Medicinal chemistry II

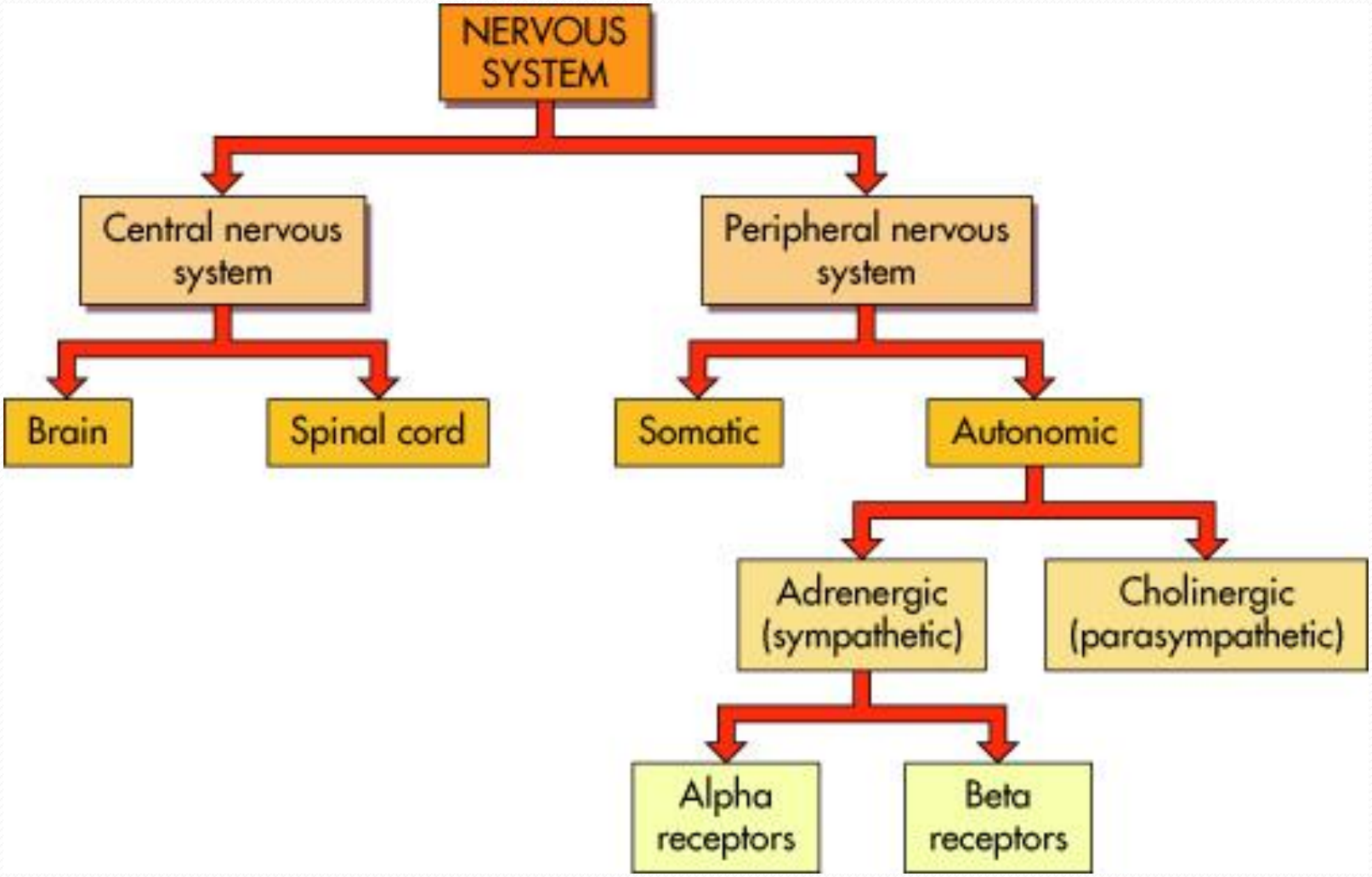
Cholinergic Drugs

4th Stage

lec1

Dr.yahya saad yaseen

Overview of Nervous System



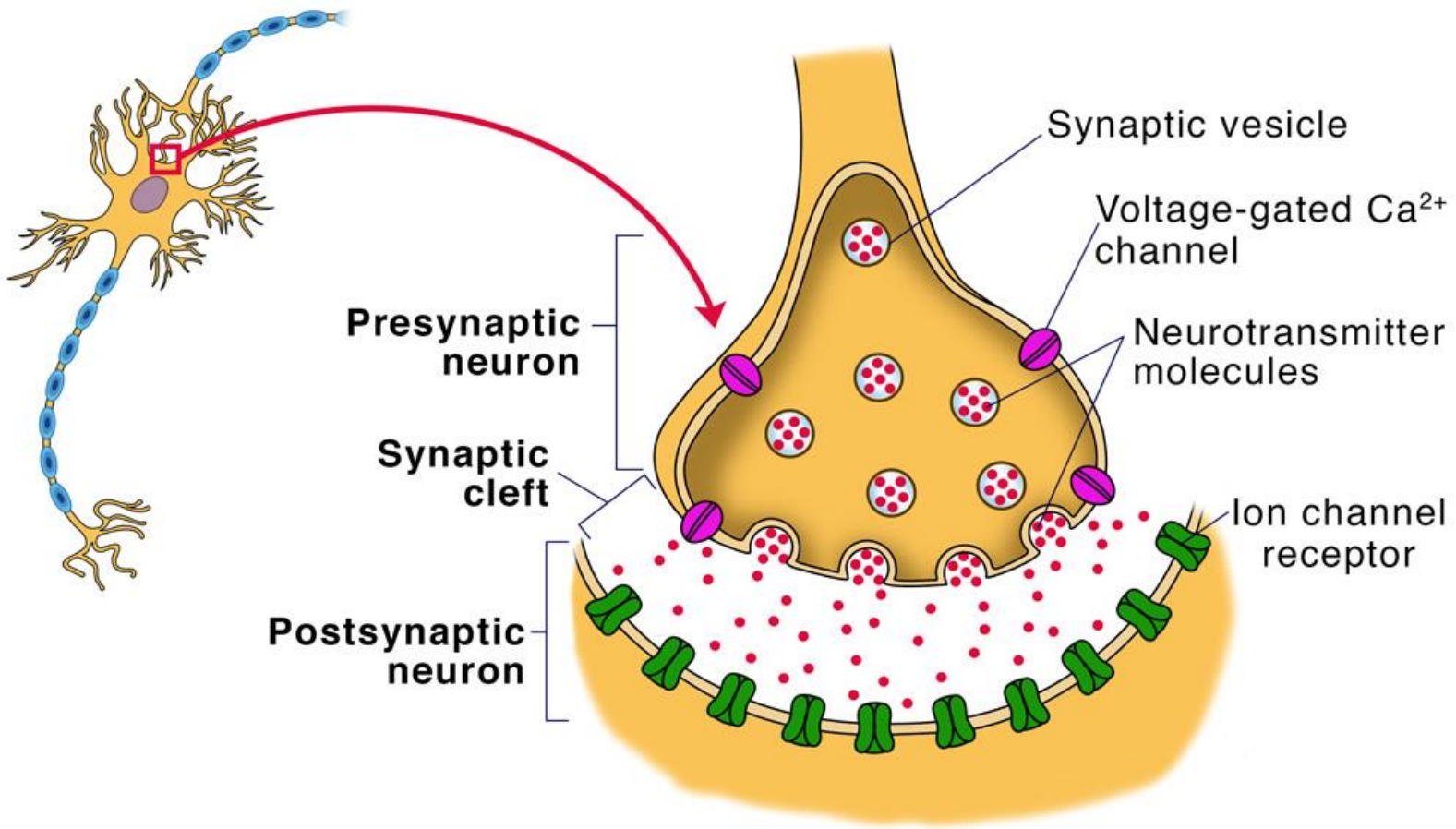
Neurotransmitters (NT)

- Neurotransmitters (NT): are endogenous (produced by body) chemicals that transmit signals across a synapse from sending presynaptic neuron to the target postsynaptic neuron
- They are synthesized and stored in neuron itself.
- There are many NTs eg: Acetylcholine, Adrenaline, serotonin, dopamine, GABA

