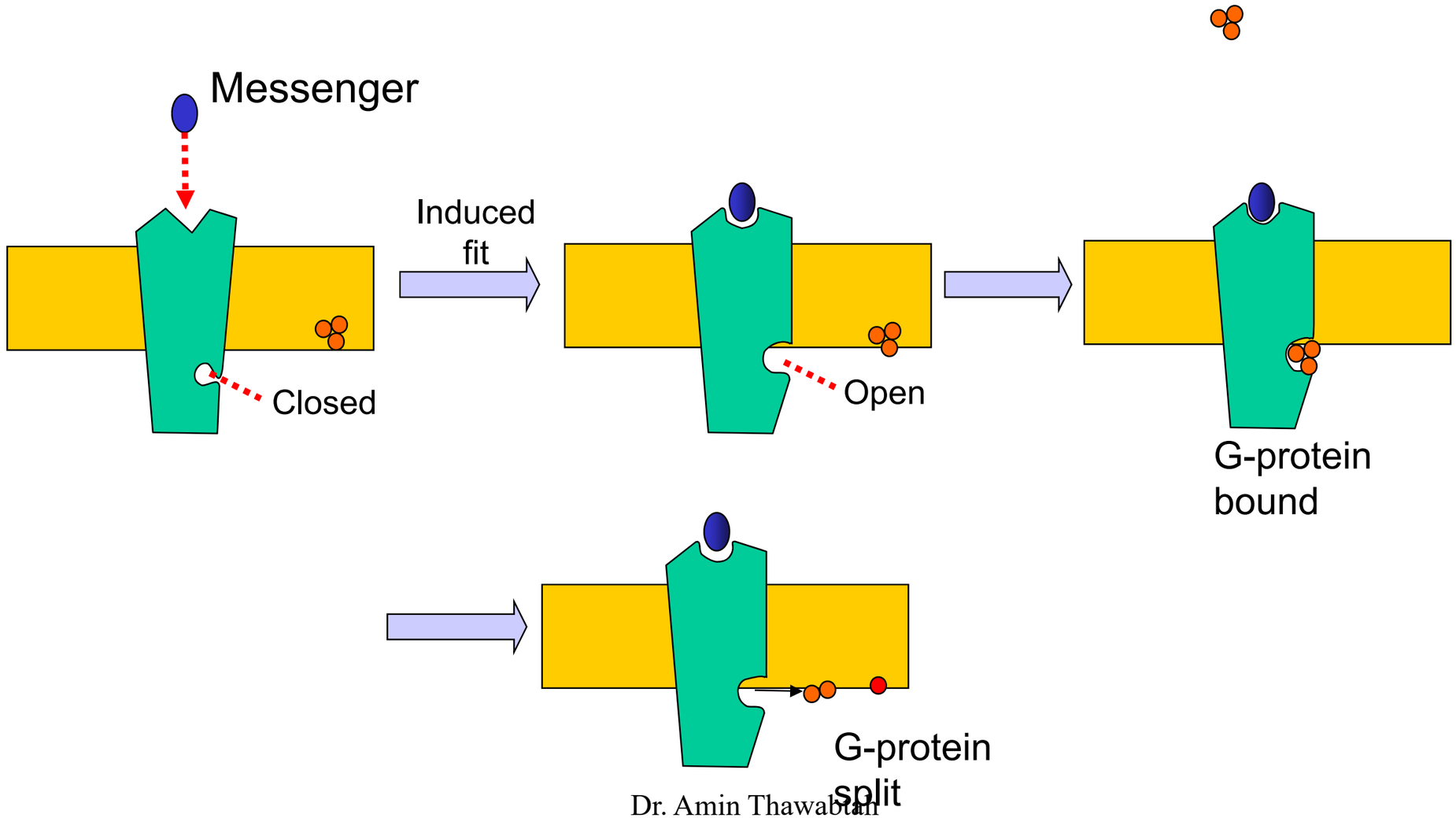
Two ligand binding sites mainly on α-subunits

5.2 Muscarinic receptor - G Protein coupled receptor

Activation of a signal protein

•Receptor binds messenger leading to an induced fit

•Opens a binding site for a signal protein (G-protein)

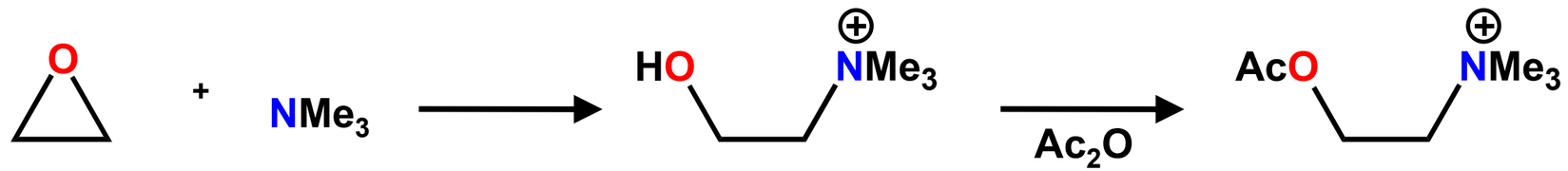


6. Cholinergic agonists

6.1 Acetylcholine as an agonist

Advantages

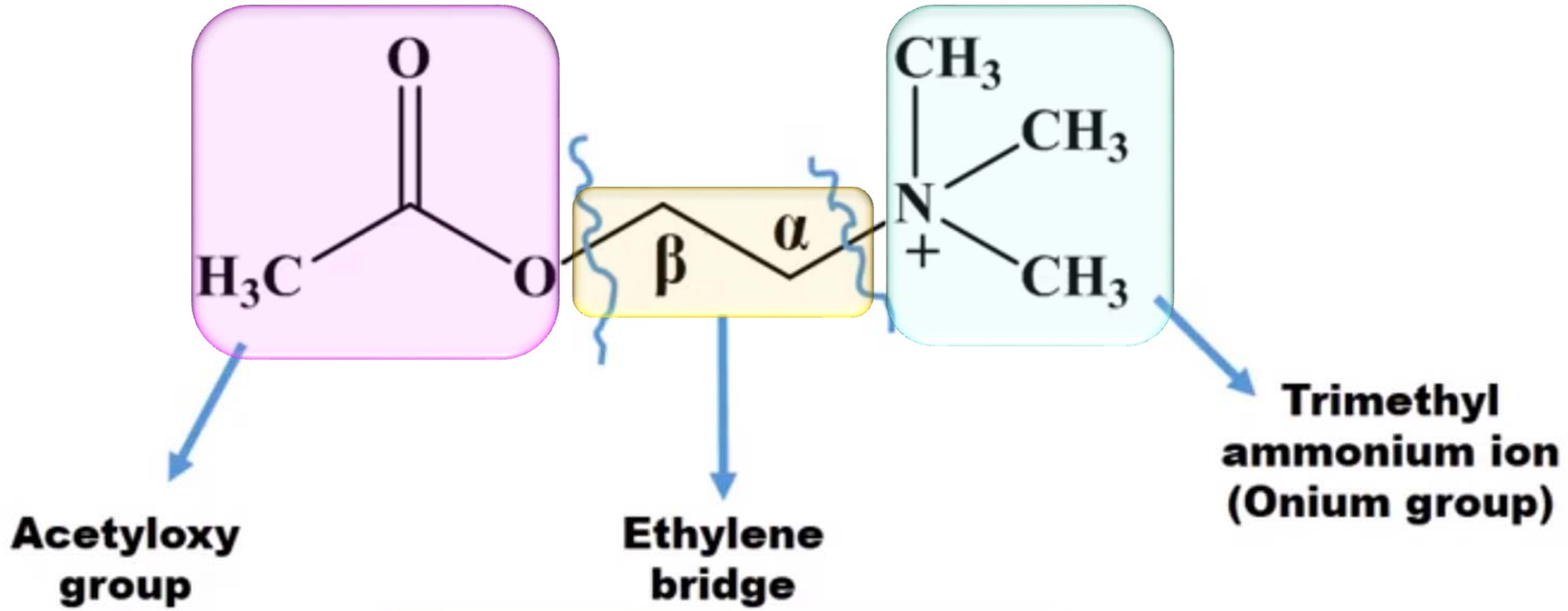
- Natural messenger
- Easily synthesised



Disadvantages

- Easily hydrolysed in stomach (acid-catalysed hydrolysis)
- Easily hydrolysed in blood (esterases)
- No selectivity between receptor types
- No selectivity between different target organs

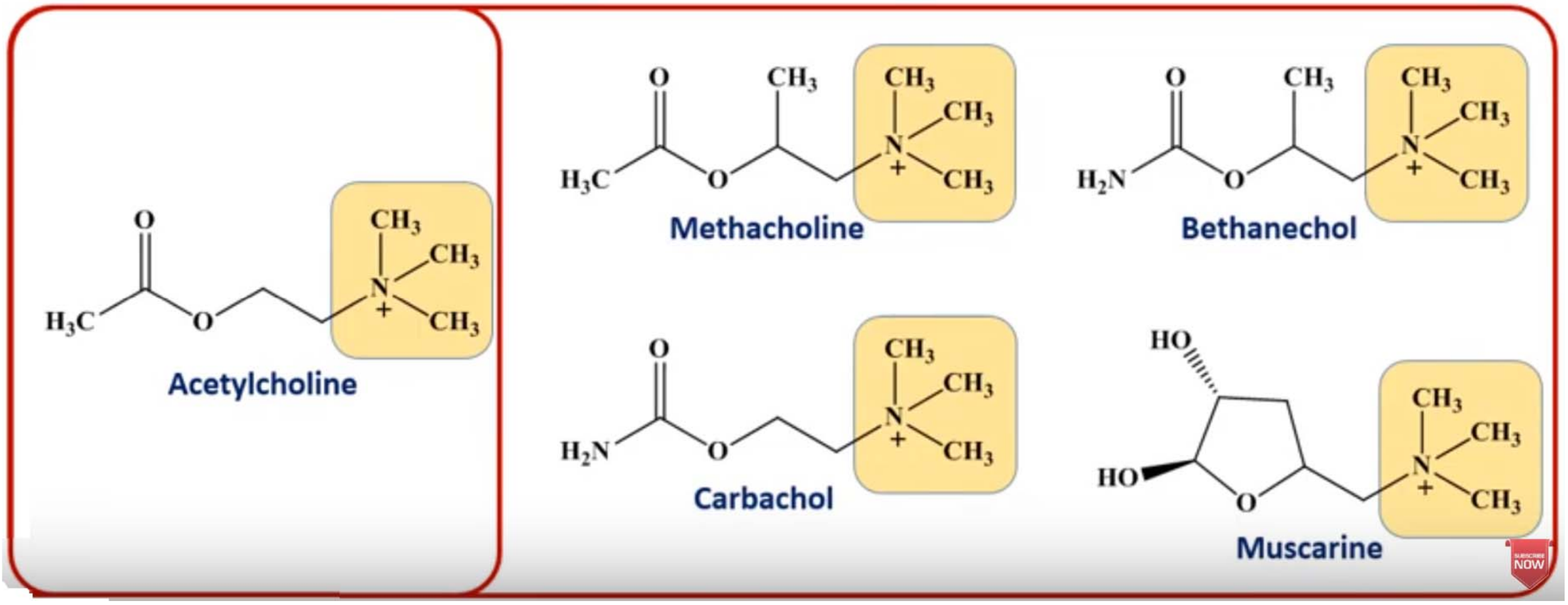
6. SAR for acetylcholine



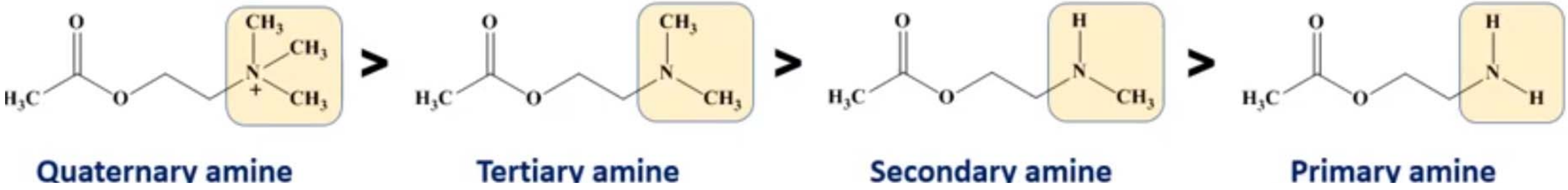
Trimethyl ammonium ion

1. For optimum activity amine group must be quaternary.

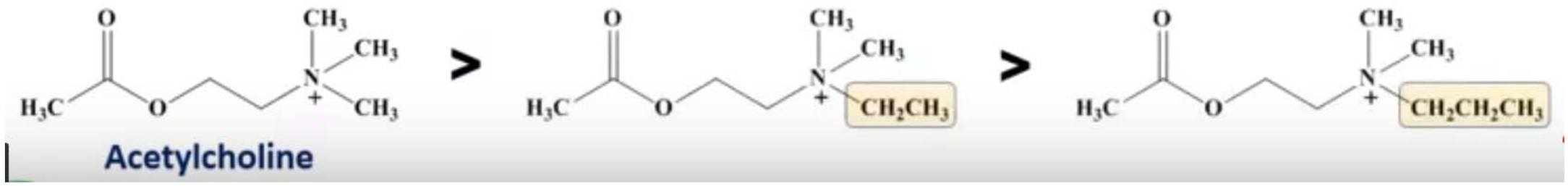
Eg. Methacoline, Bethanechol, Carbachol and Muscarine



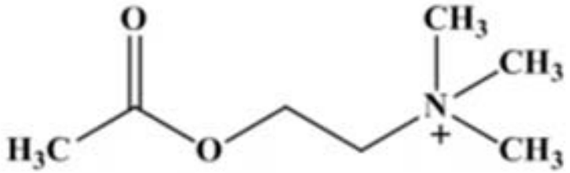
2. Primary, secondary and tertiary amines are less active than quaternary amines and there cholinergic activity is in the following order.



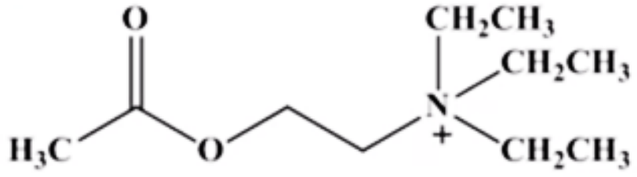
3. Substitution of one of the three methyl groups with higher alkyl group like ethyl or propyl make the molecules less active than Ach.



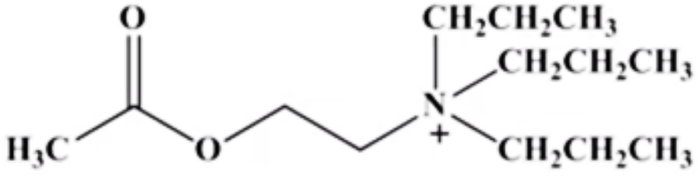
4. Substitution of all the three methyl groups with higher alkyl groups like ethyl or propyl make the molecules antagonist at cholinergic receptors.



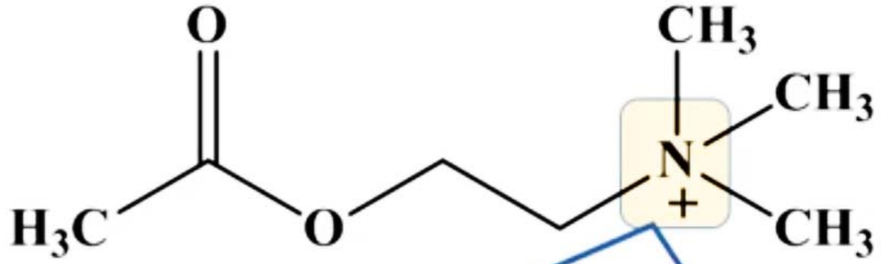
Acetylcholine
(Agonist at cholinergic receptors)



All methyls replaced by ethyls
'Antagonist at cholinergic receptors)



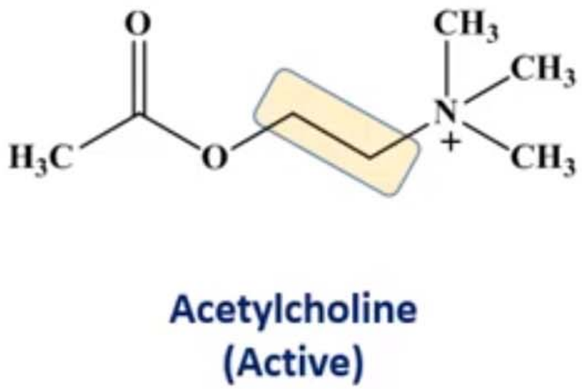
All methyls replaced by propyls
(Antagonist at cholinergic receptors)



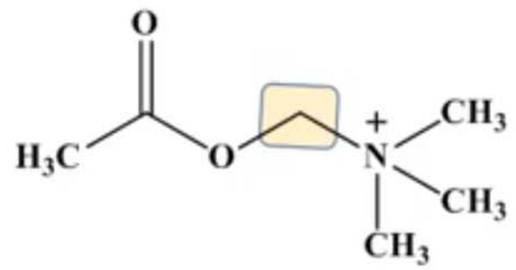
5. The substitution of nitrogen by phosphorous (P), Sulphur (S), arsenic (As) or antimony (Pb) atom decreases the cholinergic activity.

Ethylene bridge

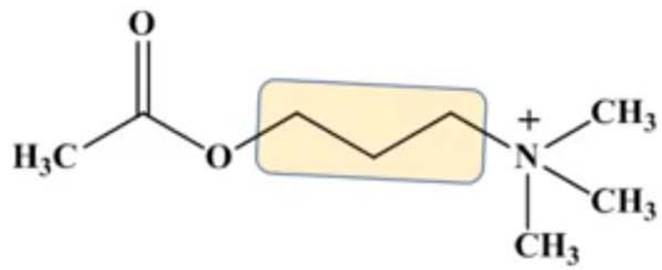
1. Chain length decreases or increases from ethylene to methylene or propylene respectively , decreases the cholinergic activity .