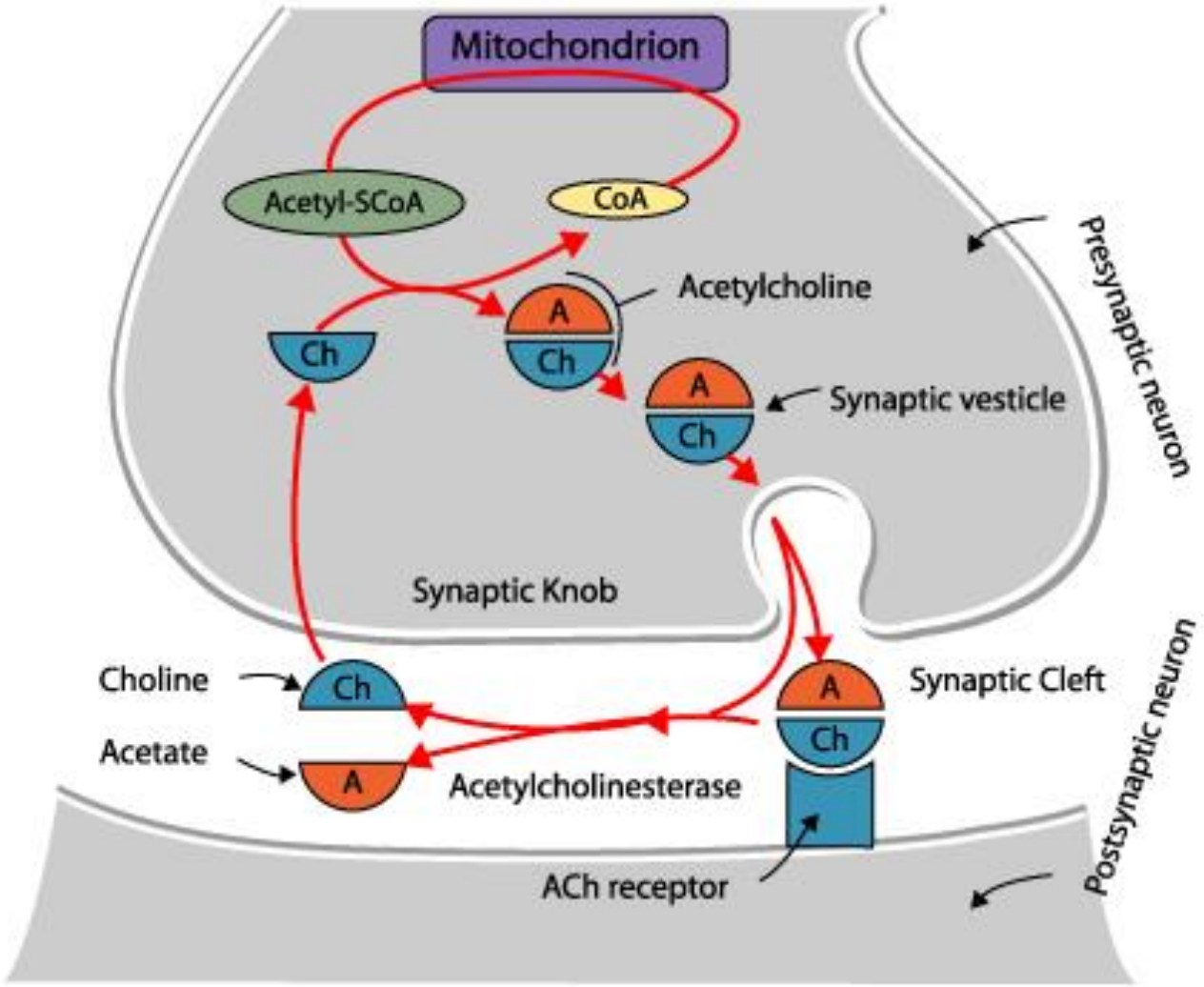
A synapse is a small gap at the end of a neuron that allows a signal to pass from one neuron to the next. Neurons are cells that transmit information between your brain and other parts of the central nervous system.

presynaptic neuron transmits the signal toward a synapse, whereas a postsynaptic neuron transmits the signal away from the synapse.

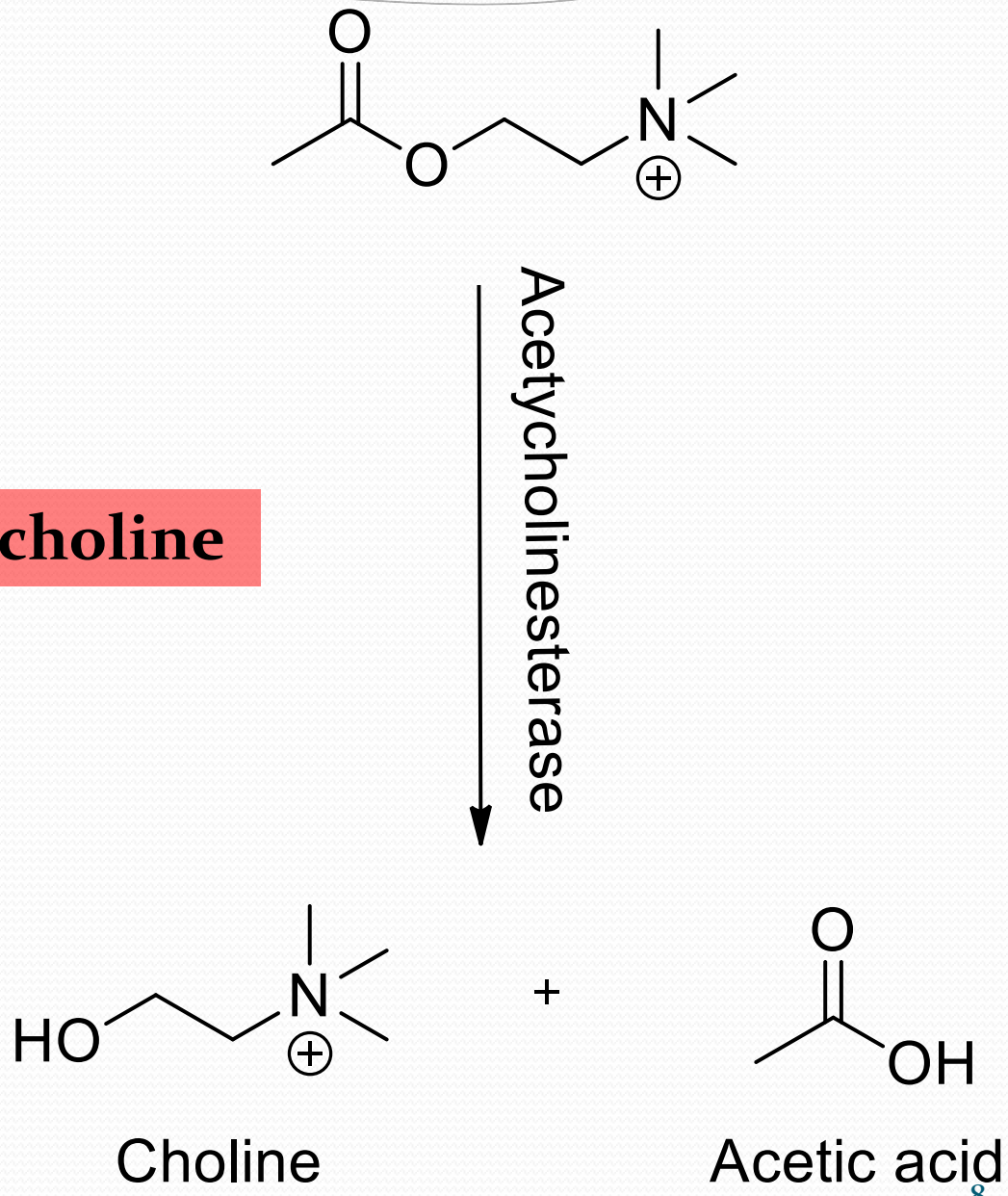
Synapse


Synaptic vesicle
Voltage-gated Ca^{2+} channel
Neurotransmitter molecules
Ion channel receptor
Presynaptic neuron
Synaptic cleft
Postsynaptic neuron

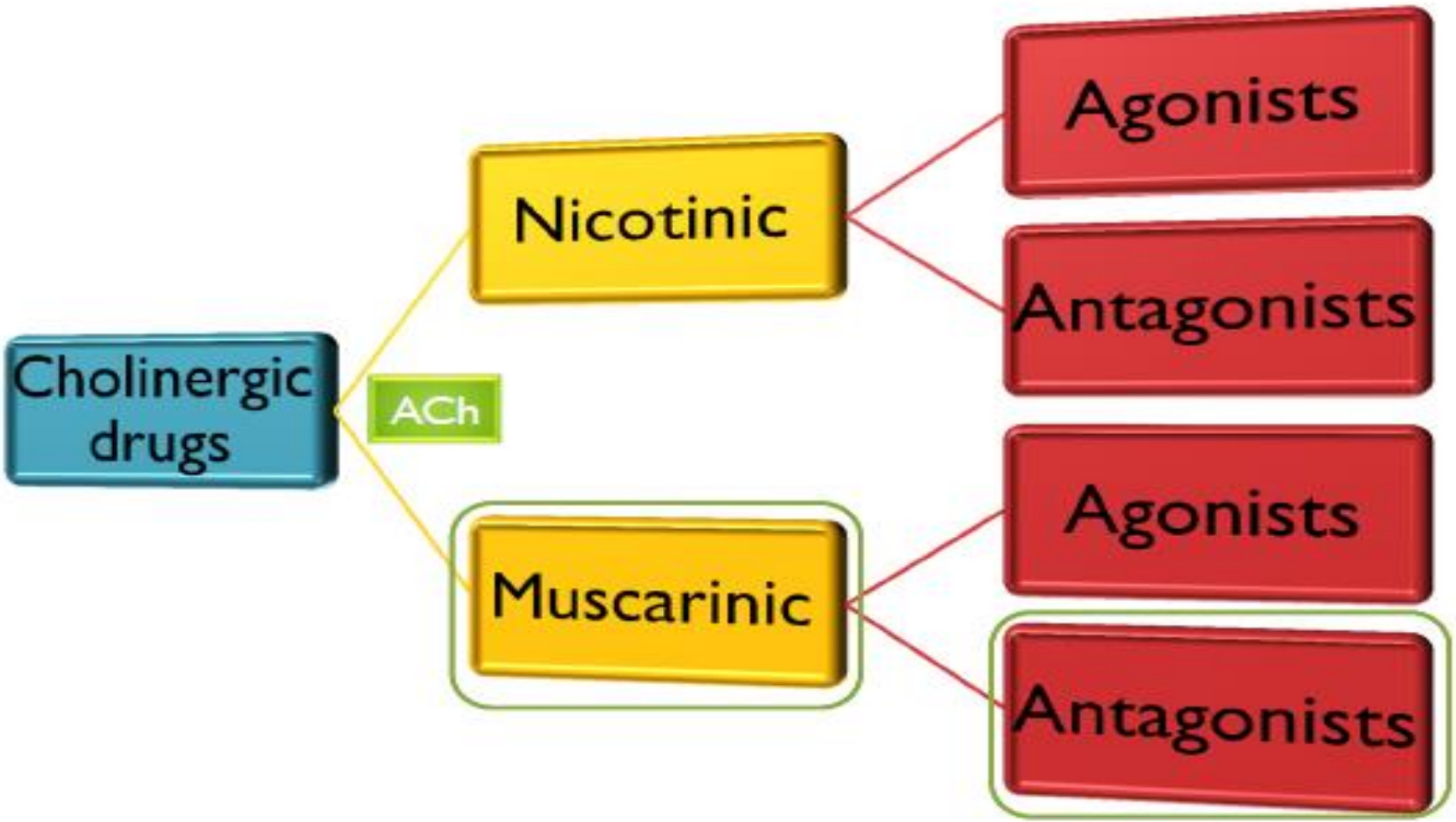
The cholinergic system


Mitochondrion
Acetyl-SCoA
CoA
Acetylcholine
A
Ch
Ch
A
Ch
Synaptic vesticle
Presynaptic neuron
Synaptic Knob
Choline
Ch
Acetate
A
Acetylcholinesterase
A
Ch
Synaptic Cleft
ACh receptor
Postsynaptic neuron

Hydrolysis of Acetylcholine



Cholinergics

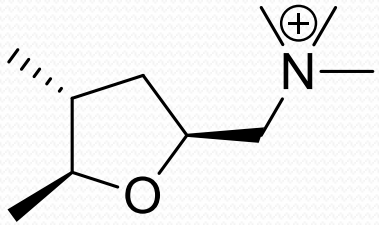


Cholinergic receptors

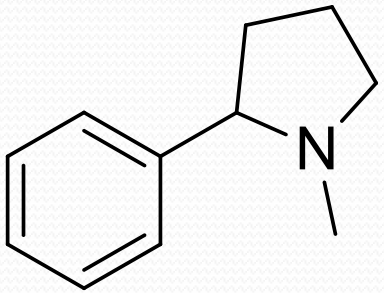
- There are two distinct receptor types for Ach.
- Nicotinic and muscarinic on the basis of their ability to be bound by the naturally occurring alkaloids nicotine and muscarine, respectively.
- Receptor subtypes
  - ✓ (α1–α10)
  - ✓ M1 - M5

Cholinergic receptors

1. Muscarinic receptors:
   - Found in smooth muscles and cardiac muscles.
   - Related to muscarine.

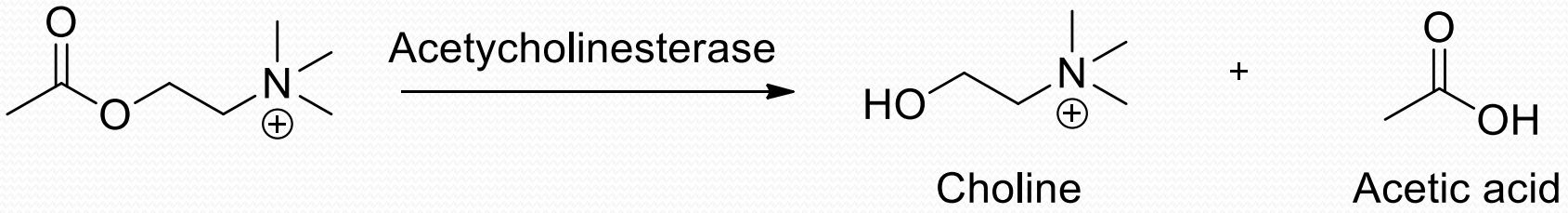


2. Nicotinic receptors:
   - Found in skeletal muscles and in synapses between neurons.
   - Related to nicotine.



Cholinergic Agents

- Direct acting - act on the receptors
- Indirect acting - inhibit the action of the enzyme Acetylcholinesterase:



- Major uses = Stimulate bladder & GI tone, constrict pupils (miosis), neuro-muscular transmission

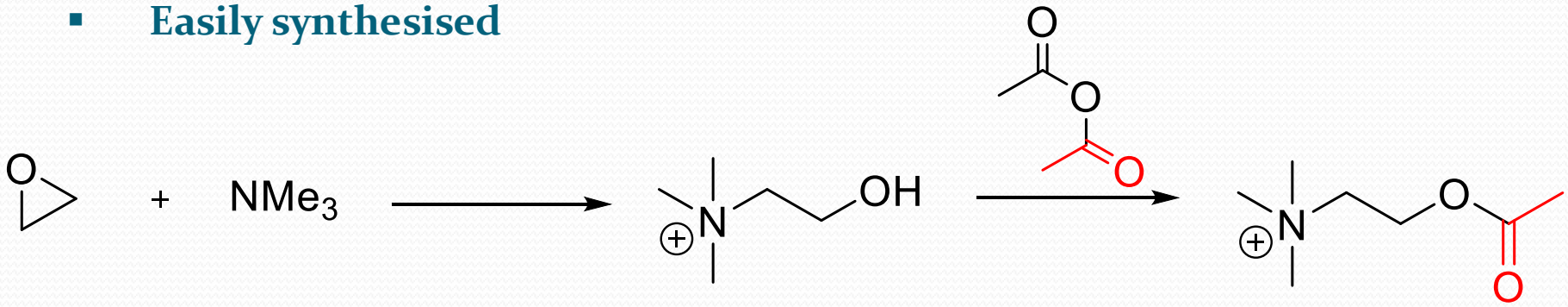
Cholinergic agonists

Cholinergic agonists

Acetylcholine as an agonist

Advantages

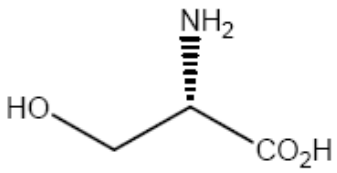
- Natural messenger
- Easily synthesised



Disadvantages

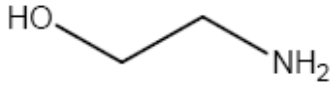
- Easily hydrolysed in stomach (acid catalysed hydrolysis)
- Easily hydrolysed in blood (esterases)
- No selectivity between receptor types
- No selectivity between different target organs

Biosynthesis of Acetylcholine:



L-serine

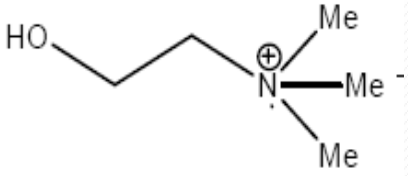
Serine Decarboxylase



ethanolamine

Choline-*N*-Methyl-Transferase

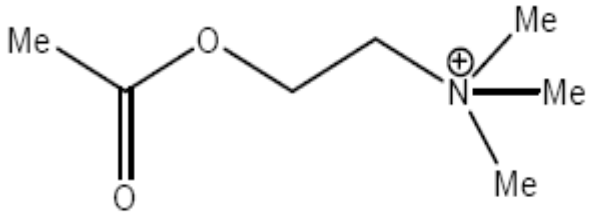
S-Adenosylmethionine (Me donor)



choline

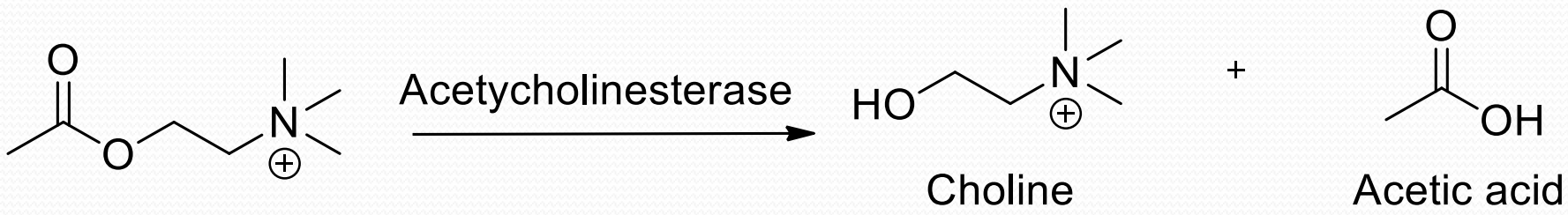
Choline Acetyl Transferase

Acetyl-*S*-Coenzyme A (Acetyl donor)