Acetylcholine
(Active)



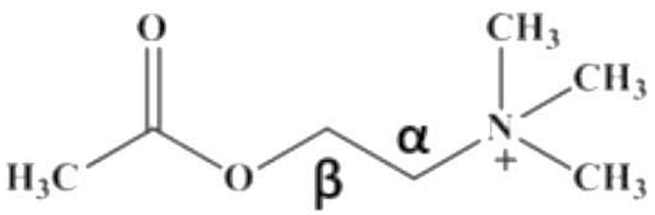
Methylene chain
(Less Active)



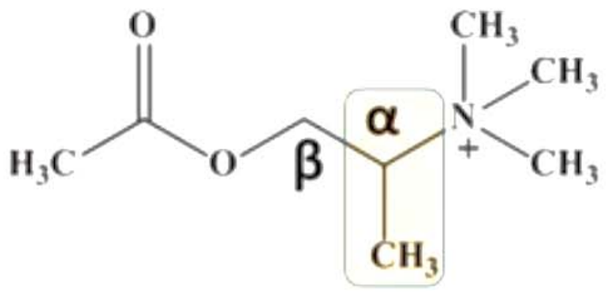
Propylene chain
(Less Active)

2 . Introduction of methyl group at alpha (α) position forms acetyl - α - methylcholine which has more selectivity towards nicotinic receptor than muscarinic (N > M)

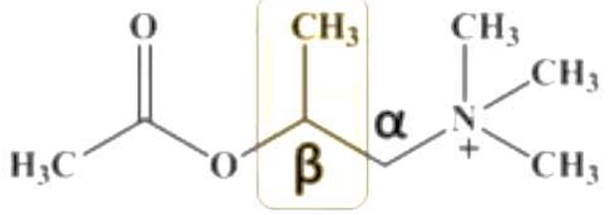
3 . Introduction of methyl group at beta (β) position forms acetyl - β - methylcholine (Methacholine) which has more selectivity towards Muscarinic receptor than Nicotinic (M > N) .



Acetylcholine
(Non selective cholinergic agonist)

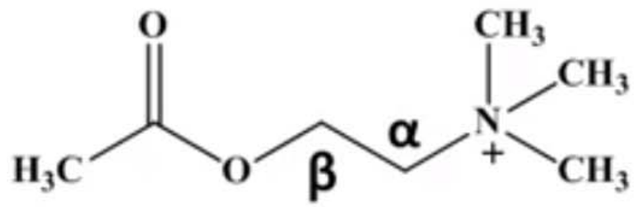


acetyl-α-methylcholine
(Nicotinic agonist)

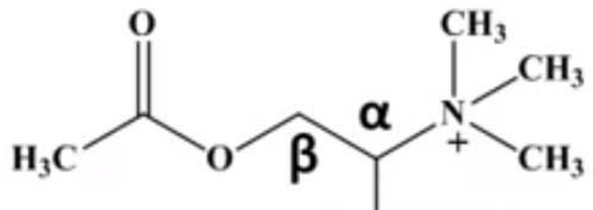


Methacholine
(Muscarinic agonist)

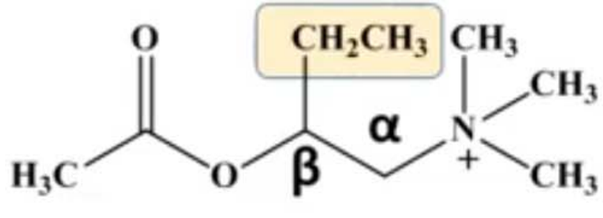
4. Introduction of alkyl groups other than methyl at alpha (α) or beta (β) position deceases the cholinergic activity .



Acetylcholine
(Active cholinergic agonist)



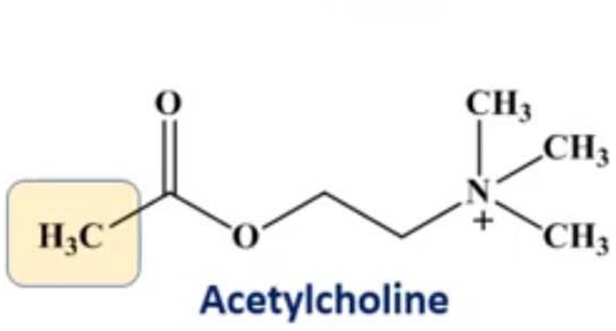
acetyl-α-ethylcholine
(Less active)



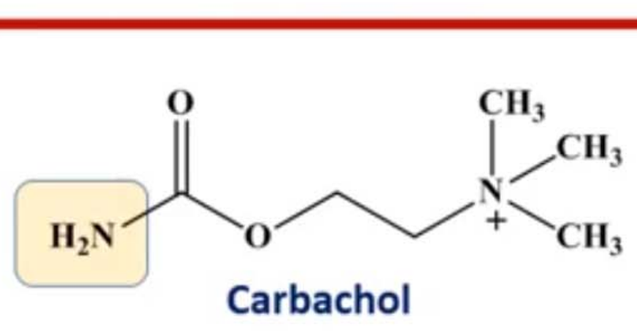
acetyl-β-ethylcholine
(Less active)

Acetyloxy group

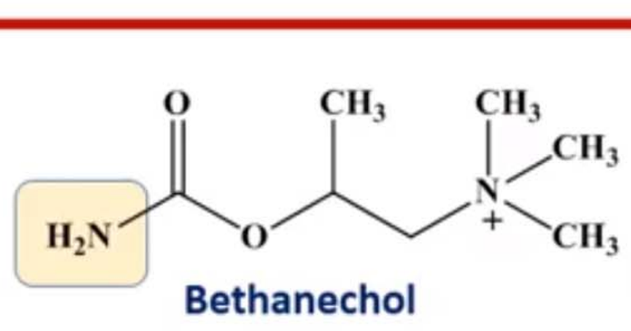
1. Conversion of acetyloxy into carbamic acid ester increases cholinergic activity . (Methyl group is replaced with amino group)



Acetylcholine



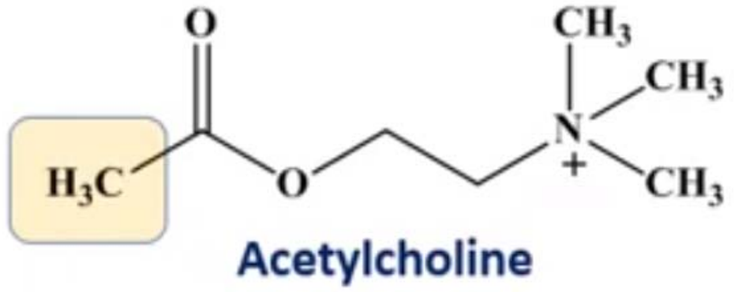
Carbachol



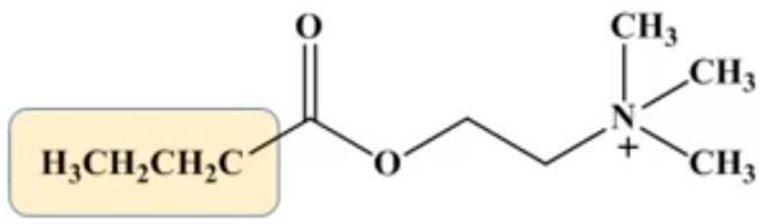
Bethanechol

More potent than acetylcholine

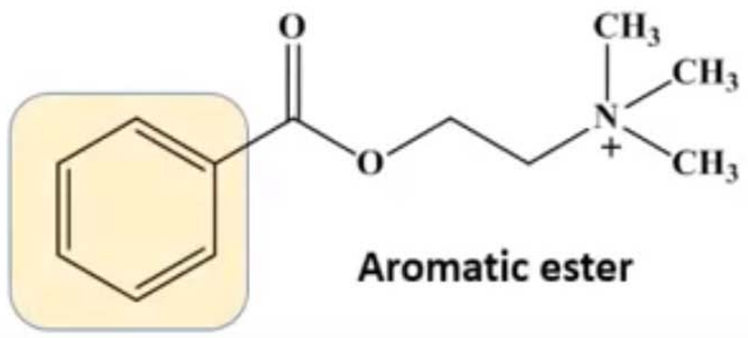
2 . Replacement of acyl group with aromatic or higher molecular weight esters makes the molecule antagonist at cholinergic receptors .



Acetylcholine



higher molecular weight esters

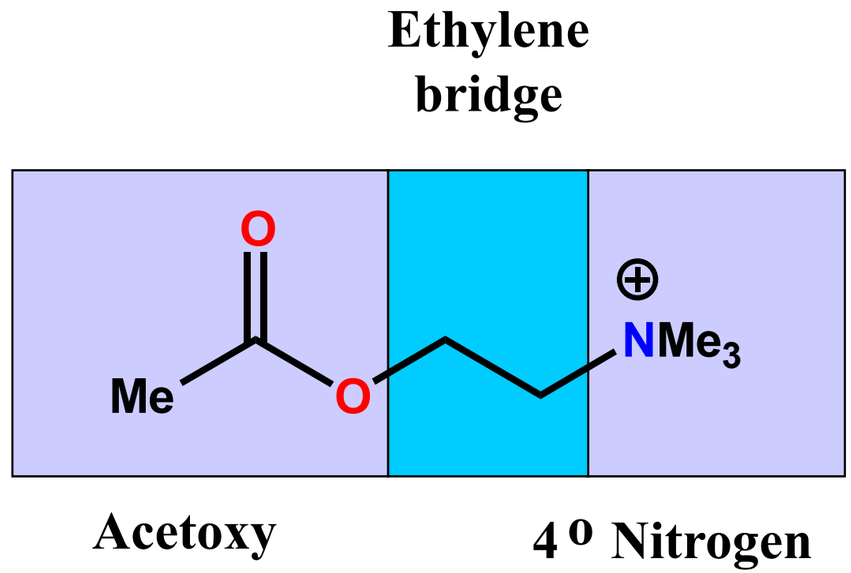


Aromatic ester

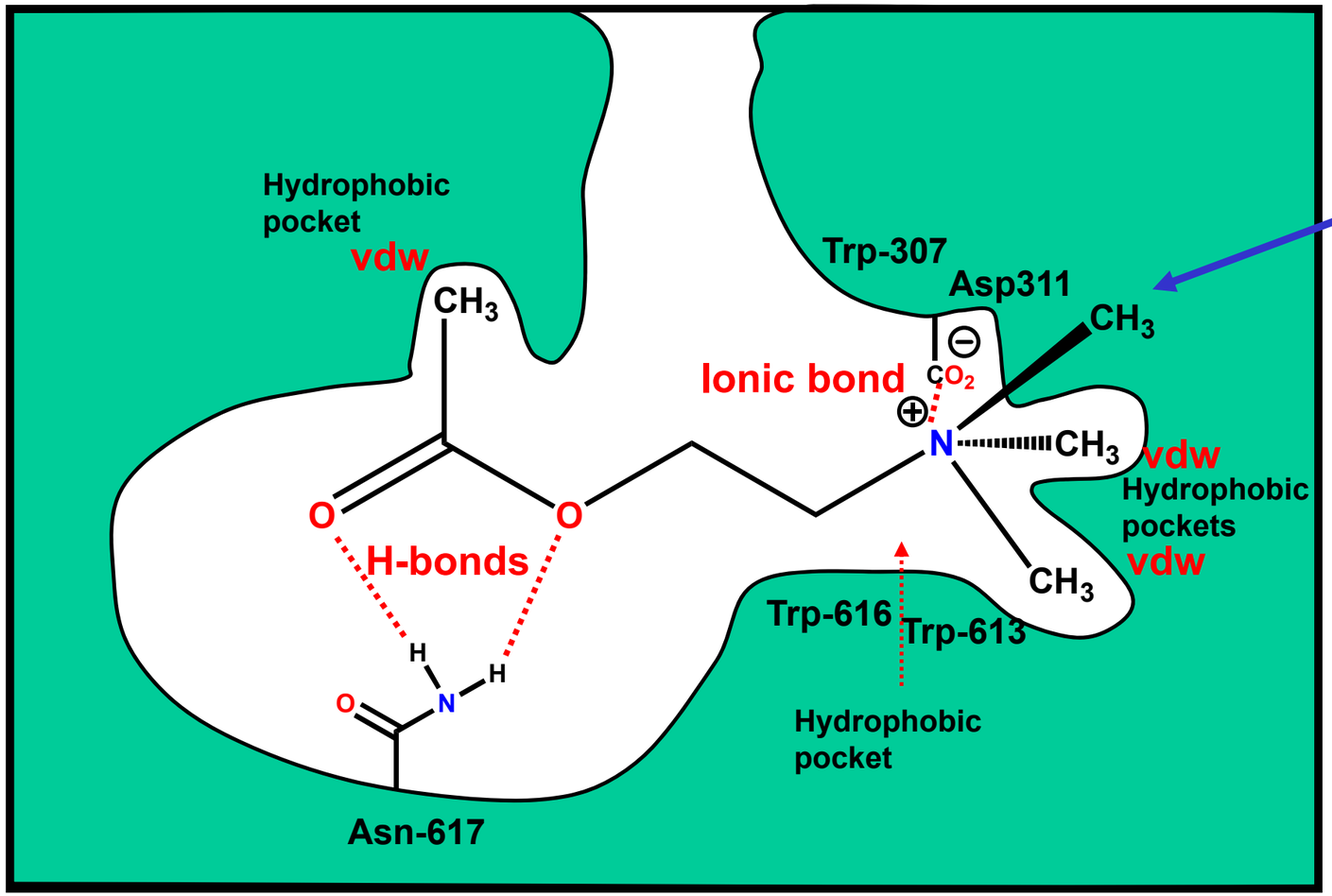
Molecules are antagonist at cholinergic receptors

Conclusions:

- Tight fit exists between Ach and the binding site
- Methyl groups fit into small hydrophobic pockets
- Ester interacts by hydrogen bonding
- Quaternary nitrogen interacts by ionic bonding



7. Binding site (muscarinic receptor)



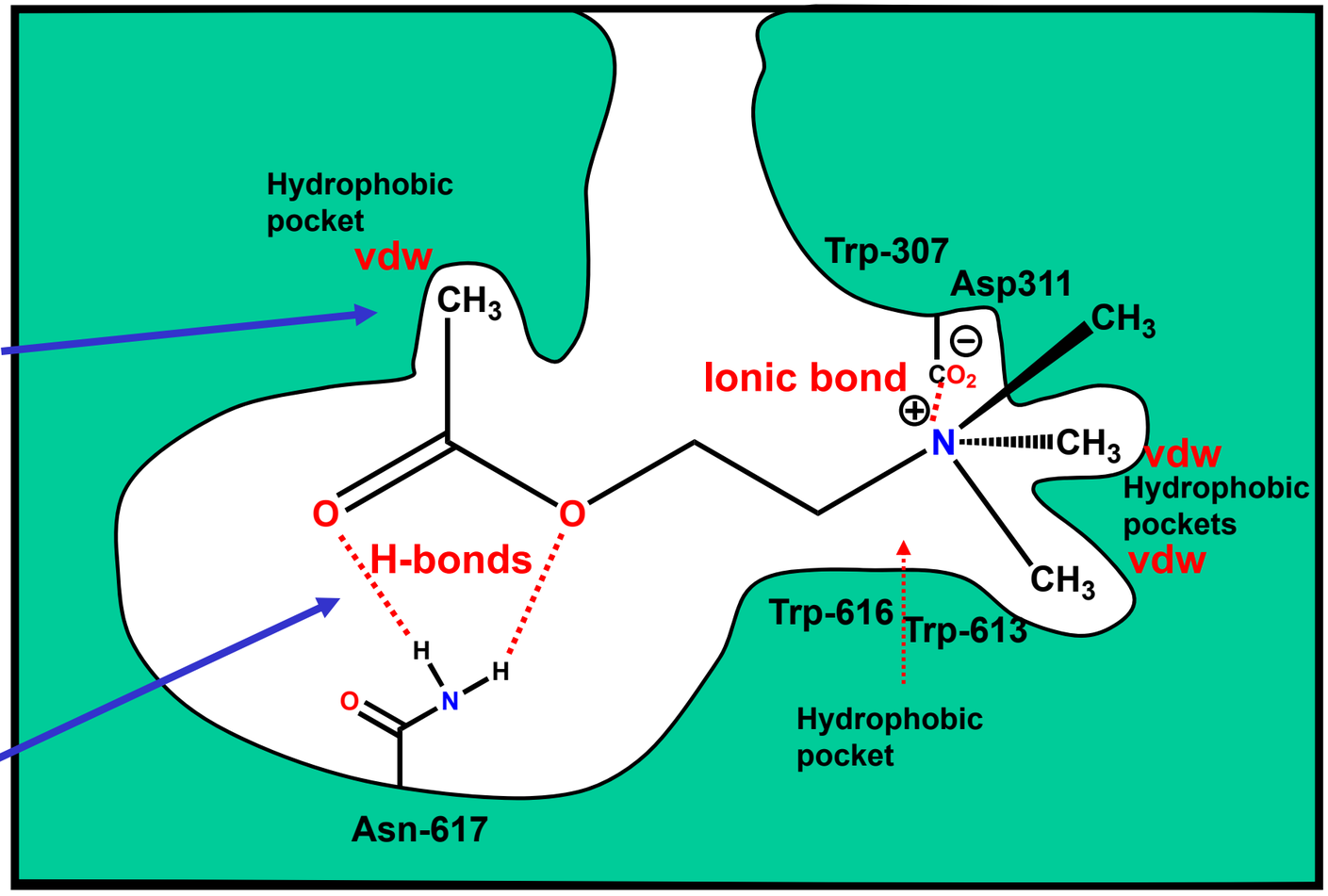
It is positioned in an open region, so it is possible to replace it with other groups

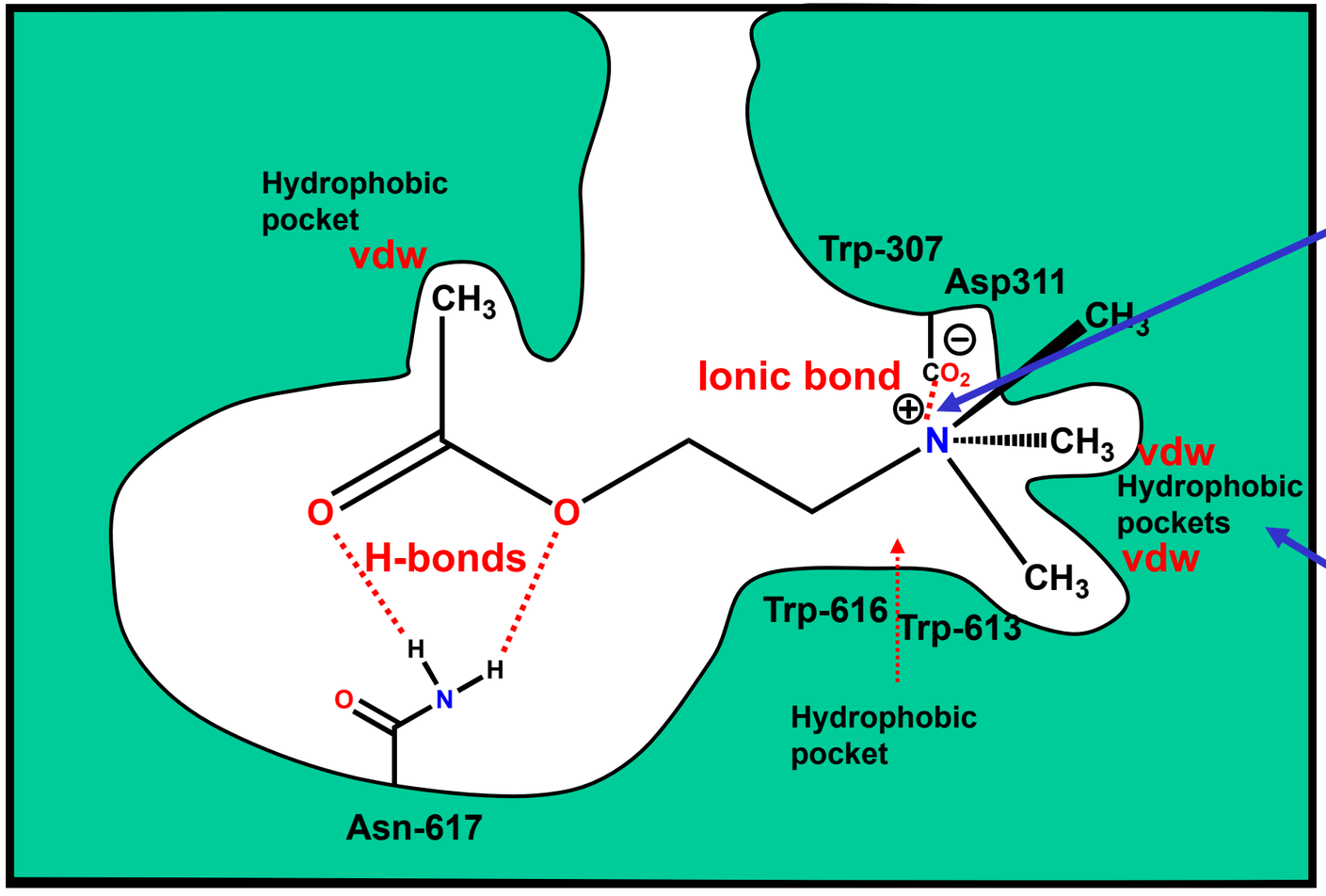
- There must be two methyl groups on the nitrogen. A larger, third alkyl group is tolerated, but more than one large alkyl group leads to loss activity.

•Bigger ester groups lead to a loss of activity.

this small hydrophobic pocket can accommodate the methyl gp of the ester, but nothing larger. This interaction is thought to be more important in the muscarinic receptor than the nicotinic receptor.

Hydrogen bonding interation exist between the ester group and an asparagine residue

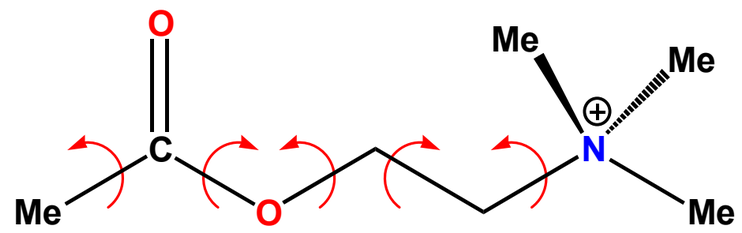




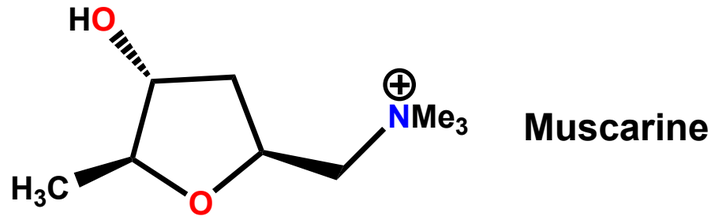
A strong ionic interaction has been proposed between the charged nitrogen atom and the anionic side group of asparate residue.

This hydrophobic pocket is lined with three aromatic amino acids.

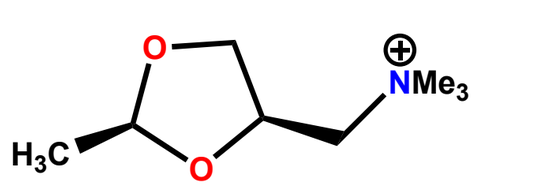
A large amount of effort has been expended trying to identify the active **conformation** of acetylcholine, because acetylcholine is a **highly flexible molecule where bond rotation along** the length of its chain can lead to **many possible stable conformations** (or shapes).

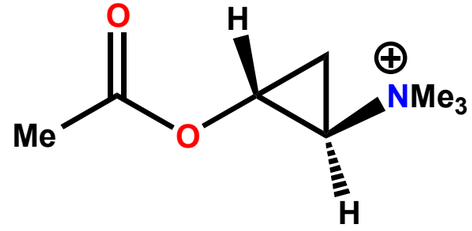


Muscarine has a **ring** that **locked** the **changing and rotation freely** and as this compound can bind to cholinergic receptor indicates that this particular conformation is "allowed" for activity.

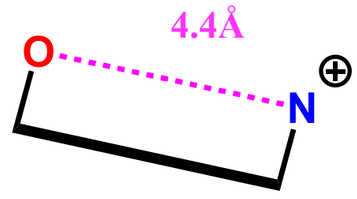


Muscarine

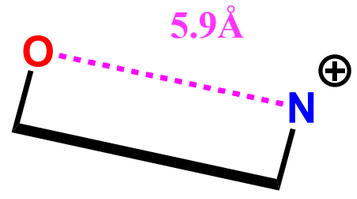




Studies have shown that the **separation** between the **ester** group and the **quaternary nitrogen** is important for binding, and that this **distance differs for the muscarinic and the nicotinic receptor**



Muscarinic receptor

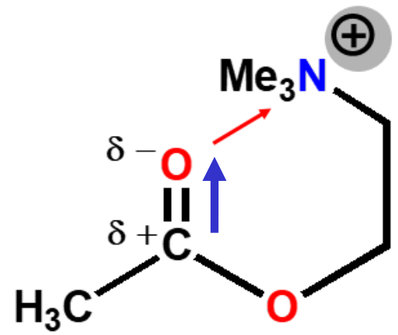


Nicotinic receptor

8. Instability of acetylcholine

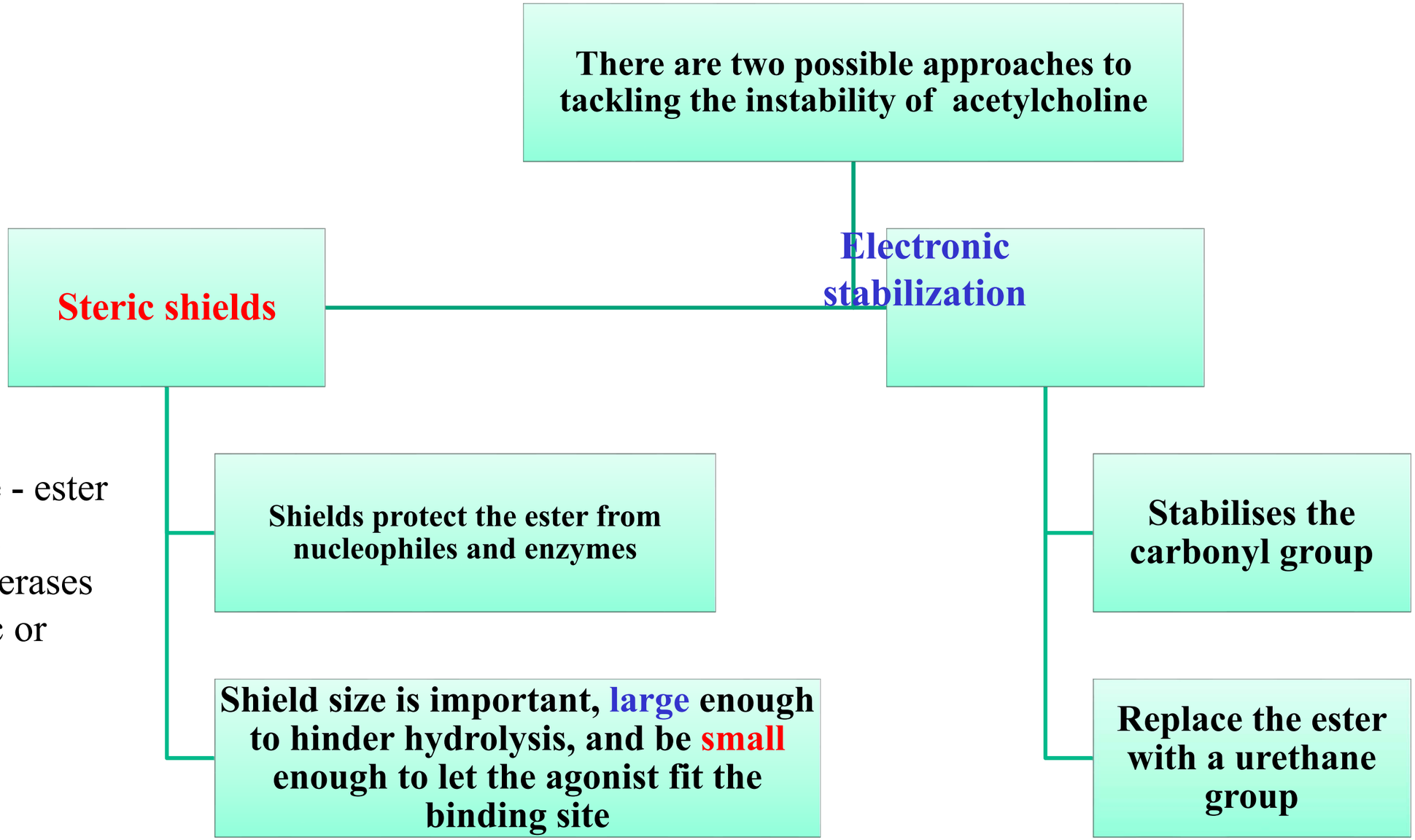
- In the conformation of the acetylcholine, the **+ve charged N** interacts with the **carbonyl oxygen** and has an electron withdrawing effect.

- the oxygen atom pulls electrons from the neighbouring carbon atom and makes that carbon atom electron deficient and more prone to **nucleophilic** attack "Increases sensitivity to nucleophiles".



- Water is a poor **nucleophile** but because the cabronyl group is more electrophilic "Increases electrophilicity of carbonyl group" , hydrolysis takes place relatively easily. This influence of the nitrogen ion is known as **neighbouring group participation** or **anchimeric assistance**.

9. Design of cholinergic agonists

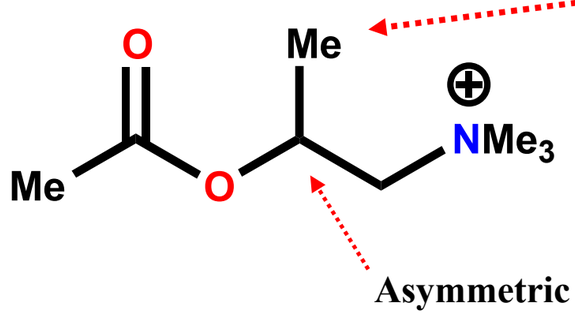


Requirements

- Correct size
- Correct pharmacophore - ester and quaternary nitrogen
- Stability to acid and esterases
- Selectivity for nicotinic or muscarinic receptors

9.1 Use of steric shields

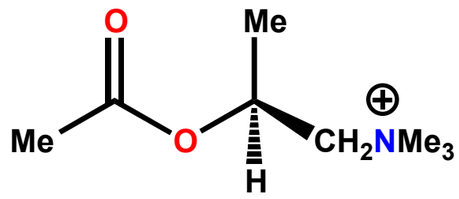
Methacholine



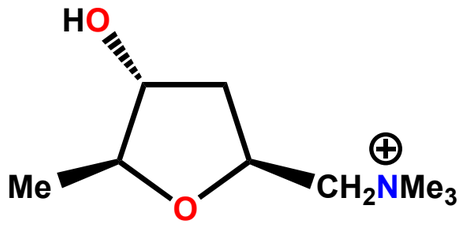
Hinders binding to esterases and provides a shield to nucleophilic attack

Properties

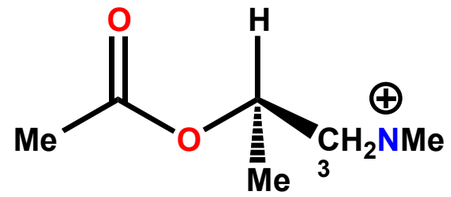
- Three times more stable than acetylcholine
- Increasing the shield size increases stability but decreases activity
- Selective for muscarinic receptors over nicotinic receptors
- *S*-enantiomer is more active than the *R*-enantiomer



(S)



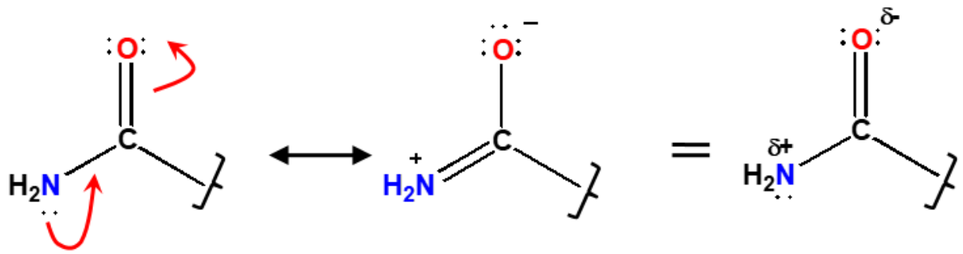
Muscarine



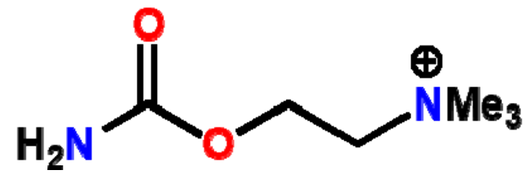
(R)

9.2 Use of electronic factors

- The use of electronic factors to stabilize functional groups has been seen in the design of **carbachol**. Replacing the methyl group with amino group NH_2.



- This functional group is more resistant to hydrolysis since the lone pair of electron on nitrogen can interact with the neighboring carbonyl group and **lower** its **electrophilic** character.



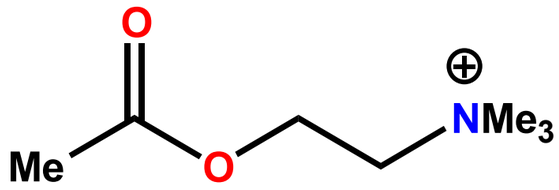
Carbachol

- Unfortunately, carbachol shows very little **selectivity** between muscarinic and nicotinic receptors.
- But it was used clinically for the treatment of **glaucoma** where it can be applied locally, thus avoiding the problem of receptor selectivity.

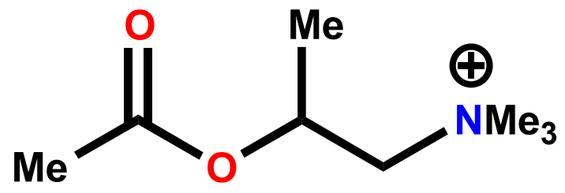
➢ Glaucoma arises when the aqueous contents of the eye cannot be drained. This raises the pressure on the eye and can lead to blindness. Agonists cause eye muscles to contract thus relieving the blockage and allowing drainage.

9.3 Steric + electronic factors

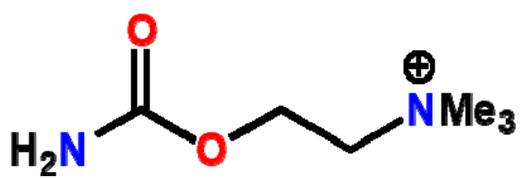
The **β-methyl group** of **methacholine** <u>increases stability and introduces receptor selectivity.</u> Therefore, it made sense to add a β-methyl group to carbachol. The resulting compound is bethanechol which is both stable to hydrolysis and selective in its action. It is occasionally used therapeutically in stimulating the GI tract and urinary bladder after surgery.