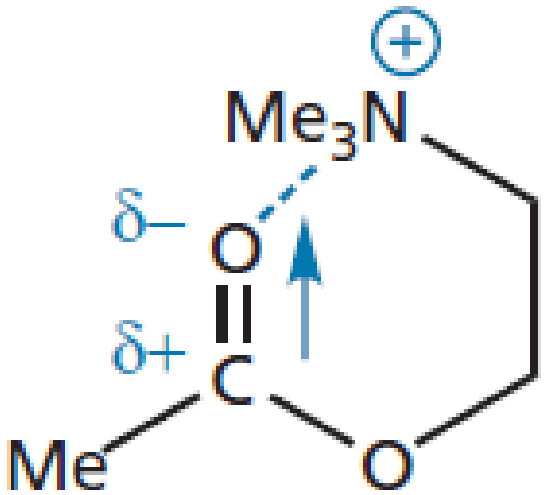
Acetylcholine

Biological hydrolysis of Ach



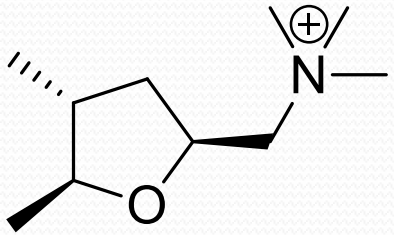
What makes acetylcholine exceptionally prone to hydrolysis is the possibility of folding to form an intramolecular dipole bond that will increase the positive charge of the ester carbonyl

Neighbouring group participation.
The arrow indicates the inductive pull of oxygen which increases the electrophilicity of the carbonyl Carbon.

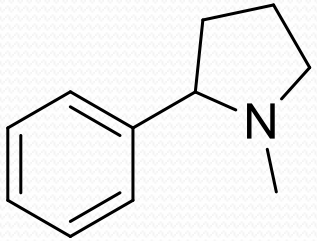


Cholinergic agonists

Nicotine and muscarine as cholinergic agonists



Muscarine



Nicotine

Advantages

- More stable than Ach
- Selective for main cholinergic receptor types
- Selective for different organs

Disadvantages

- unsuitable as medicines because they have undesirable side effects resulting from their interactions with other receptors.

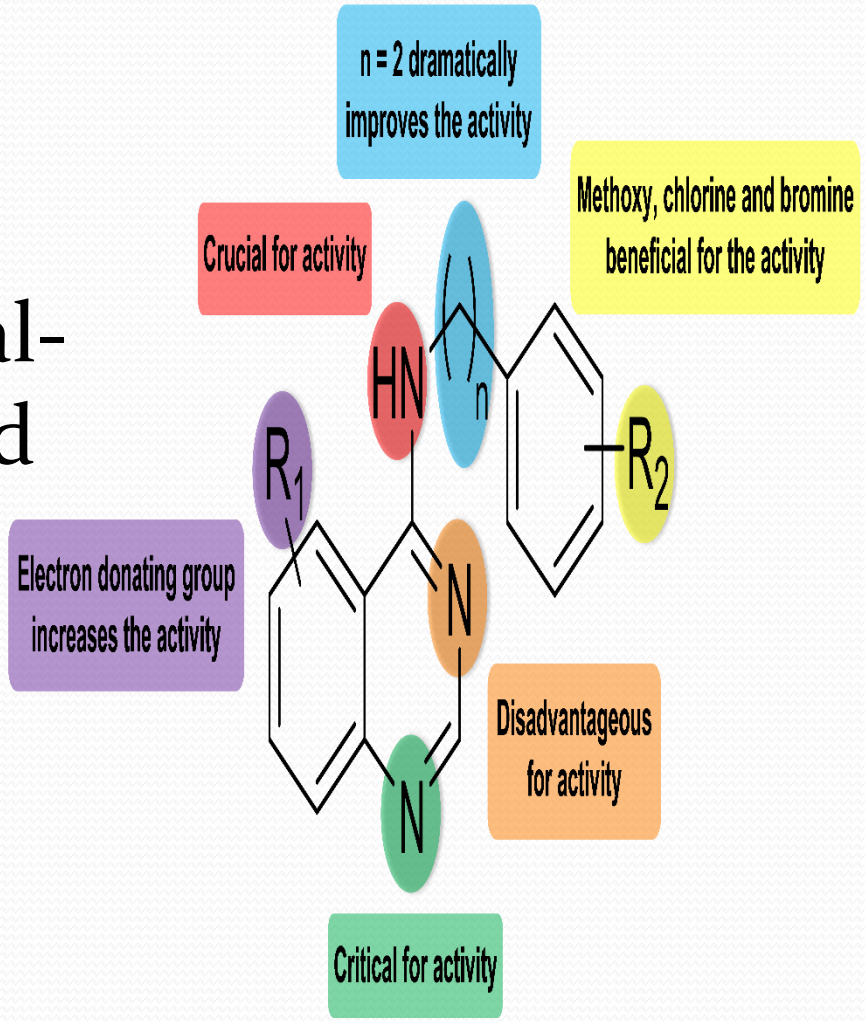
Cholinergic agonists

Requirements for cholinergic agonists

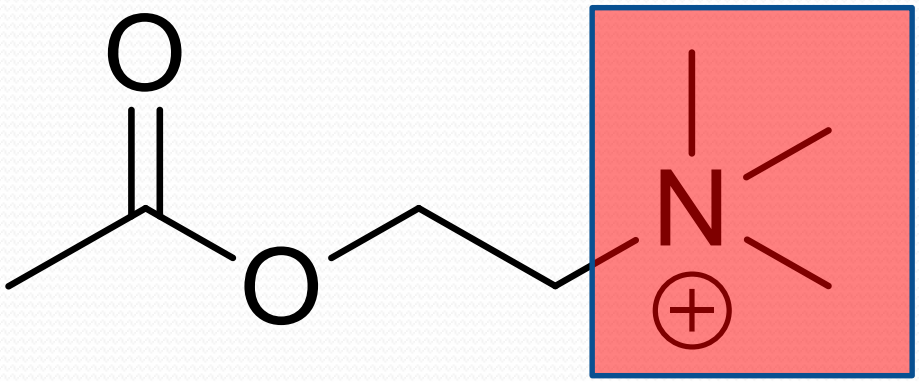
- Stability to stomach acids and esterases
- Selectivity for cholinergic receptors
- Selectivity between muscarinic and nicotinic receptors
- Knowledge of binding site
- SAR for acetylcholine

Acetylcholine: structure, structure–activity relationships, and receptor binding

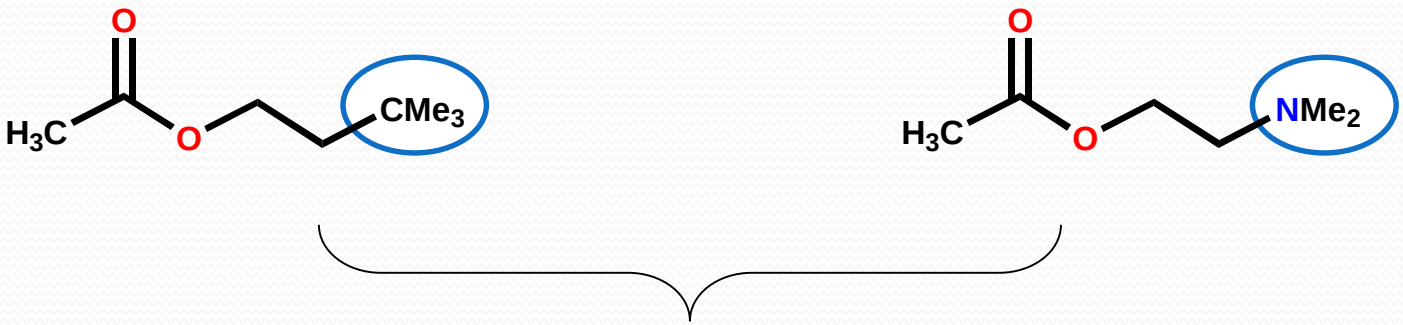
Structure-Activity Relationship (SAR) is an approach designed to find relationships between chemical structure (or structural-related properties) and biological activity (or target property) of studied compounds



SAR for acetylcholine

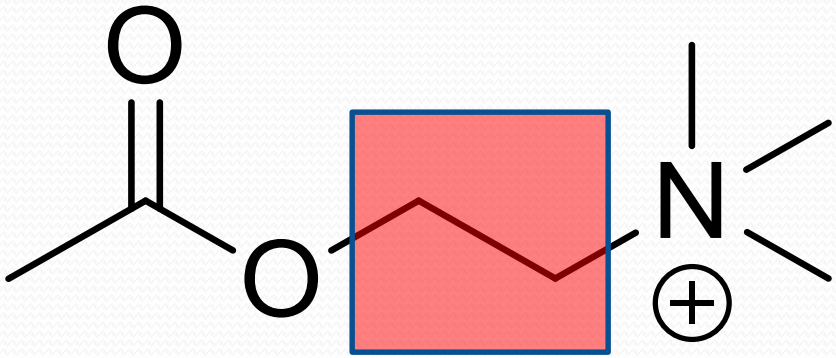


- Quaternary nitrogen (positively charged nitrogen atom) is essential to activity.
- Replacing it with a neutral carbon atom eliminates activity.

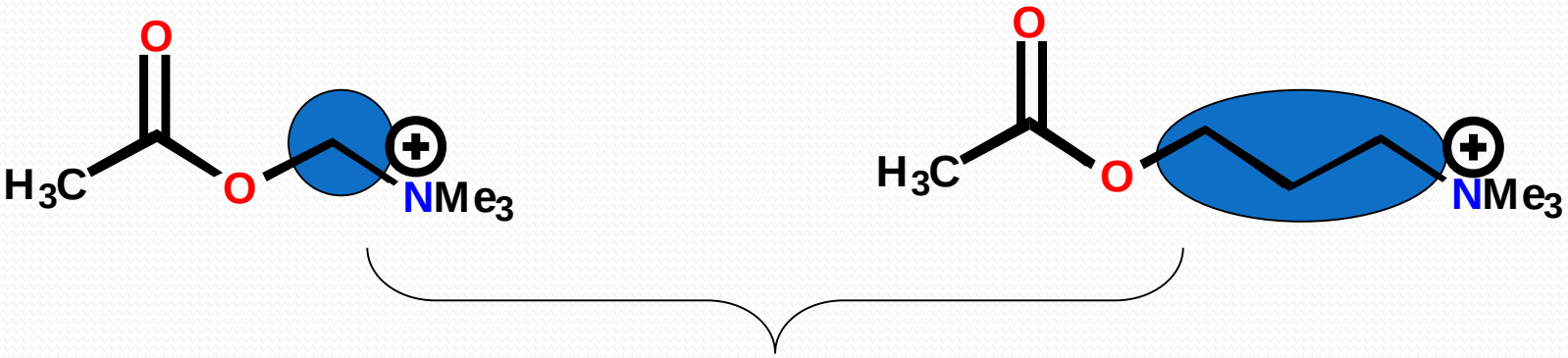


Lower the activity

SAR for acetylcholine

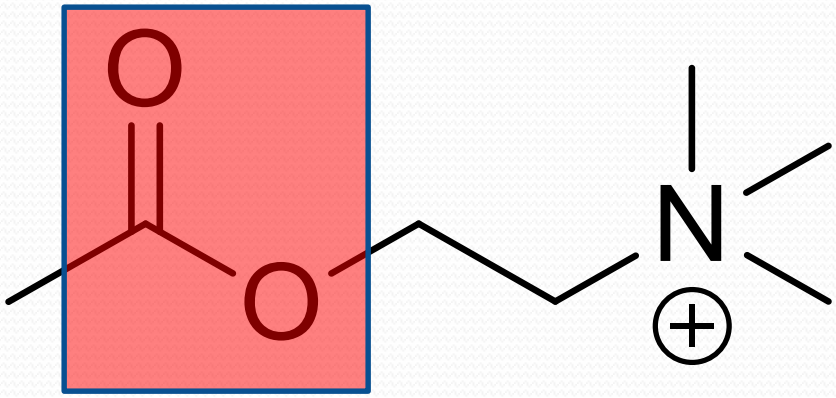


- Distance from quaternary nitrogen to ester is important
- Ethylene bridge must be retained