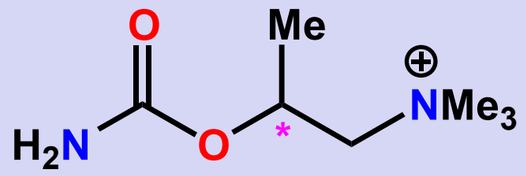
Ach



Methacholine



Carbachol



Bethanechol

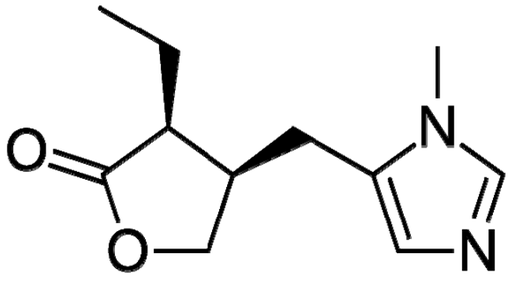
10. Clinical uses for cholinergic agonists

❑ Muscarinic agonists

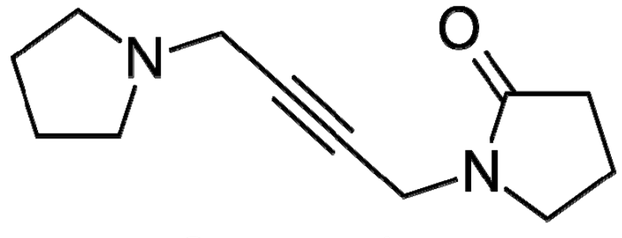
- treatment of glaucoma
- switching on the GIT and urinary tract after surgery
- treatment of certain heart defects by decreasing heart muscle activity and heart rate.

➢ **Pilocarpine** "it is an alkaloid" is an example of a muscarinic agonist which is used in the treatment of glaucoma. The separation between nitrogen and oxygen is 4.4 A.

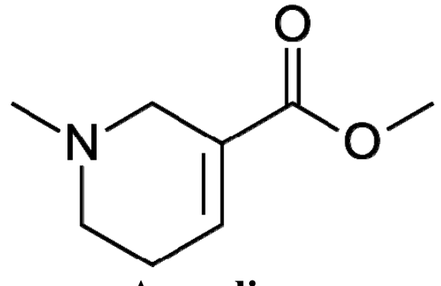
➢ Pilocarpine is also being considered for the treatment of Alzheimer's disease, as are other muscarinic agonists such as oxotremorine and various arecoline analogues



Pilocarpine



Oxotremorine



Arecoline

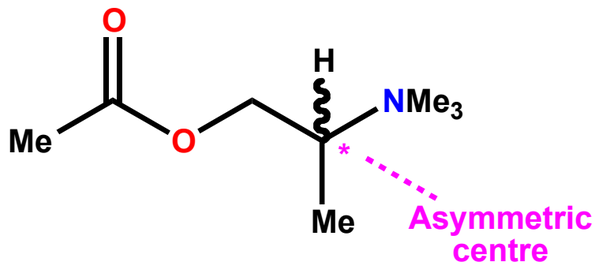
❑ Nicotinic agonists

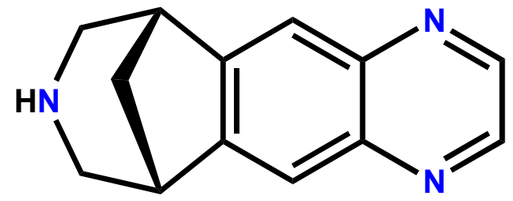
➢they are used in the treatment of myasthenia gravis. This is an autoimmune disease where the body has produced antibodies against its own cholinergic receptors.

➢So the number available receptors drops and so fewer messages reach the muscle cells. This leads to severe muscle weakness and fatigue.

➢Administering an agonist **increases the chance of activating what few receptor remains**. The following agent is selective **nicotinic agonist** has similar structure of methacoline but differs in methyl position.

➢Despite that, this particular compound is not used clinically and anticholinesterases are the preferred treatment. Varenicline is used clinically, however. It is a partial agonist at nicotinic receptors and was approved in 2006 as an aid to stop smoking.



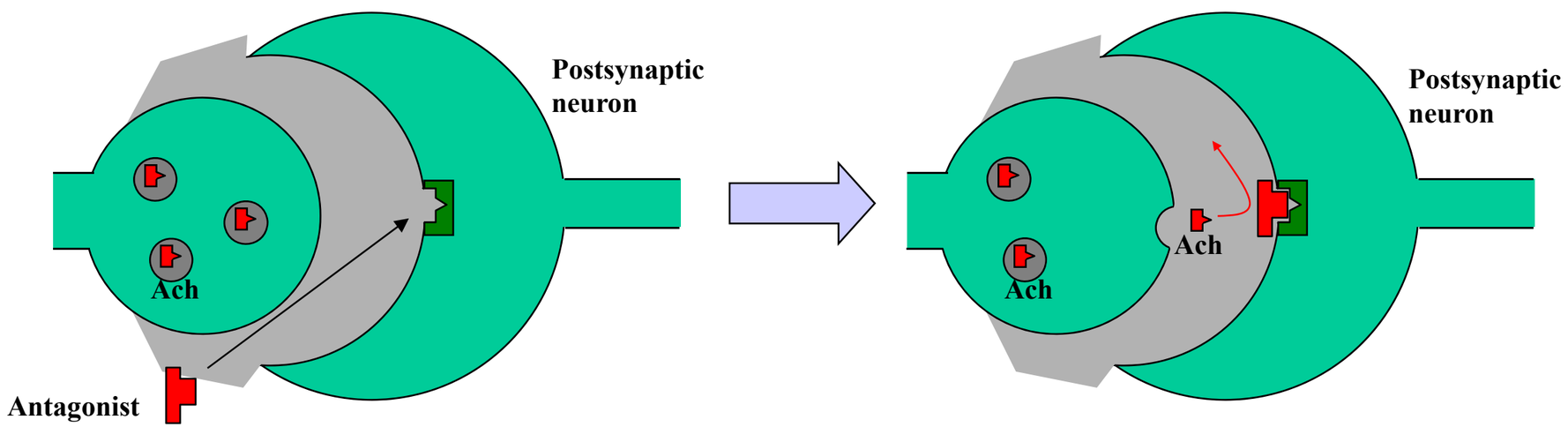


Varenicline

11. Antagonists of the muscarinic cholinergic receptor

Actions and uses of muscarinic antagonists

- Drugs which bind to cholinergic receptor but do not activate it were **Prevent acetylcholine from binding** cause **lowering in the activity** of acetylcholine and opposite clinical effect to agonists



Physiological effects

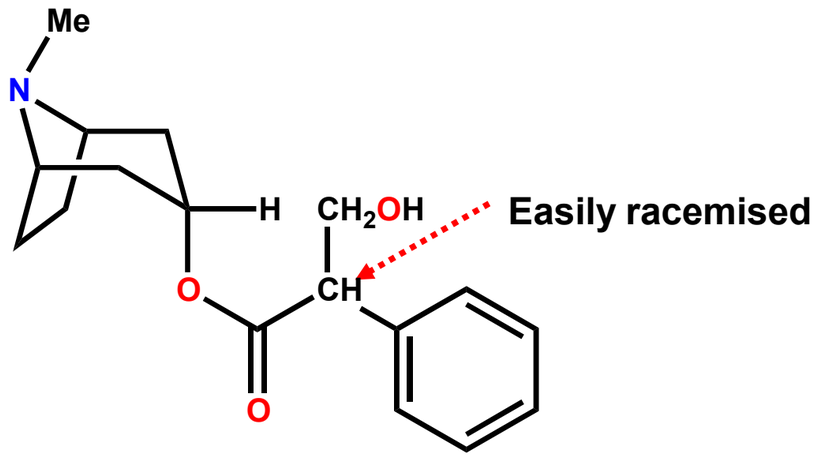
- Decrease of saliva and gastric secretions
- Relaxation of smooth muscle
- Decrease in motility of GIT and urinary tract
- Dilatation of eye pupils

Clinical uses

- Shutting down GIT and urinary tract for surgery
- Treatment of incontinence
- Ophthalmic examinations
- Relief of peptic ulcers
- Treatment of Parkinson's Disease
- Treatment of anticholinesterase poisoning
- Treatment of motion sickness
- Treatment of chronic obstructive pulmonary disease

Muscarinic antagonists

11.1 Atropine

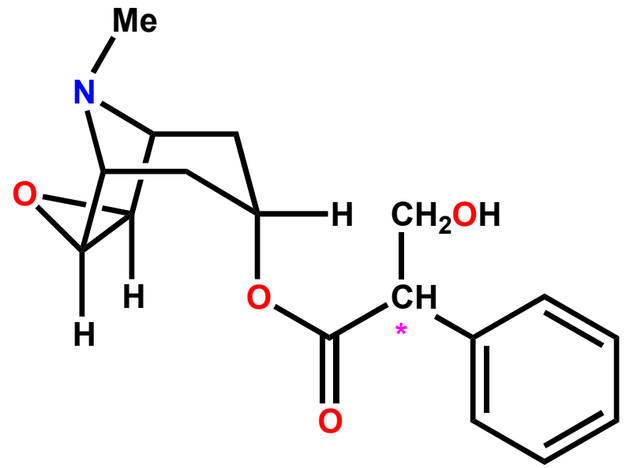


- Racemic form of hyoscyamine
- Source - roots of *Atropa belladona* (1831)
- Used as a poison
- Used as a medicine

- decreases GIT motility
- antidote for anticholinesterase poisoning
- dilation of eye pupils

- CNS side effects - hallucinations

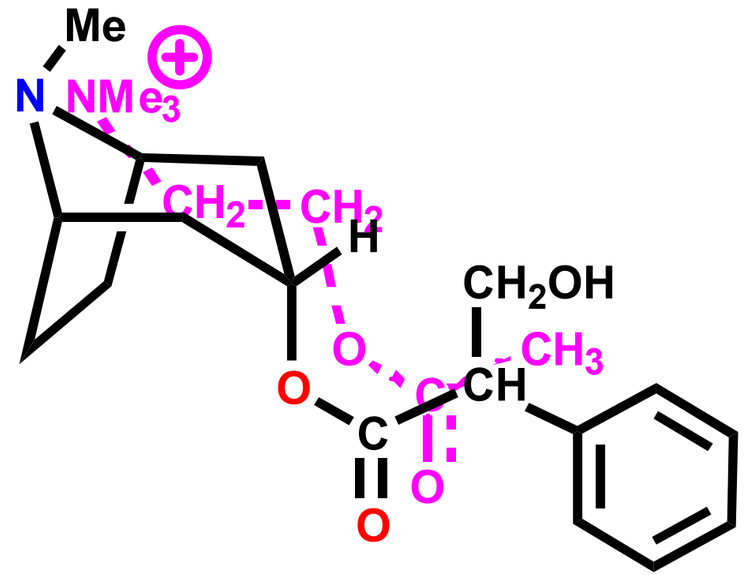
11.2 Hyoscine (scopolamine)



•Source - thorn apple
•Medical use - treatment of motion sickness
•Used as a truth drug (CNS effects)

11.3 Comparison of atropine with acetylcholine

- If we look closely structurally to the two compounds we can see a basic **nitrogen and an ester group are present** and if we superimpose the acetylcholine skeleton on to the atropine skeleton, **the distance between the ester and the nitrogen groups are similar in both molecules**.

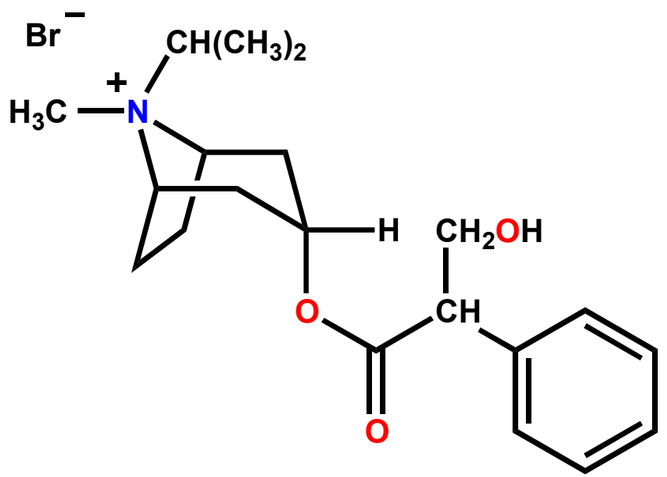


<u>Why atropine unable to switch it on the receptor??</u>

- because atropine is a larger molecule than acetylcholine, it is capable of binding to other binding regions within the binding site which are not used by acetylcholine itself.
- So it interacts differently with the receptor and doesn't induce the same conformational changes as acetylcholine.
- Since is it a tertiary amine rather than quaternary salts they are able to cross **the BBB** as the free base. Once they are in the brain they can become protonated and antagonize muscarinic receptors in the CNS, this leads to CNS effects such as hallucinogenic activity.

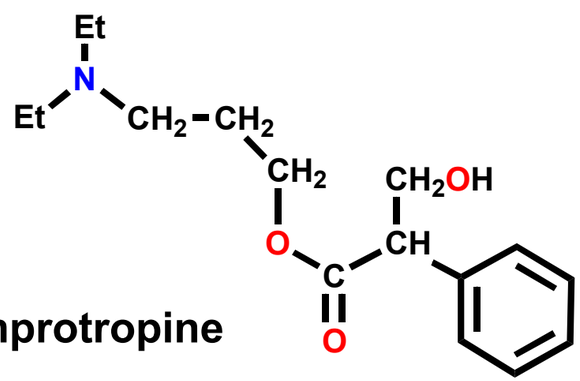
11.4 Analogues of atropine

- In order to reduce CNS side effects, **quaternary salts** of atropine and atropine analogues are used clinically. E.g. ipratropium is used as a bronchodilator, Atropine methonitrate acts at the intestine to relieve spasm.

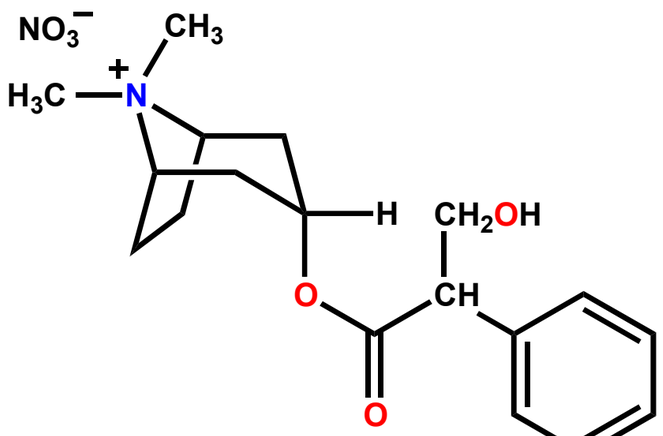


Ipratropium
(bronchodilator & anti-asthmatic)

- it was further discovered that the **complex ring** system was not necessary for antagonist activity so simplification could be carried out. Example **amprotropine is active and has ester separated from amine by three carbon atoms**.

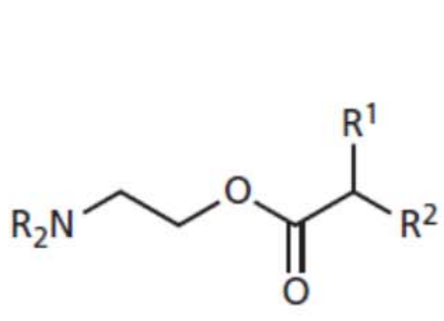


Amprotropine

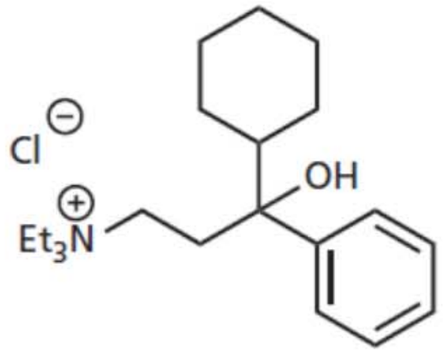


Atropine methonitrate
(lowers GIT motility)

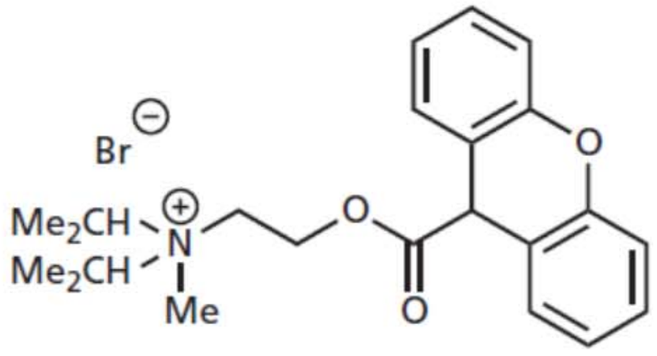
- A large variety of active antagonists like **propantheline bromide** have been prepared and having the general formula:



R^1 and R^2 = Aromatic or heteroaromatic



Tridihexethyl chloride



Propanthreline bromide

❑ these studies came up with the following generalizations:

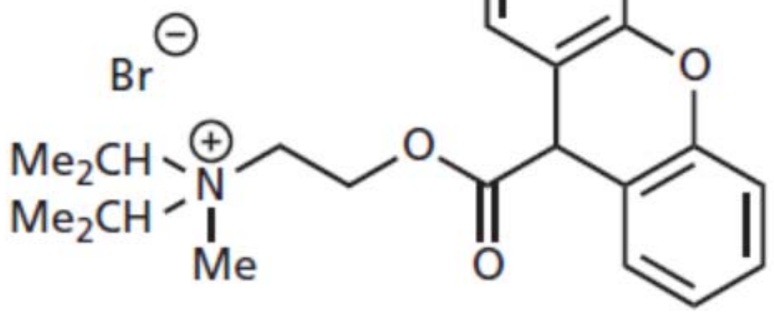
✓ The **alkyl groups** on nitrogen can be larger than methyl "in contrast to agonists"
✓ The nitrogen can **be tertiary or quaternary**, whereas agonists must have a quaternary nitrogen.
✓ Very large **acyl groups are allowed**. This in contrast to agonists were only the acetyl group is permitted.

- the **large acyl groups is the most crucial in determining whether a compound will act as an antagonist or not**. It should be bulky but arranged in a certain manner with some sort of branching.