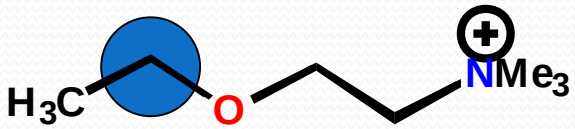


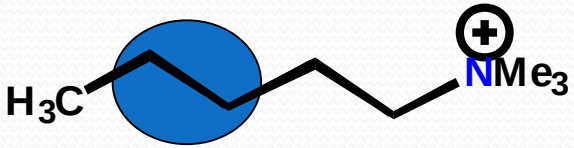
Lower the activity

SAR for acetylcholine



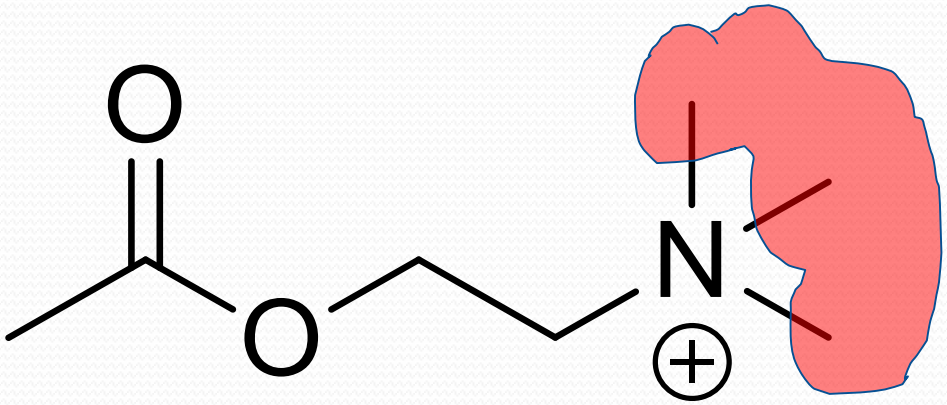
Ester is important



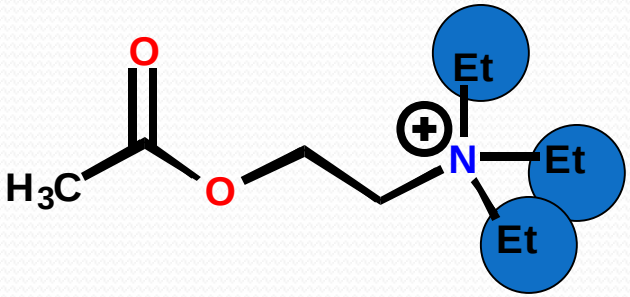


Lower the activity

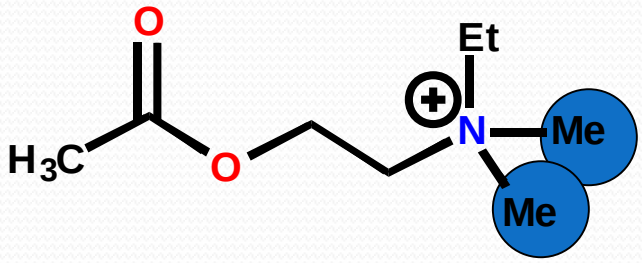
SAR for acetylcholine



Minimum of two methyl groups on quaternary nitrogen

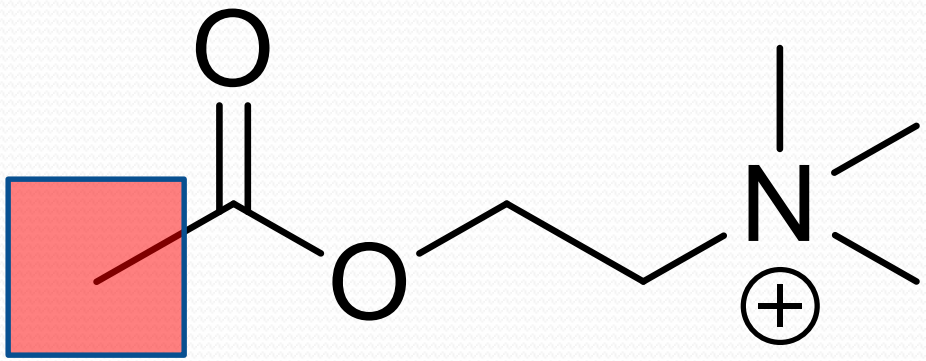


Lower activity

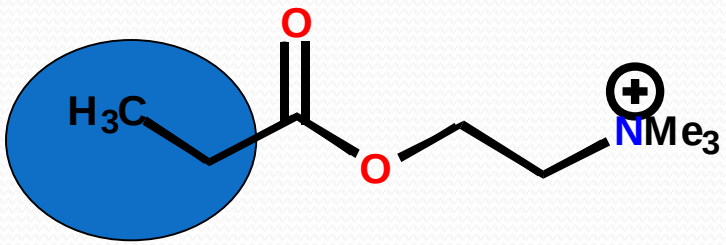


Active

SAR for acetylcholine

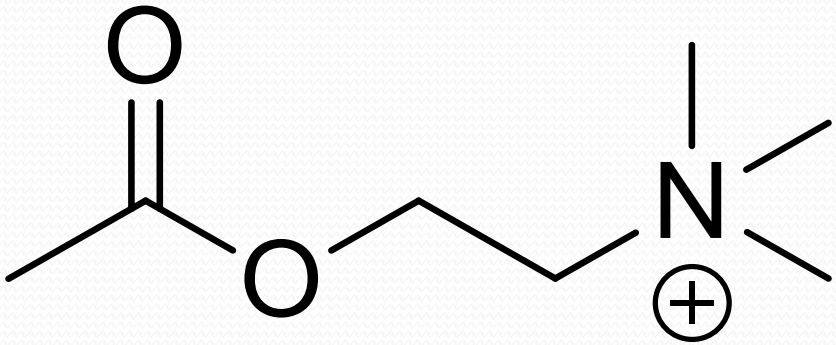


Methyl group of acetoxy group cannot be extended



Much lower activity

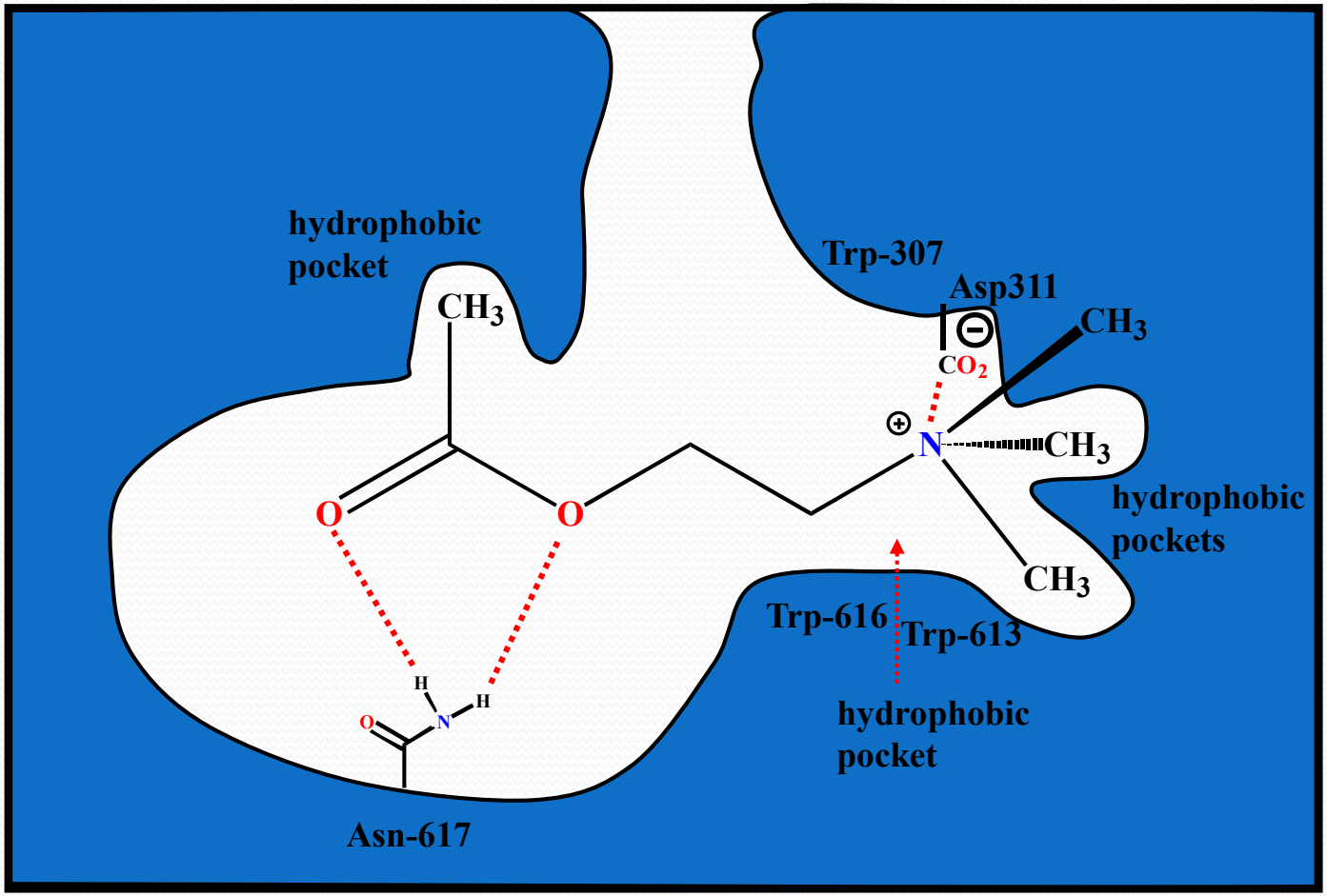
SAR for acetylcholine



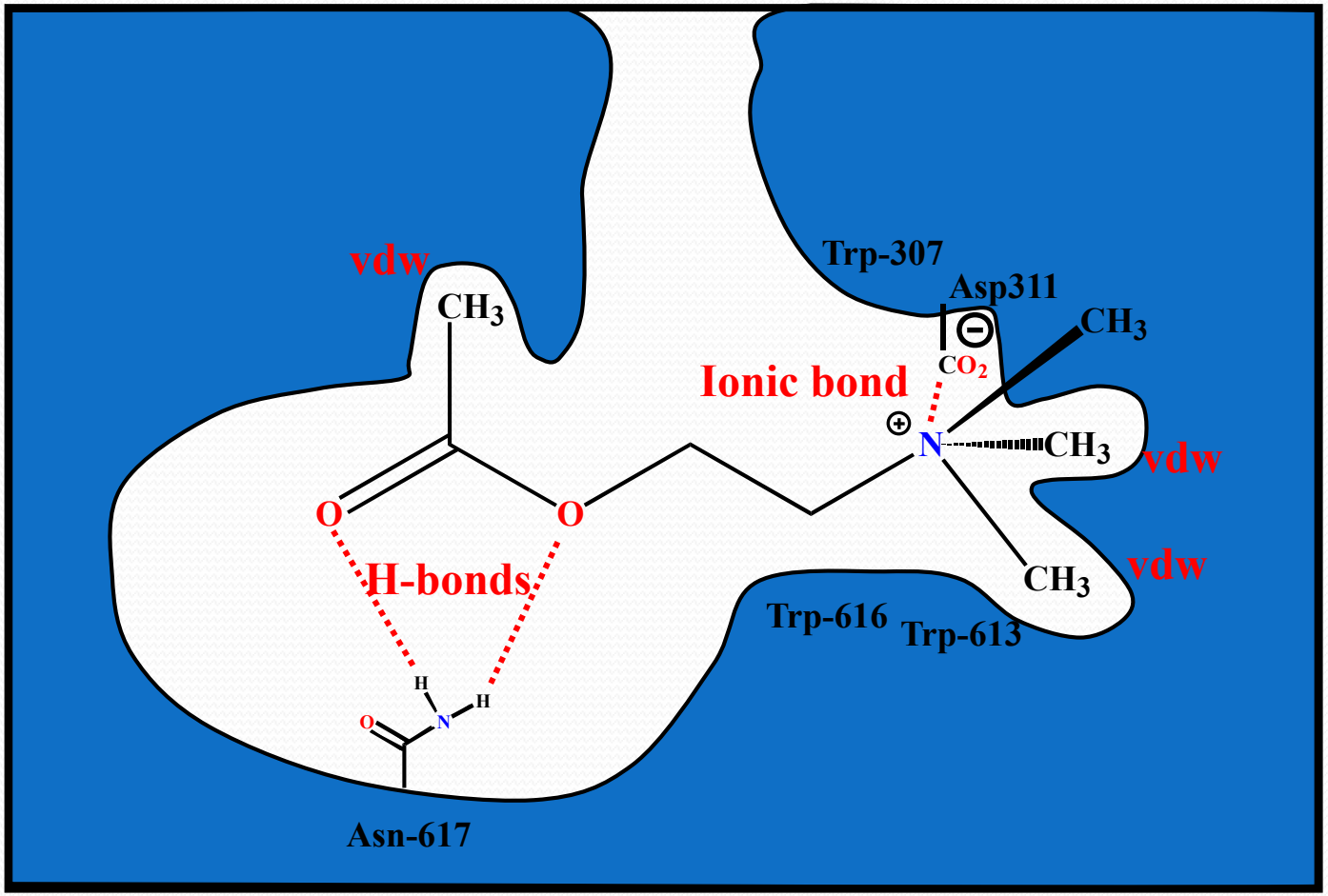
Conclusions:

- **Tight fit between Ach and binding site**
- **Methyl groups fit into small hydrophobic pockets**
- **Ester interacting by H-bonding**
- **Quaternary nitrogen interacting by ionic bonding**

Binding site (muscarinic)



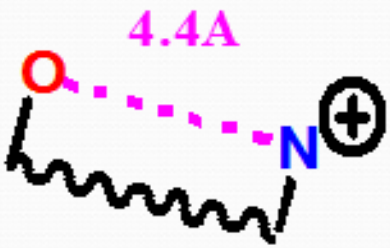
Binding site (muscarinic)



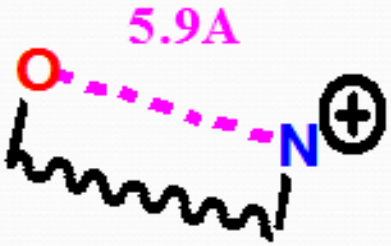
- Study have shown that the distance between the ester group and the quaternary nitrogen is important for binding.
- This distance differ for the muscarinic and the nicotinic receptors.