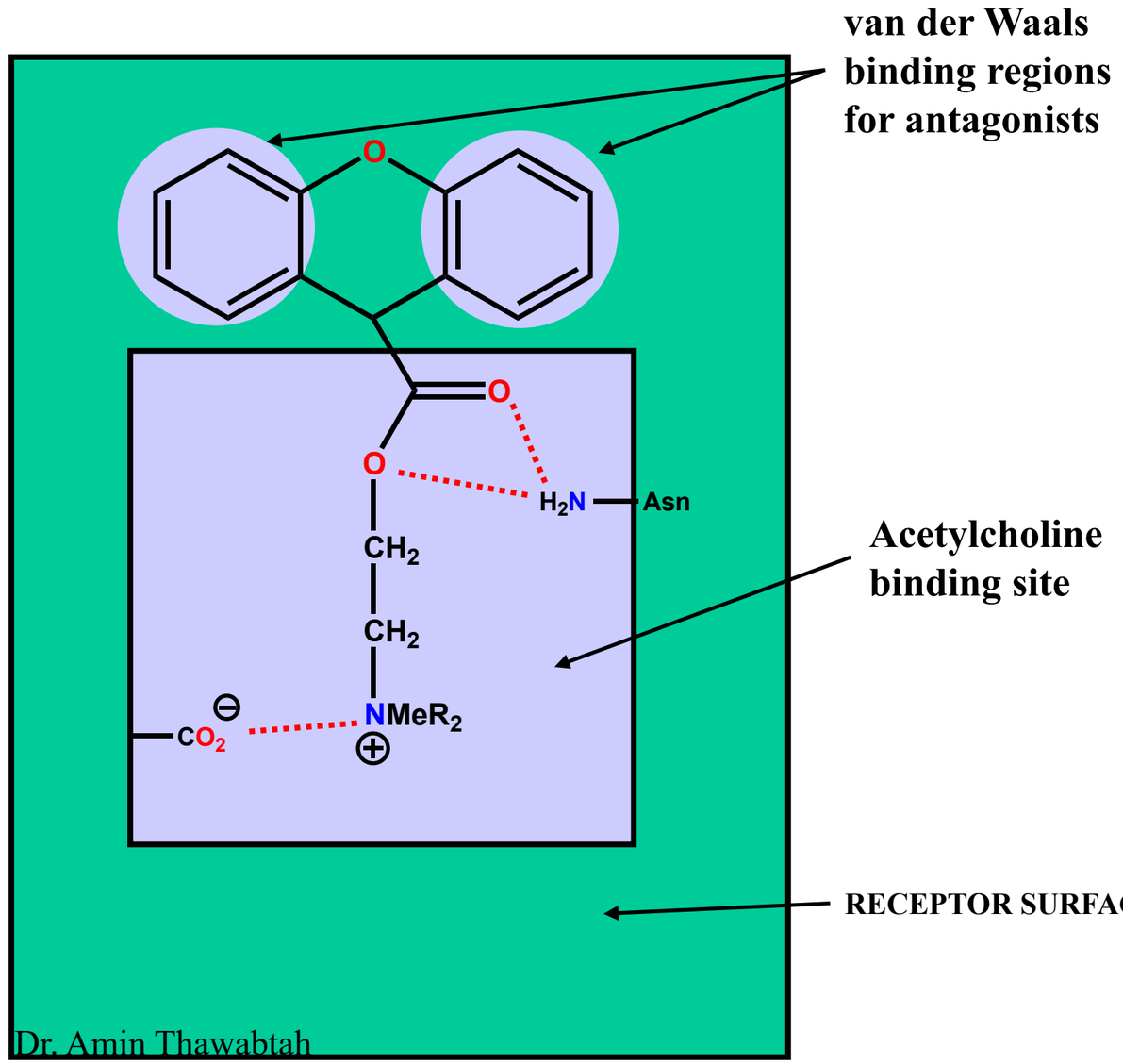
- **The conclusion that there must be hydrophobic binding region next to the normal Ach binding site**. The overall shape of the Ach binding site plus the extra binding regions would have to be T-shaped or Y- shaped.

- example of that is the structure of **propantheline** which contains the complete acetylcholine skeleton as well as the hydrophobic acyl side chain.

11.6 Binding site for antagonists



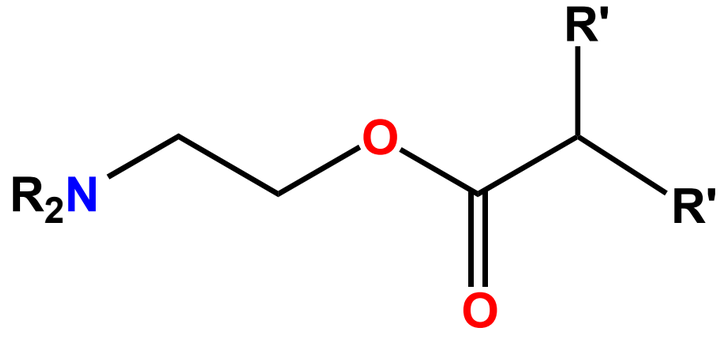
Propanthreline bromide



11.5 SAR for antagonists

Important features

- Tertiary amine (ionised) or a quaternary nitrogen
- Aromatic ring
- Ester
- *N*-Alkyl groups (R) can be larger than methyl (unlike agonists)
- Large branched acyl group
- R’ = aromatic or heteroaromatic ring
- Branching of aromatic/heteroaromatic rings is important



R' = Aromatic or heteroaromatic

SAR for antagonists vs. agonists

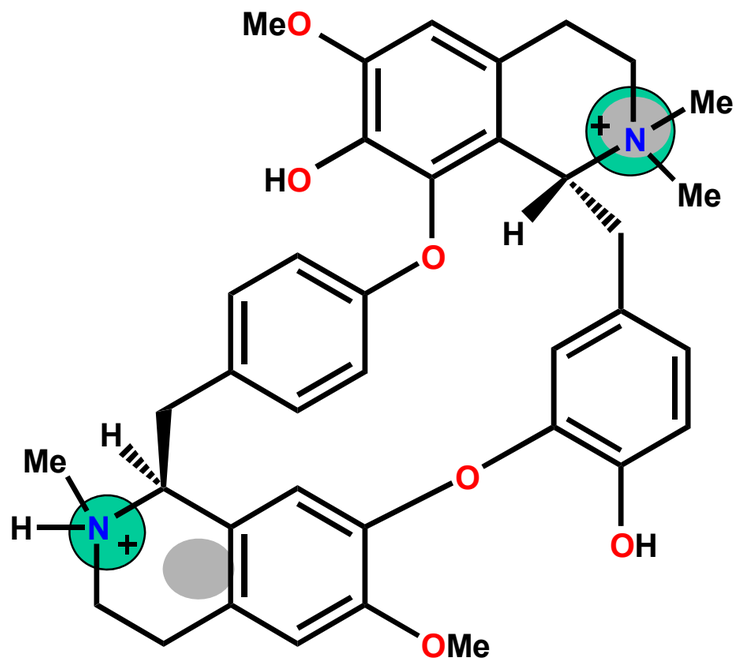
SAR for antagonists	SAR for agonists
•Tertiary amine (ionised) or quaternary nitrogen •Aromatic ring •Ester •*N*-Alkyl groups (R) can be larger than methyl •R' = aromatic or heteroaromatic •Branching of Ar rings important	•Quaternary nitrogen •Aromatic ring •Ester •*N*-Alkyl groups = methyl •R' = H

12. Cholinergic Antagonists (nicotinic receptor)

12.1 Curare and tubocurarine

- Curare is a mixture of compounds that the active principle is an antagonist of acetylcholine "**which stop the heart and cause paralysis".**

- The main application in medicine is in **the relaxation of abdominal muscles in preparation for surgery with the right dose.**

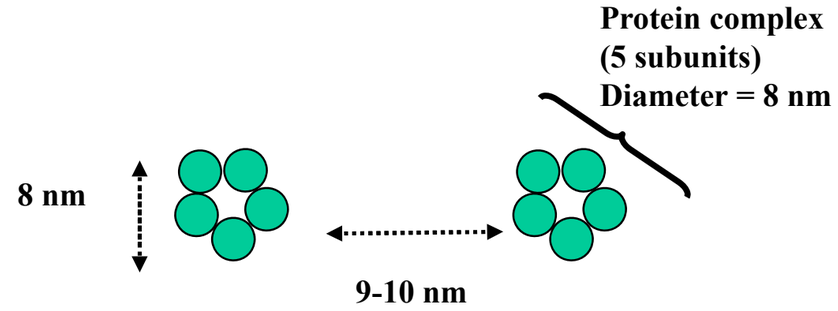


Tubocurarine

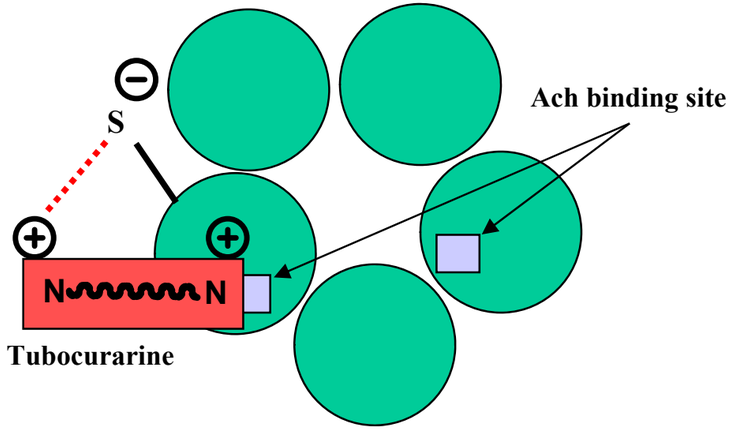
The structure of tubocurarine presents a **problem to our theory**. It has a couple of charged nitrogen centers and there is no ester to interact with the acetyl binding region. So how it bind to the receptor??

- The molecule has **two positively charged nitrogen** atom and the distance between the two centers "11.5 A" might be equivalent to the distance between two separate cholinergic receptors and the tubocurarine could act as a bridge between the two receptor sites.

But the dimensions of the nicotinic receptor make this impossible as the receptor is a protein dimer of two identical protein complexes separated by 9-10 nm far too large to be bridged by tubocurarine.



a) Receptor dimer

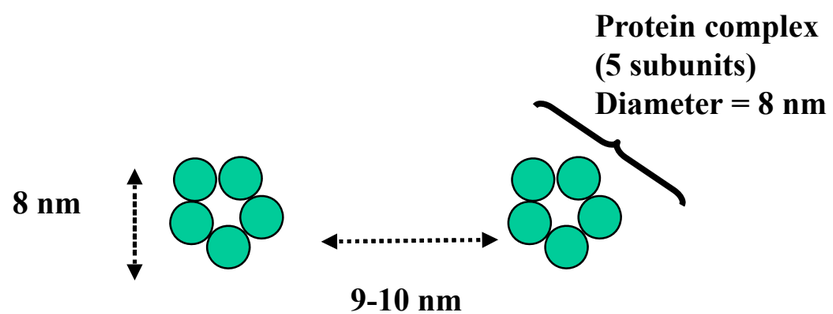


b) Interaction with tubocurarine

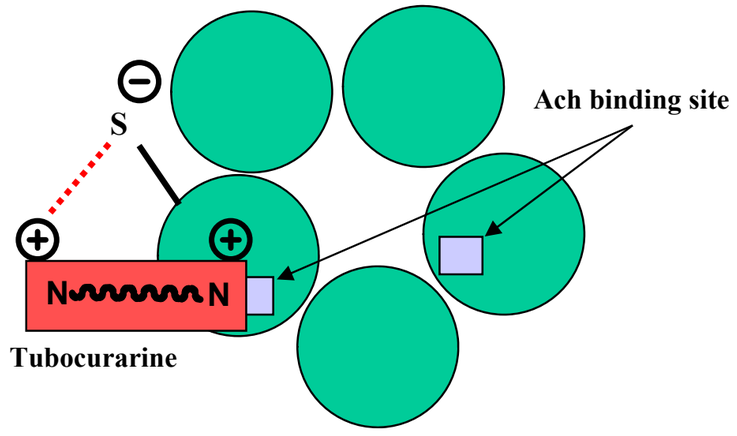
- So another possibility is that the tubocurarine bridges two acetylcholine binding sites within the one protein complex. ***(MOA)***

- however the two sites are more than 1.15 nm apart so they proposed that one positively charged nitrogens binds to the anionic binding region of the acetylcholine binding site and the other binds to a nearby cysteine residue 0.9-1.2 nm away.

- Such an interaction is extremely strong and would more than make up for the lack of the ester binding interaction.



a) Receptor dimer



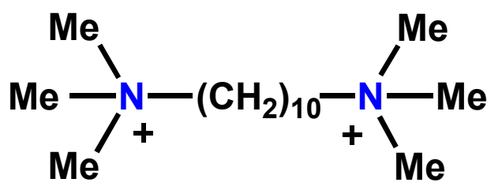
b) Interaction with tubocurarine

12.2 Analogues of tubocurarine

❑ Decamethonium and suxamethonium

Decamethonium is a simple an analogue of tubocurarine as one could imagine. **It is a flexible, straight-chain molecule and is capable of a large number of conformations**. The fully extended conformation places the nitrogen atoms 1.4 nm apart, but there are other more folded conformations that position the nitrogen centres 1.14 nm apart, which compares well with the equivalent distance in tubocurarine (1.15 nm)

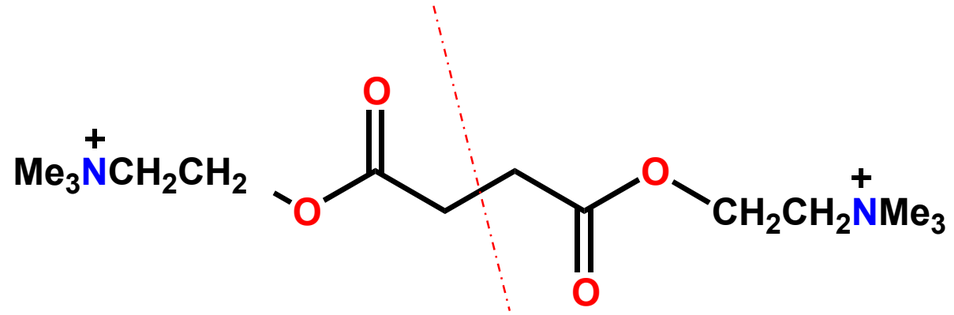
The drug binds strongly to cholinergic receptors and has proved a useful clinical agent, but it suffers from several disadvantages. For example, when it binds initially to nicotinic receptors, it acts as an agonist rather than an antagonist. Another disadvantage is that it binds too strongly, so patients take a long time to recover from its effects.



Decamethonium

- **Suxamethonium** where two ester groups are incorporated into the chain in such a way that the distance between the charged nitrogens remains the same. The ester groups are susceptible to chemical and enzymatic hydrolysis and, once this takes place, the molecule can no longer bridge the two binding regions on the receptor and is inactivated.

- Suxamethonium has a fast onset and short duration of action (5–10 minutes), but suffers from various side effects.



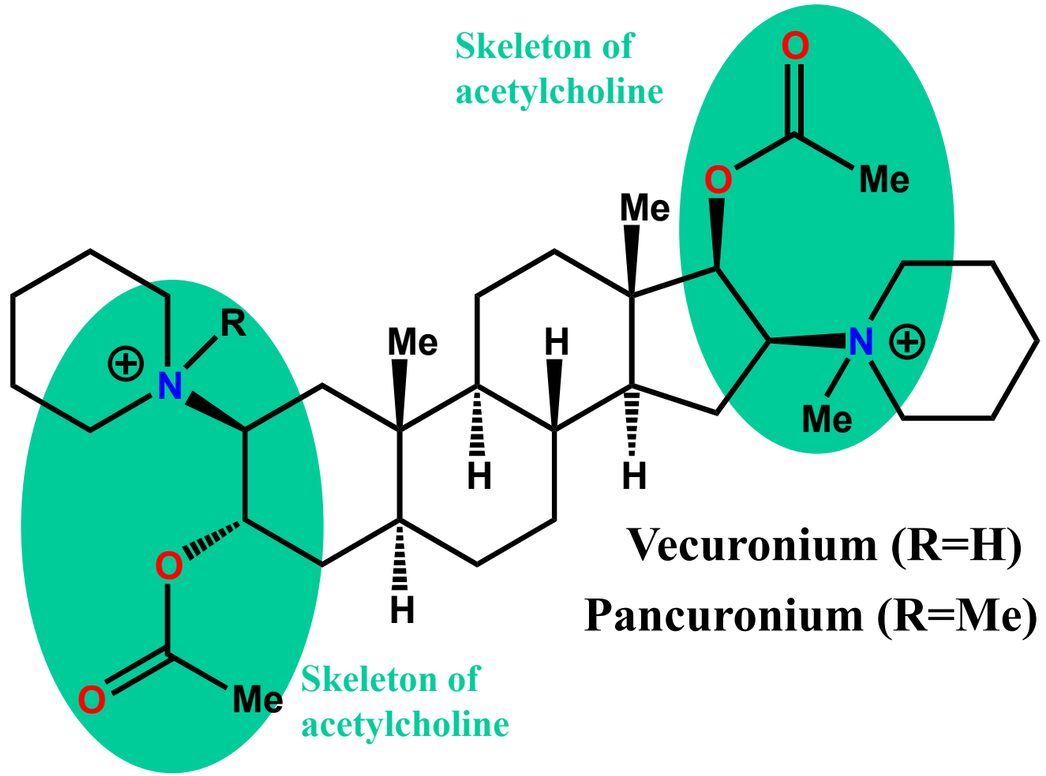
Suxamethonium

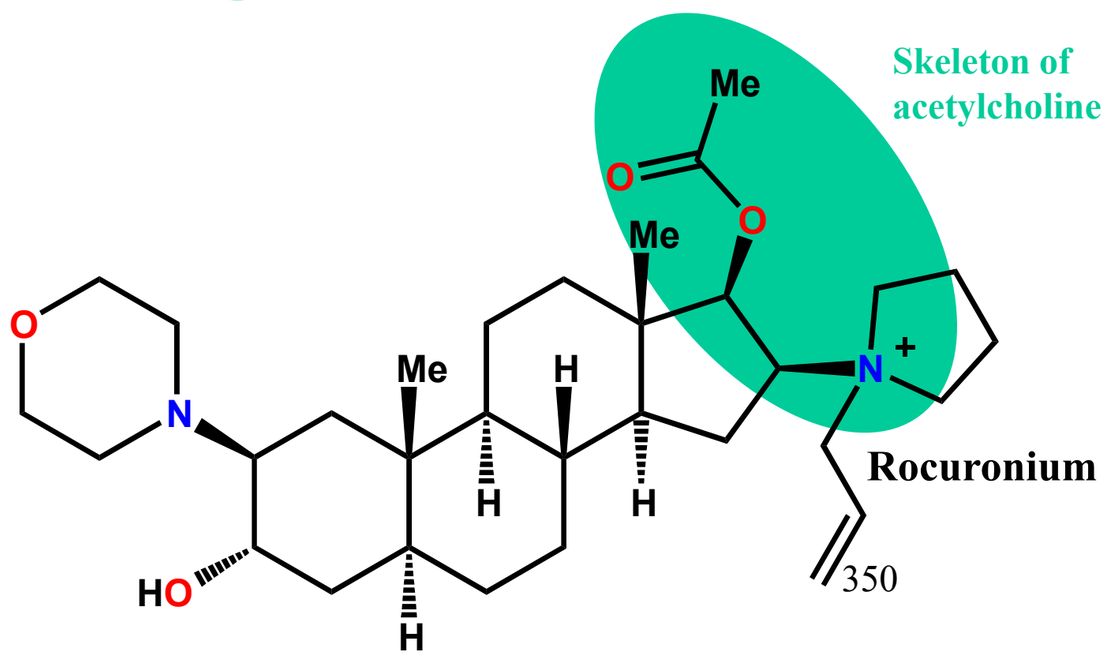
❑ Steroidal neuromuscular blocking agents

➢**Pancuronium, vecuronium, and rocuronium was design** based on tubocurarine but involved a steroid nucleus acting as a spacer between the two nitrogen groups. The distance between the nitrogens is 1.09 nm compared to 1.15 nm in tubocurarine.

➢***One or two Acyl groups were also added to introduce two acetylcholine skeletons into the molecule in order to improve affinity for the receptor sites.***

➢this compound has faster onset of action and don't affect blood pressure. They are not as rapid in onset as suxamethonium and have a longer duration of action (45 minutes)



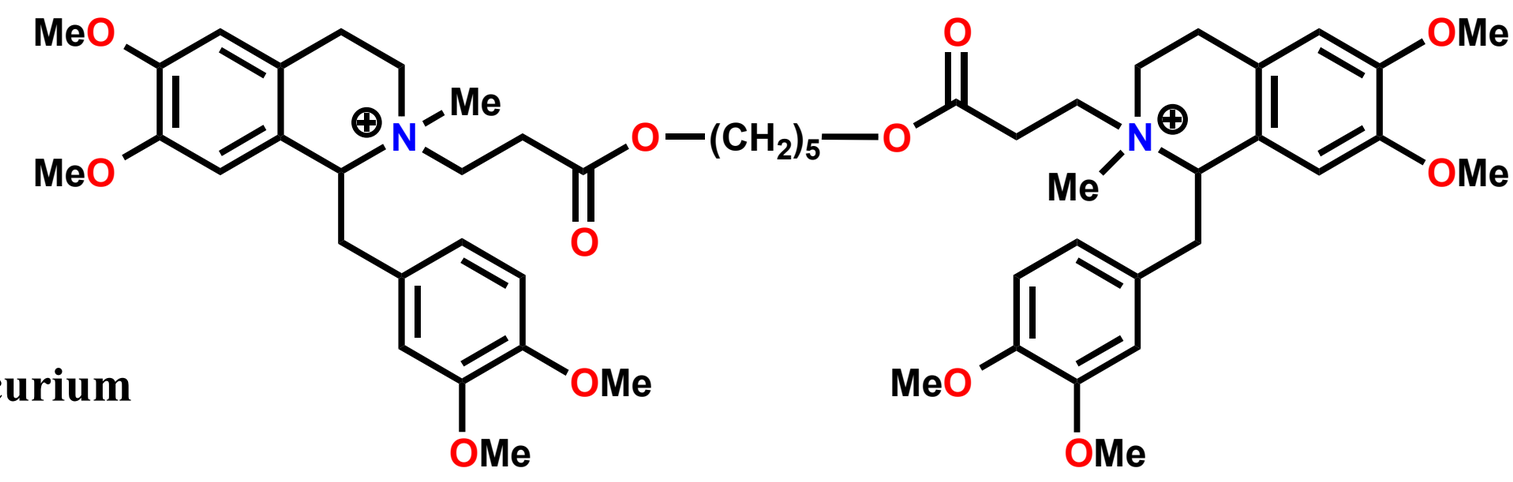


❑ Atracurium and mivacurium

- **Atracurium** was based in the structure of tubocurarine and it lacks cardiac side effects and is rapidly broken down in blood.

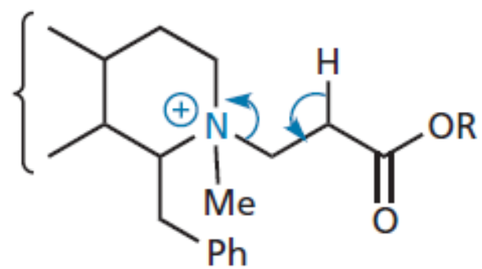
- at the slightly alkaline pH of blood "pH = 7.4" the molecule can undergo a **Hofmann elimination**. Once this happens the compound is inactived coz the positive charge on nitrogen is lost.

Atracurium

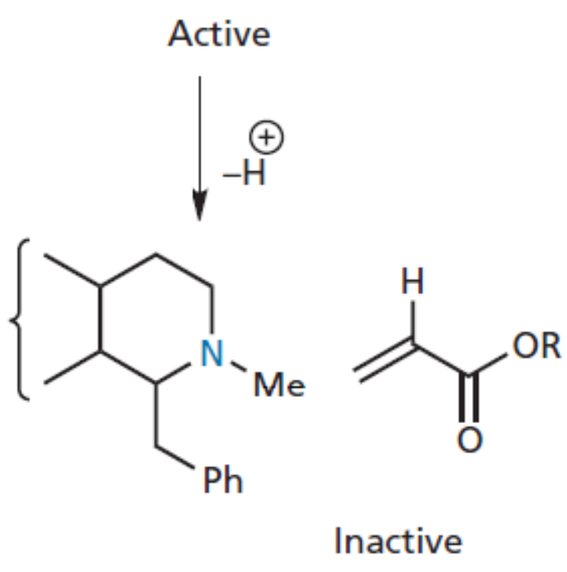


➢ **<u>The important features of atracurim are:</u>**

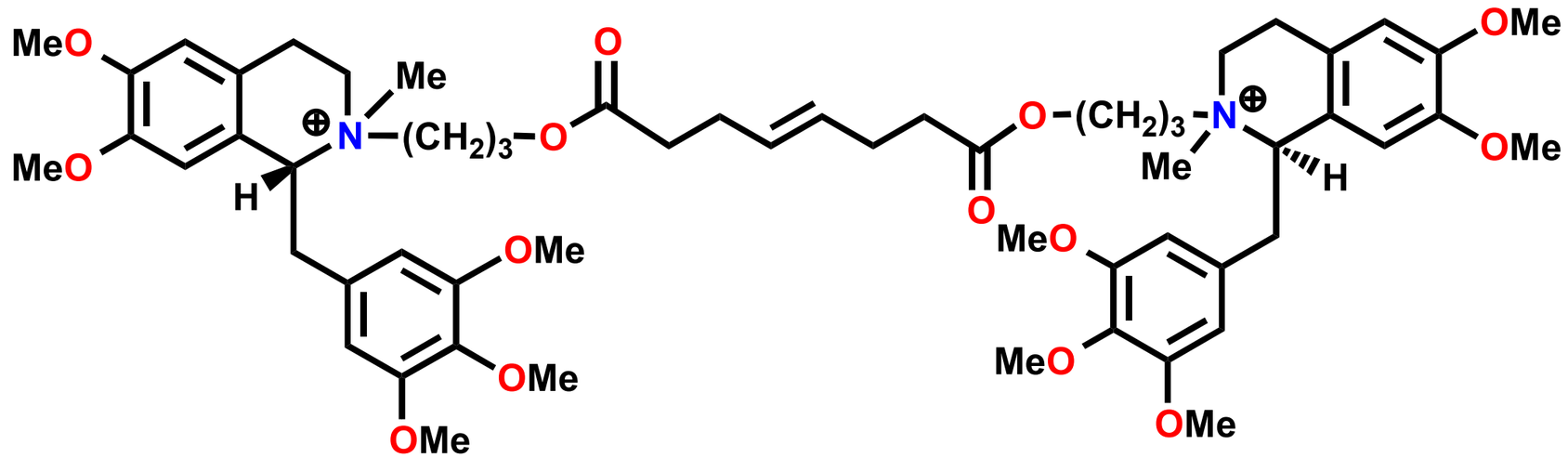
- **the sapcer**: a 13 atom chain connects the two quaternary centers.
- **the blocking units**: the cyclic structures at either end of the molecule block the binding site from acetylcholine.
- **the quaternary centers**: these are essential for receptor binding.



- **the Hofmann elimination**: It is normally require strong alkaline conditions but with a good electron-withdrawing gp it allows the reaction to proceed under milder conditions. Stable at a pH of 3-4 and can be stored in refrigerator. Since the drug acts very briefly "~ 30 mins" it is added intravenously for as long as needed. When surgery is over the IV drip is stopped and antagonism ceases. The deactivation doesn't need enzymes.



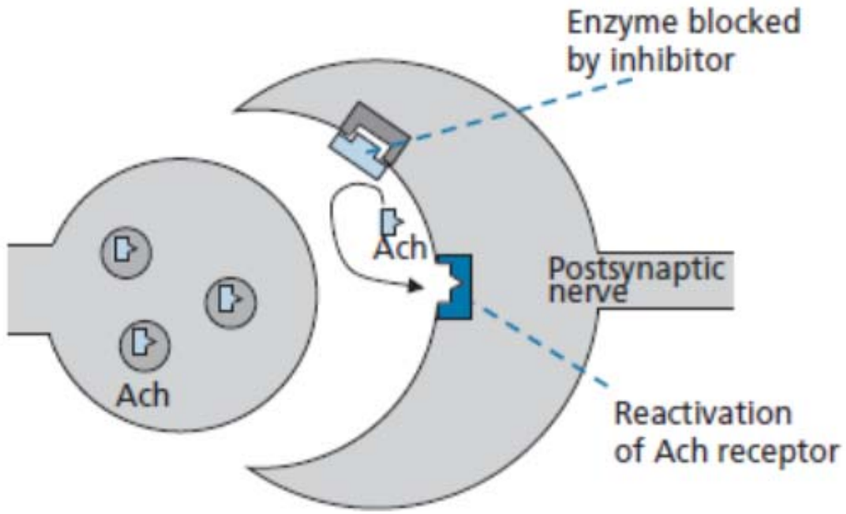
Mivacurium



Faster onset (2 min)
Shorter duration (15 min)

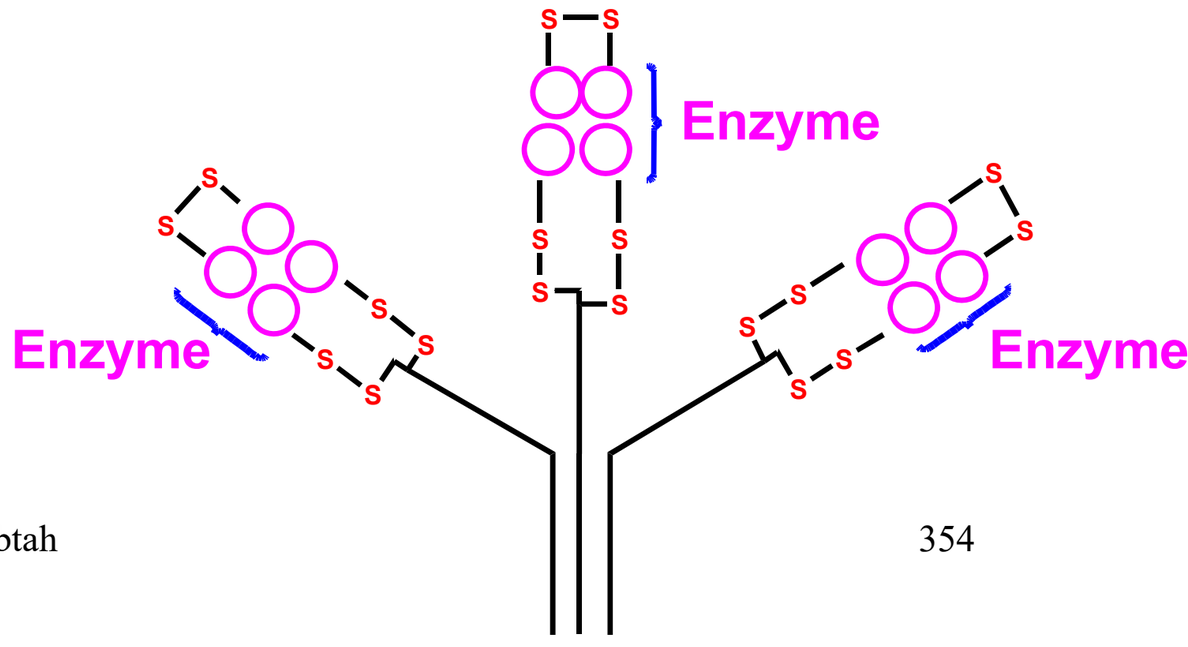
13. Anticholinesterases & acetylcholinesterase

- Anticholinesterases are **inhibitors** of acetylcholinesterase - the enzyme that hydrolyses acetylcholine. If acetylcholine is not destroyed, it can return to reactivate the cholinergic receptor and increase cholinergic effects.



- So acetylcholinesterase inhibitor will have the same biological effect as a cholinergic agonist.

- The design of anticholinesterases depends on the shape of the enzyme active site, the binding interactions involved with acetylcholine and the mechanism of hydrolysis.



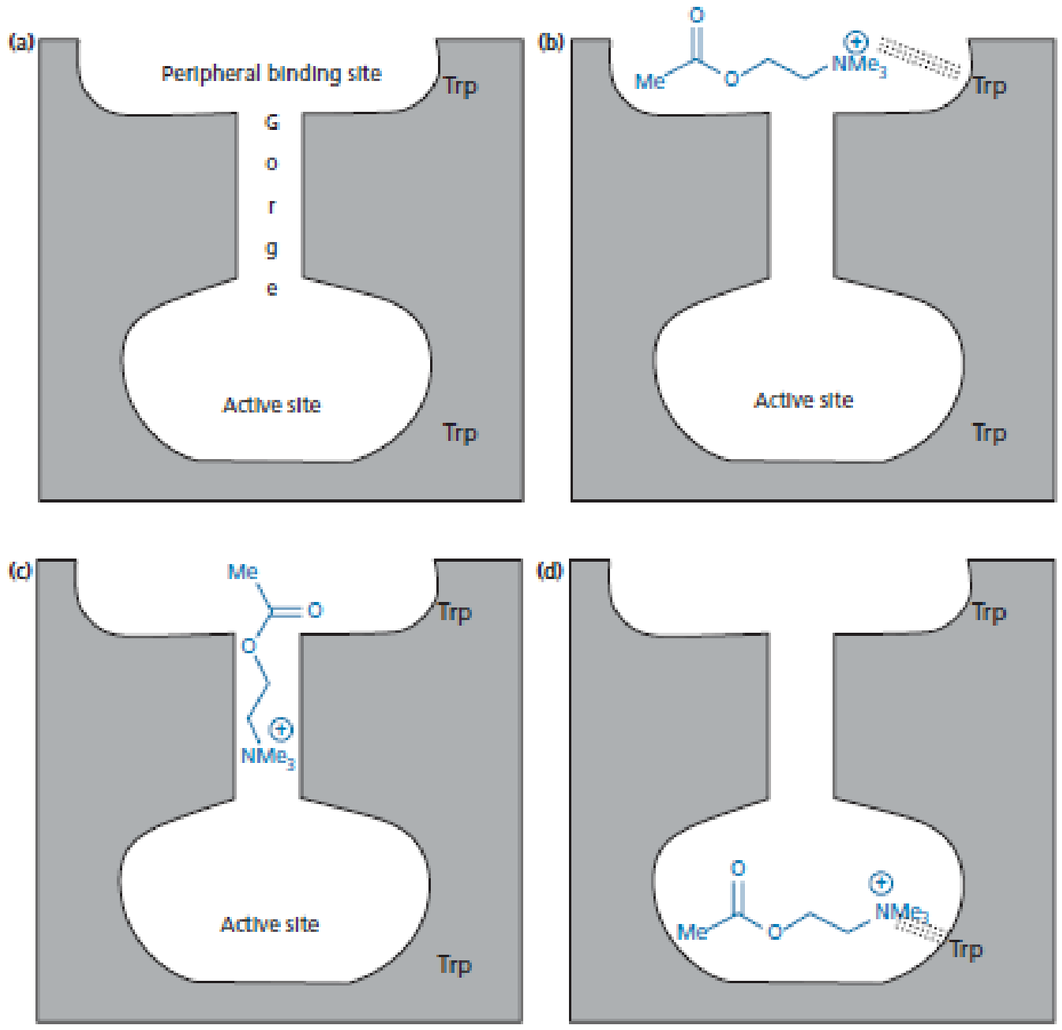
The active site of acetylcholinesterase

(a): The active site itself is at the foot of a narrow gorge and, at the entrance to the gorge, there is a peripheral binding site.