Active conformation of acetylcholine

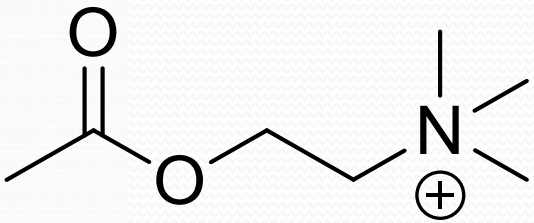
Defines separation of ester and N



Muscarinic receptor



Nicotinic receptor



Uses of cholinergic agonists

Nicotinic selective agonists

- Treatment of myasthenia gravis

  - Skeletal muscle causing weakness

Muscarinic selective agonists

- Treatment of glaucoma
- Treatment of certain heart defects. Decreases heart muscle activity and decreases heart rate

Design of cholinergic agonists

- Ethylene group control selectivity
- Acyloxy group controls stability

Rationale

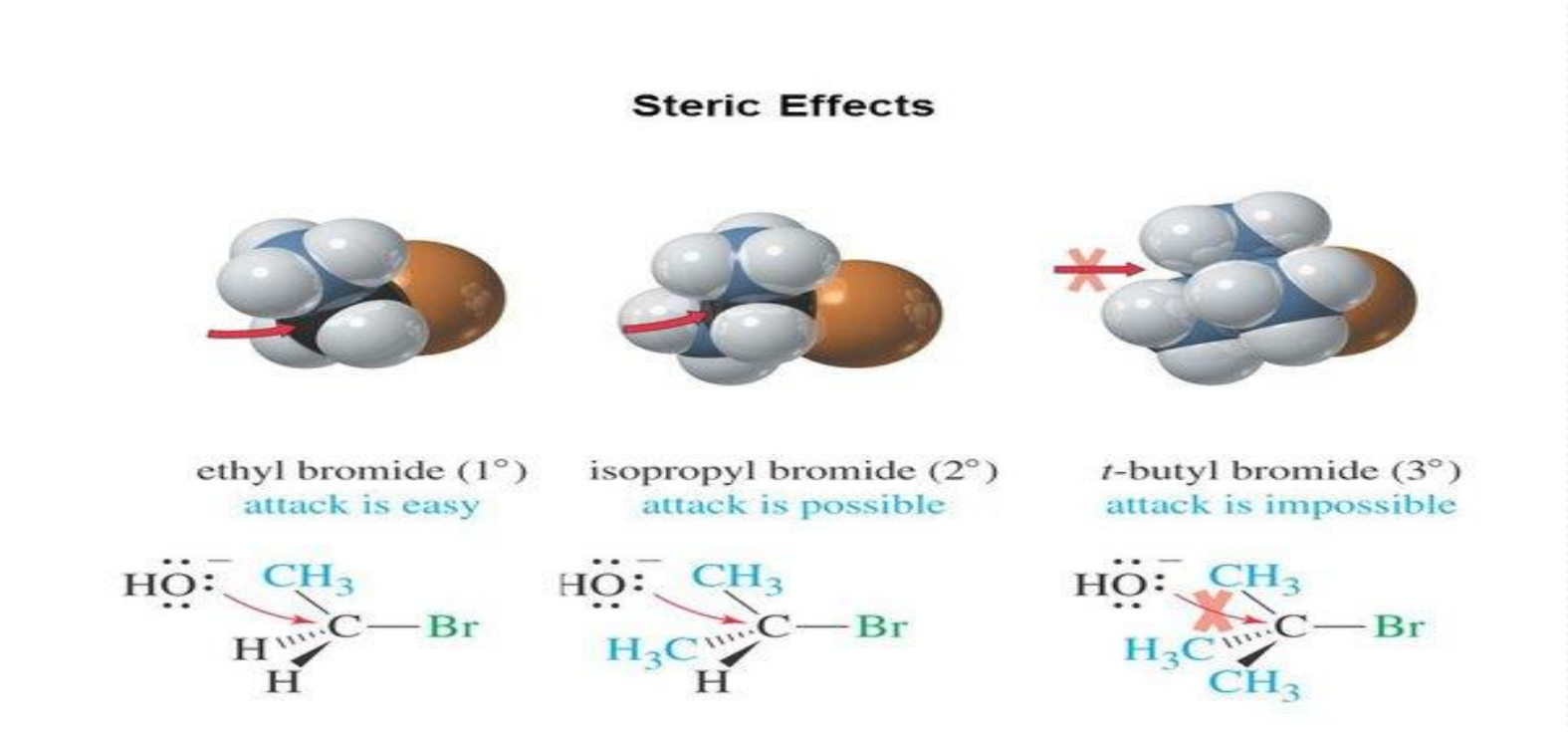
- Shields protect ester from nucleophiles and enzymes
- Shield size is important
- Must be large enough to hinder hydrolysis
- Must be small enough to fit binding site

Design of acetylcholine analogues

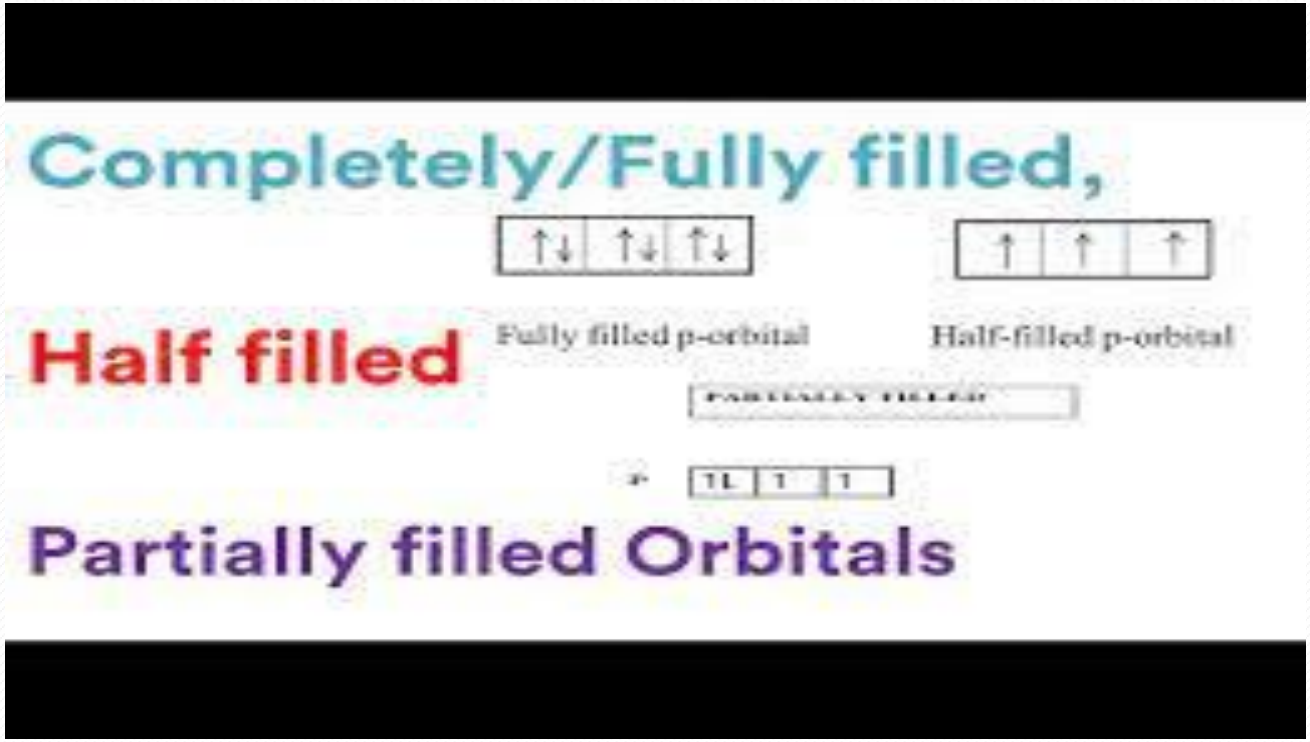
There are two possible approaches to tackling the inherent instability of acetylcholine:

- Steric shields and
- Electronic stabilization.