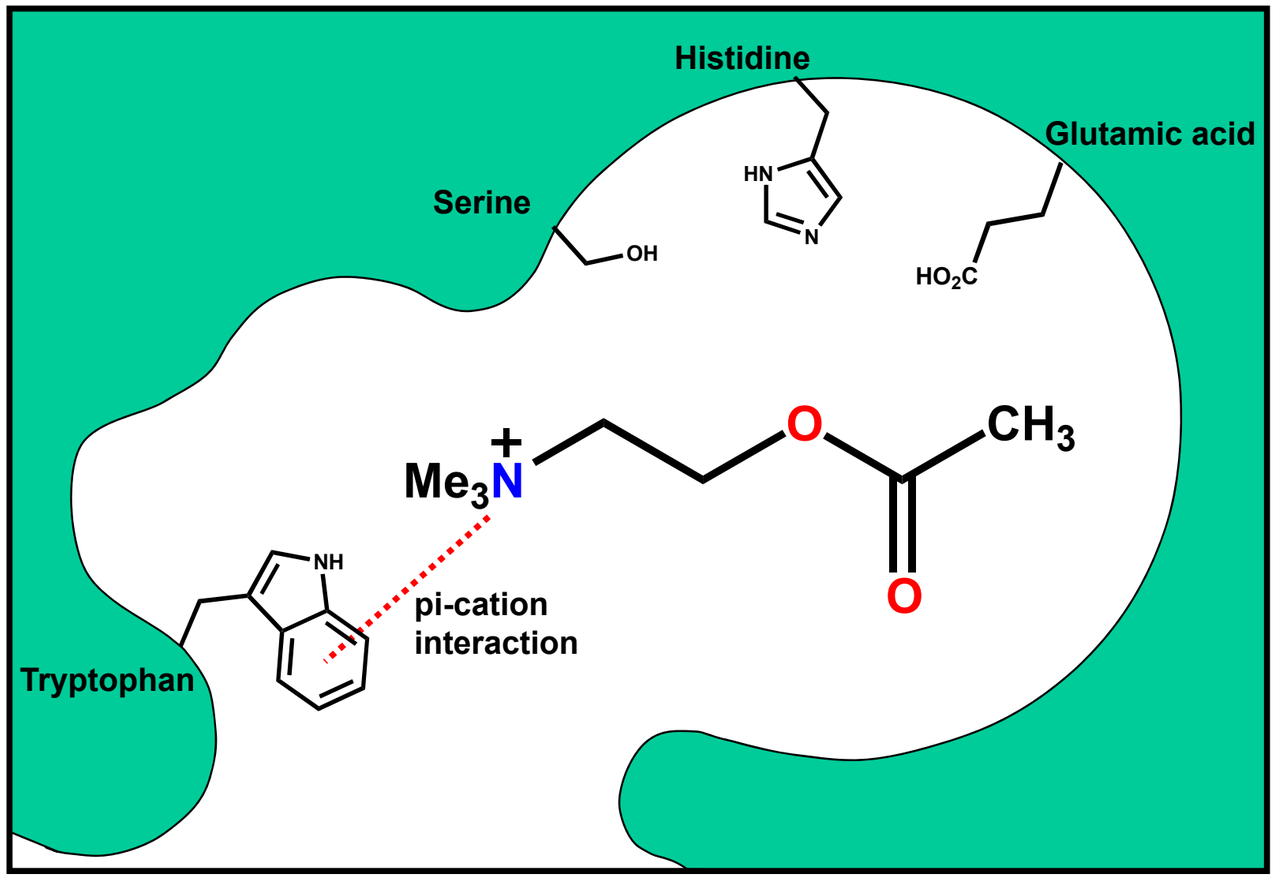
(b): One of the key interactions is a weak π-cation interaction between the heteroaromatic ring of a tryptophan residue and the charged quaternary nitrogen of acetylcholine.

(c): After acetylcholine has been 'captured' it is rapidly transferred down the gorge to the active site. This process is aided by the fact that the gorge is lined with 14 conserved aromatic residues, which can also form π-cation interactions with acetylcholine and thus channel the substrate down the gorge into the active site.

(d): Once acetylcholine enters the active site, another tryptophan residue forms yet another π-cation interaction.



Active site - important amino acid residues



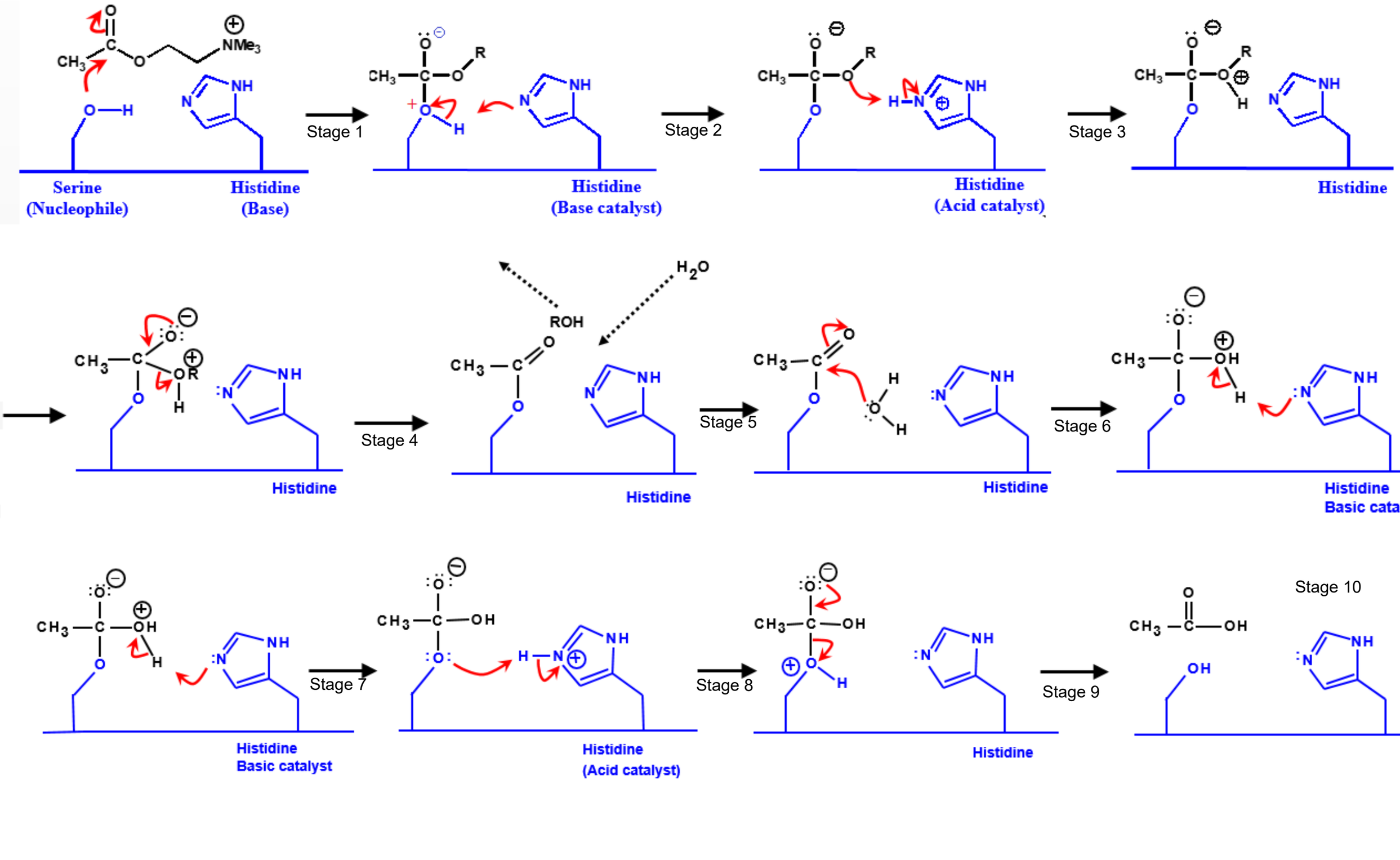
- Binding and induced fit strains Ach and weakens bonds
- Important pi-cation interaction with tryptophan
- Molecule is positioned for reaction with serine residue

❑ Mechanism of hydrolysis

- the histidine residue acts as an acid-base catalyst through out the mechanism, while serine acts as a nucleophile.

- the aliphatic alcohol of serine is a poor nucleophile and is unable to hydrolyze an ester but the acid/base catalysis provided by histidine overcomes that disadvantage.

- the aspartate residue interacts with the histidine residue and serves to orientate and activate the ring.


Serine
(Nucleophile)
Histidine
(Base)
Stage 1
Histidine
(Base catalyst)
Stage 2
Histidine
(Acid catalyst)
Stage 3
Histidine
Stage 4
Histidine
ROH
H2O
Stage 5
Histidine
Stage 6
Histidine
Basic catalyst
Stage 7
Histidine
Basic catalyst
Histidine
(Acid catalyst)
Stage 8
Histidine
Stage 9
Stage 10

14. Anticholinesterase drugs

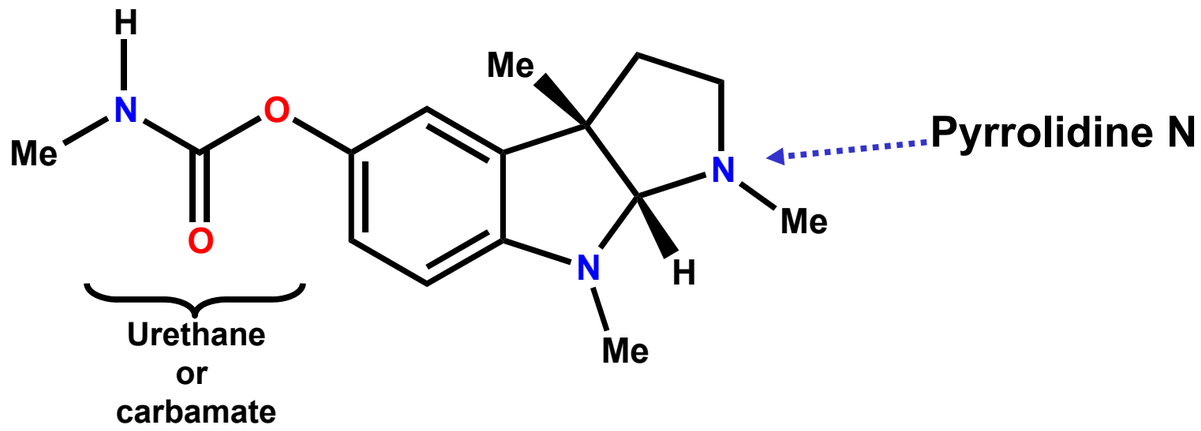
- They act as inhibitors of the enzyme acetylcholinesterase. This inhibition can be either reversible or irreversible depending on how the drug interacts with the active site.

Two main groups are considered here – carbamates and organophosphorus agents.

❑ Carbamates

➢ Physostigmine

- It is a natural product that provided the lead for the carbamate inhibitors.
- Physostigmine is still used clinically to treat glaucoma.

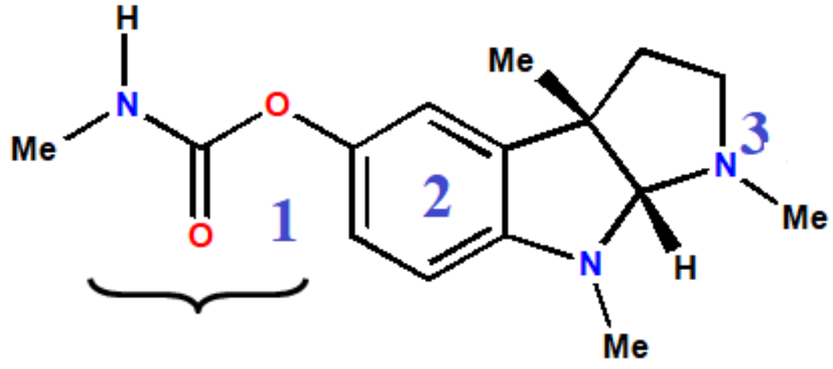


➢**SAR studies demonstrate that:**

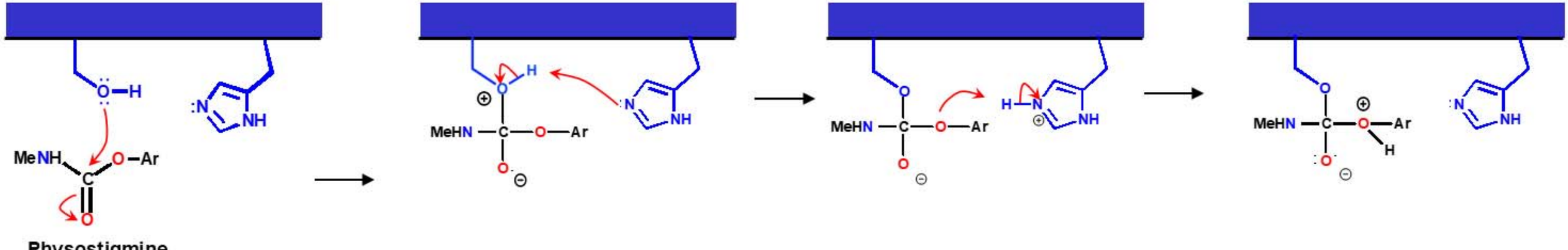
1.the carbamate gp is essential to activity; responsible for physostigmine's inhibitory properties.

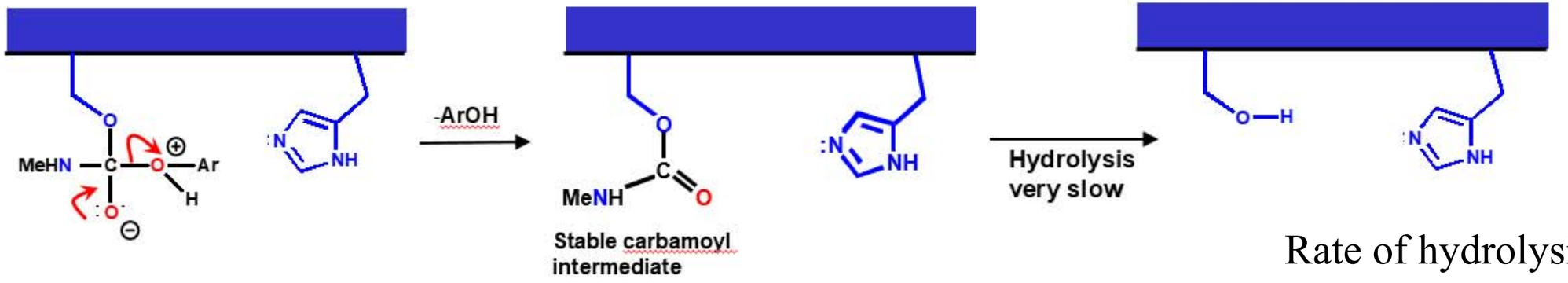
2.the benzene ring is important; to involved in some extra hydrophobic bonding with the active site and it may be important in the mechanism of inhibition as it provides a good leaving group.

3.the pyrrolidine nitrogen is important and is ionized at blood pH. "the positively charged pyrrolidine nitrogen is important because it binds to the anionic binding region of the enzyme"



❑ Mechanism of action





Rate of hydrolysis slower by 40 x 10^6

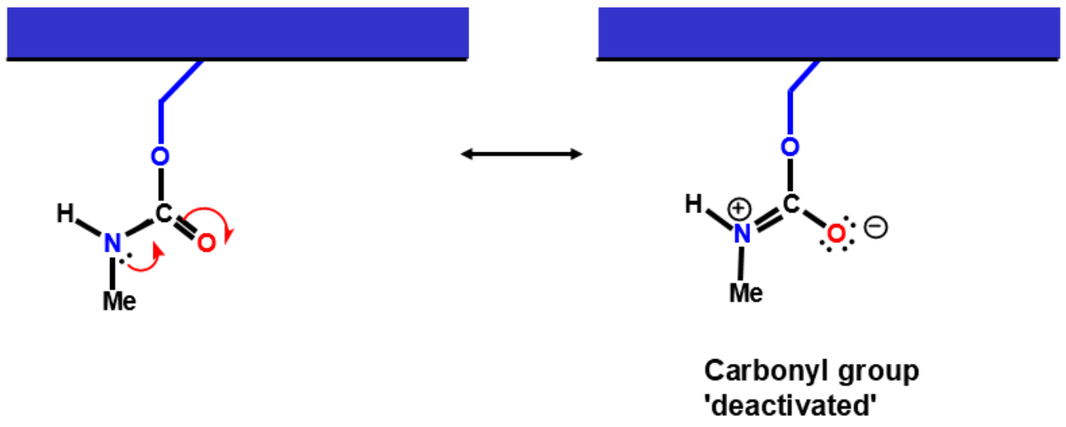
- the first four stages proceed as normal as the acetylcholine, with histidine catalyzing the nucleophilic attack of the serine residue on physostigmine “stage 1 & 2”.

- the leaving gp “phenol” is expelled with the aid of acid catalysis from histidine “stage 3 & 4”, and departs the active site to be replaced by a water molecule.

- the next stage turns out to be extremely slow. Despite the fact that histidine can still act as a basic catalyst, water finds it difficult to attack the carbamoyl intermediate.

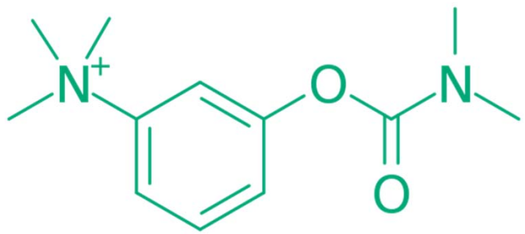
- as a result the cholinesterase active site becomes blocked and is unable to react with acetylcholine.

- the nitrogen in the carbamoyl-enzyme complex can feed a lone pair of electrons into the carbonyl gp reduce its electrophilic character drastically



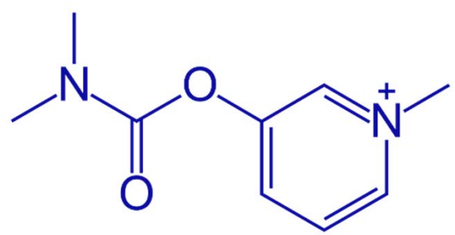
Physostigmine analogues

- physostigmine has limited use because of serious side effects and it has only been used in the treatment of glaucoma or as antidote of atropine poisoning.
- analogues like neostigmine & pyridostigmine are in use today to reverse the actions of nueromuscular blockers or used orally in the treatment of myasthenia gravis.
- they don't cross the BBB so don't have CNS side effects also the compound is more stable to chemical hydrolysis.



Neostigmine

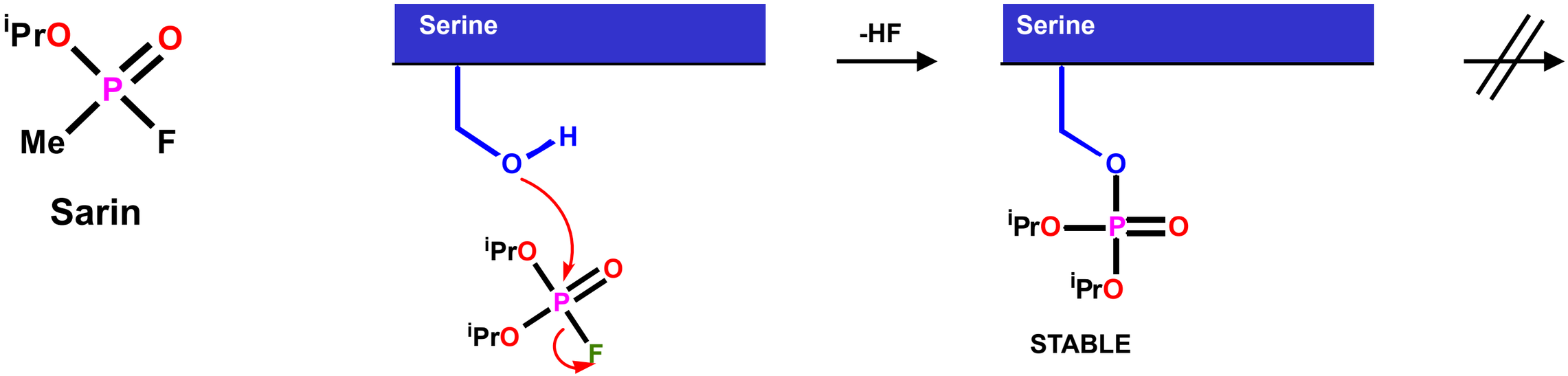
- by analyzing the structure, a quaternary nitrogen atom is present so that there is no chance of the free base being formed. Since the molecule is permanently charged, it cannot cross the BBB & so the drug is free of CNS side effects.
- increased stability is achieved by using a dimethylcarbamate group rather than a methylcarbamte gp.



Pyridostigmine

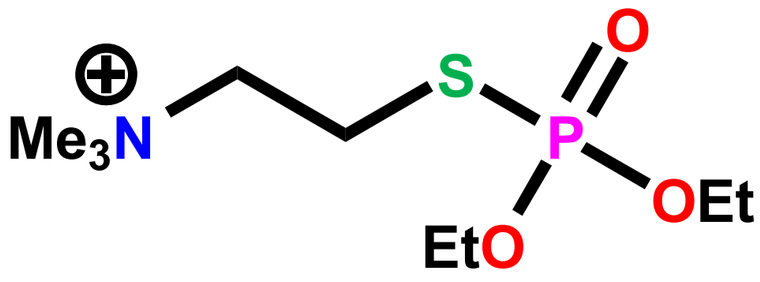
Organophosphates – Nerve agents

The nerve gas sarin inhibit acetylcholinesterase by irreversibly phosphorylating the serine residue at the active site.



- the phosphorylated adduct which is formed is extremely resistant to hydrolysis. The enzyme is permanently inactivated.
- As Ach can't be hydrolyzed the cholinergic system is continually stimulated. This results in permanent contraction of skeletal muscle, leading to death.

Organophosphates – Medicinal organophosphate

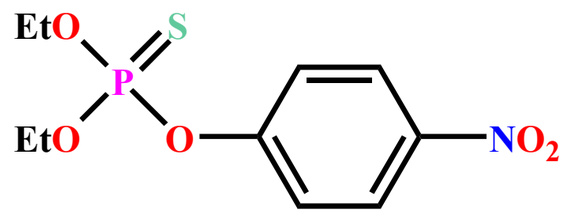


Ecothiopate

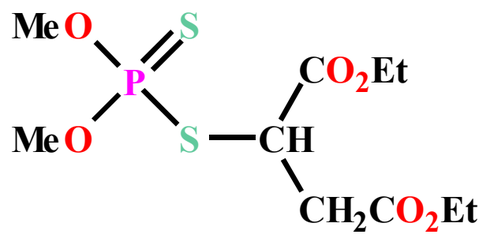
- **Used to treat glaucoma**
- **Topical application**
- **Quaternary N is added to improve binding interactions**
- **Results in better selectivity and lower, safer doses**

Organophosphates – Insecticides

- Relatively non-toxic compared to nerve gases since
- P = S double bond prevents these molecules from inhibiting acetylcholinesterase enzymes.
  - The equivalent compounds containing a P = O double are lethal compounds.
  - Fortunately, there are no metabolic pathways in mammals which can convert the P = S double bond to a P = O double bond.
  - Such a pathway does exist in insects
- Preparations of malathion are used medicinally for the treatment of head lice and scabies
- They should not be used too frequently or over prolonged periods.

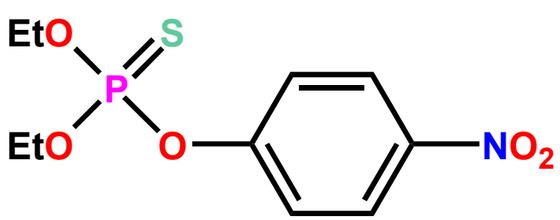


Parathion



Malathion

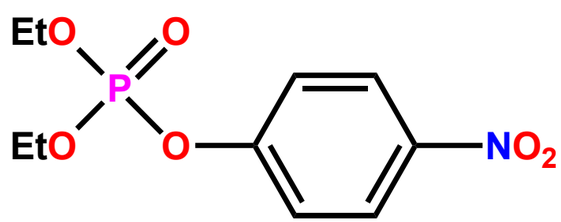
MAMMALS



Parathion
(Inactive prodrug)

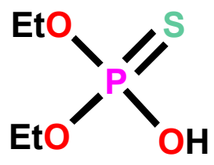
Insect oxidative desulphurisation

INSECTS



Active drug

Mammalian metabolism



Inactive and excreted

Phosphorylates enzyme

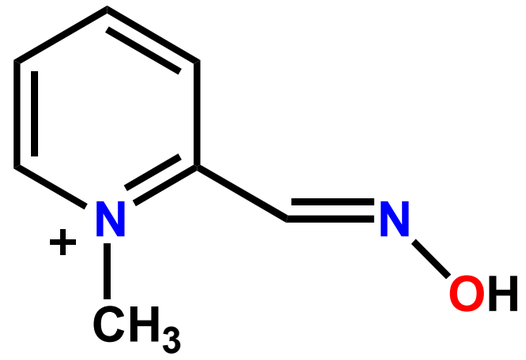
DEATH

❑ Pralidoxime: an organophosphate antidote

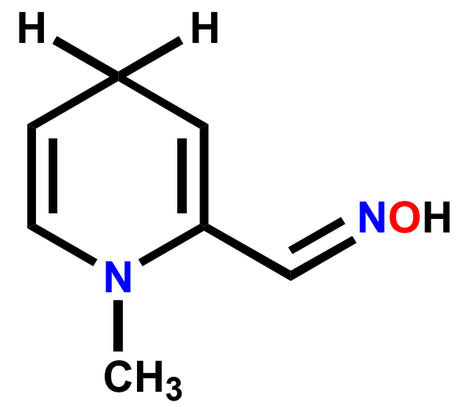
- it is an antidote to organophosphate poisoning, and it has to displace the organophosphate moiety from serine. As it is a strong bond it should use a stronger nucleophile than water.

- phosphate can be hydrolyzed with hydroxylamine, but it is a toxic compound to use on humans, so the next stage was to design an equally reactive nucleophilic group which would specifically target the acetylcholinesterase enzyme.

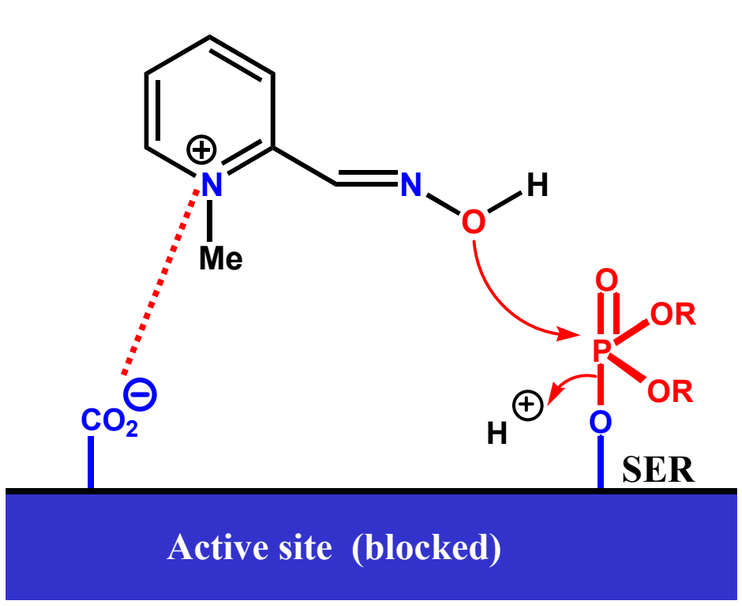
- As the anionic binding region is vacant when organophosphate group binds and doesn´t fill it. So the obvious thing is to find a suitable gp to bind in the anionic centre and attach a hydroxylamine moiety to it.

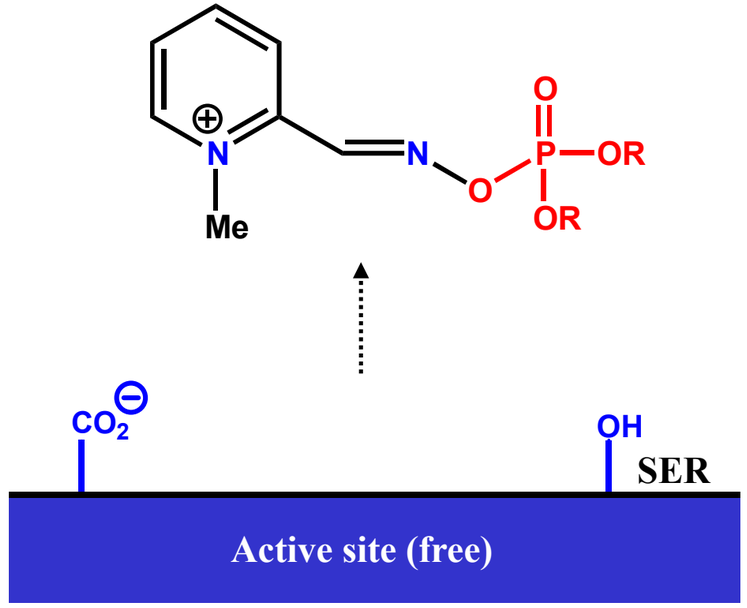


- Pralidoxime was the result. The positive charge is provided by a methylated pyridine ring and the nucleophilic side group is attached to the *ortho* position.
- the results were spectacular, with pralidoxime showing a potency as an antidote 10^6 times greater than hydroxylamine.
- As it has a quaternary nitrogen it is fully charged so cannot pass through BBB this means the antidote can´t work on any enzymes inhibited in the brain. **Pro-2-PAM** is a prodrug which avoid this problem as a tertiary amine it can pass through BBB and is converted to pralidoxime once it has entered the CNS.



Pro-2-PAM





❑ Anticholinesterases as “smart drugs”

- In recent years, it has been proposed that the memory loss, intellectual deterioration and personality changes associated with Alzheimer´s disease may in part be due to loss of cholinergic nerves in the brain.

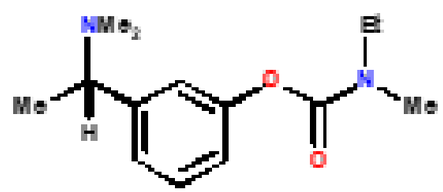
- Research has been carried out into the use of anticholinesterases for the treatment of Alzheimer´s disease the so called “smart drugs”.

- the treatment does not offer a cure for Alzheimer´s disease but it can alleviate the symptoms by allowing the brain to make more use of the cholinergic receptors still surviving.

- “smart drugs” have to cross the blood-brain barrier and so structures containing quaternary nitrogen atoms are not suitable.

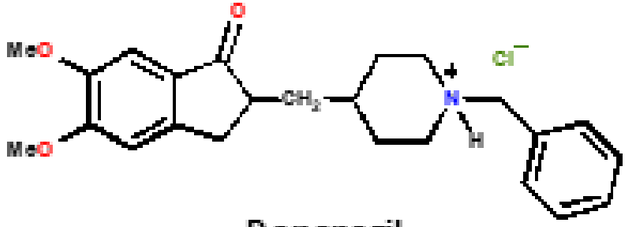
- Various agents have been introduced include **donepezil** in 1997, and **rivastigmine** in 2000.

- Rivastigmine was the first drug to be approved in all countries of the European Union.



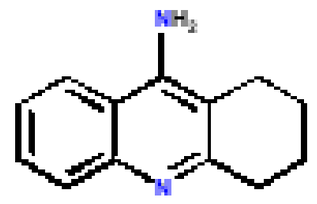
Rivastigmine (Exelon)
(Analogue of physostigmine)

- they have various disadvantages as they increase Ach levels all around the body, this leads to GIT side effects, and the other problem increases Ach levels result in an increased activation of presynaptic cholinergic receptors which act as a feedback control to lower the amounts of Ach released.

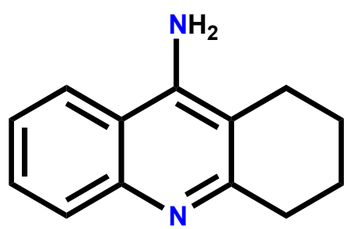


Donepezil

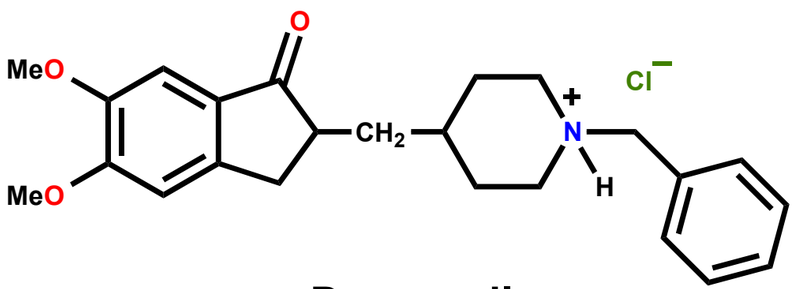
- As a result, there has been research into finding selective cholinergic agonists.



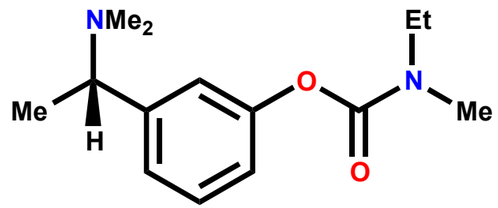
Tacrine (Cognex)
Toxic side effects



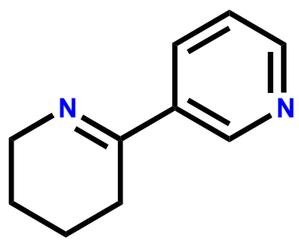
Tacrine (Cognex)
Toxic side effects



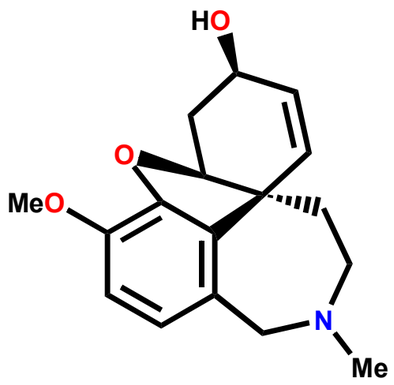
Donepezil



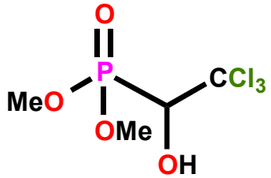
Rivastigmine (Exelon)
(Analogue of physostigmine)



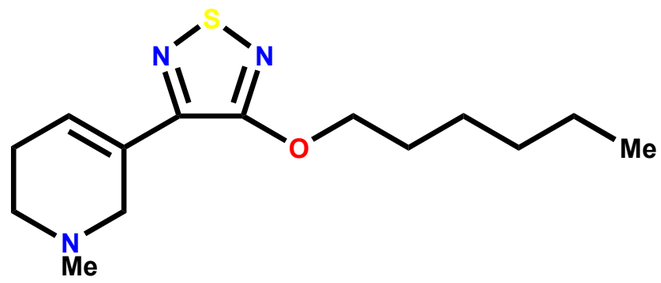
Anabaseine
(ants and marine worms)



Galantamine
(Daffodil and snowdrop bulbs)



Metrifonate
(Organophosphate)



Xanomeline

Homework: answer the end of chapter question 1, 4, and 6, page 608

Take Home exam through the link: https://forms.gle/zzMgVX2SPBK48kdA7

DL: 4\1\2021