Steric shielding refers to the effect of the size and shape of a molecule on the electron density of its substituents. Essentially, larger or more complex groups of atoms in a molecule can "shield" certain regions of the molecule from the influence of other atoms or groups of atoms. This can affect the reactivity of a molecule and its interactions with other molecules.

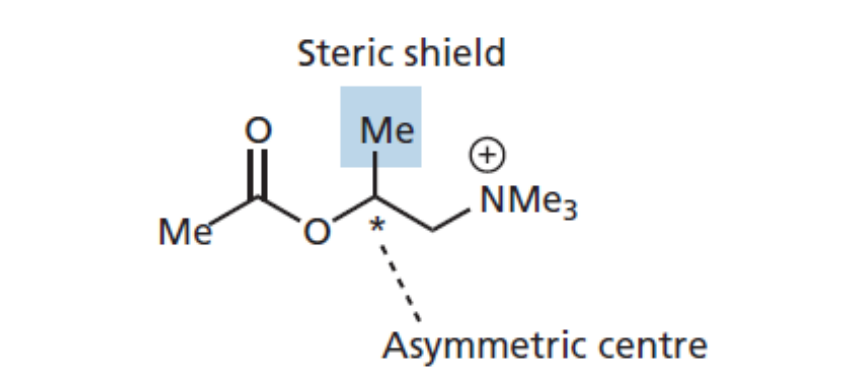


A stable electron configuration is when an atom has a filled or nearly filled outermost electron shell. This means that the atom is not very reactive and is unlikely to form chemical bonds with other atoms



Steric shields

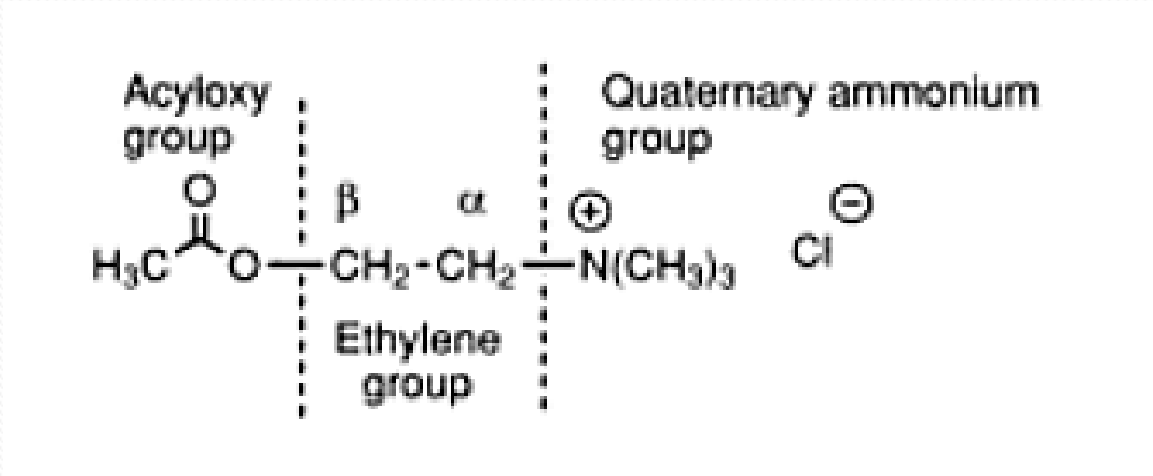
- An extra methyl group placed on the ethylene bridge as a steric shield → protect the carbonyl group.
- The shield hinders the approach of any potential nucleophile and also hinders binding to esterase enzymes, thus slowing down chemical and enzymatic hydrolysis.
- As a result, methacholine is three times more stable to hydrolysis than acetylcholine.



Methacholine (racemic mixture)

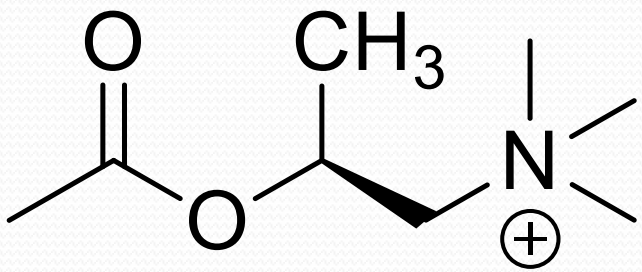
*Hydrolysis by AChE is more affected by substitutions on the β- than the α-carbon.

The hydrolysis rate of racemic acetyl β -methyl acetylcholine is about 50% of that of Ach.



Acetylcholine analogues

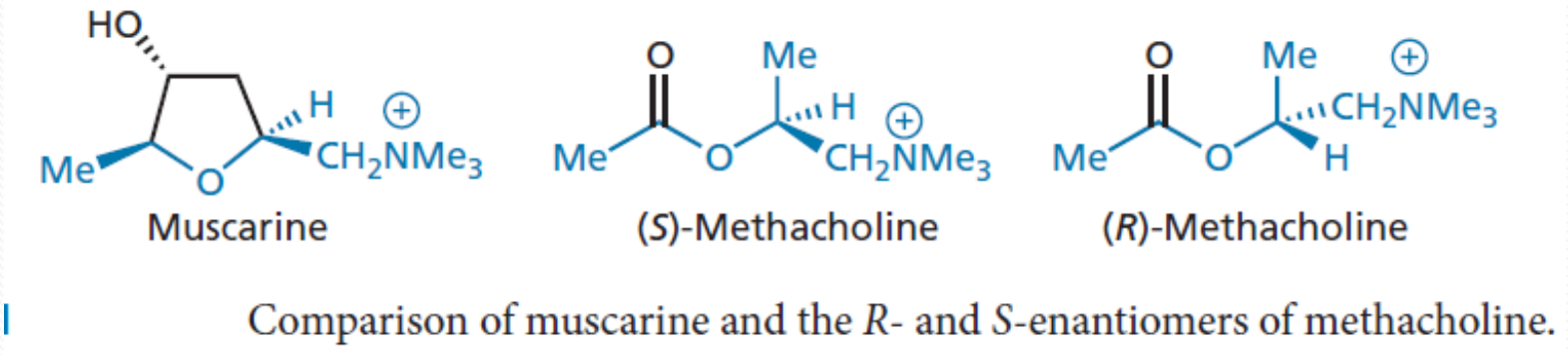
- To overcome the instability of Ach:
  - Steric shield: add large group to change the conformation of Ach:



S-methacoline

  - 3X more stable than Ach.
  - More selective on muscarinic over nicotinic receptors.
  - *S*-enantiomer is more active than the *R*-enantiomer

- Selectivity of methacholine for the muscarinic receptor?

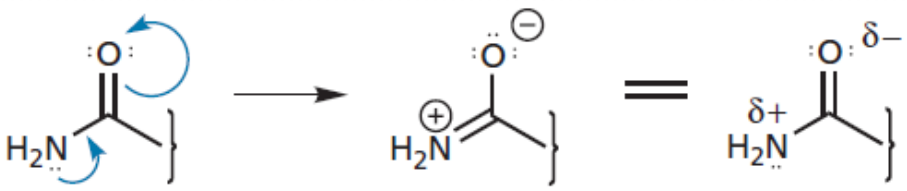


Comparison of muscarine and the *R*- and *S*-enantiomers of methacholine.

Design of cholinergic agonists

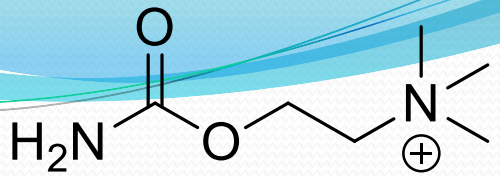
Use of electronic factors

- The acyl methyl group has been replaced by NH2 which means that the ester has been replaced by a urethane or carbamate group.
- This functional group is more resistant to hydrolysis because the lone pair of electrons on nitrogen can interact with the neighboring carbonyl group and lower its electrophilic character
- Replace ester with urethane (Carbamate) group will stabilises the carbonyl group
- The inclusion of the electron-donating amino group greatly increases chemical and enzymatic stability.



Resonance structures of carbachol.

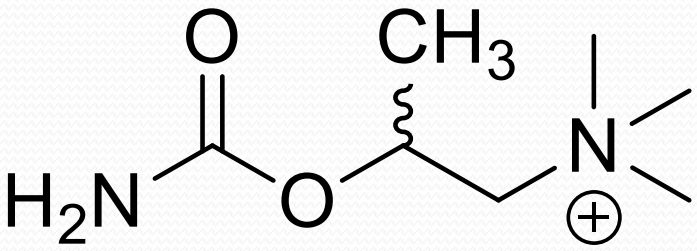
Acetylcholine analogues



Carbacol

- Carbamate more stable ester toward hydrolysis
- NH_2 and CH_3 are equal sizes. Both fit the hydrophobic pocket
- Long acting cholinergic agonist.
- Can be administered **orally**.
- Not selective…… > Just used topically in glaucoma.
- It is used clinically for the treatment of glaucoma where it can be applied locally thus avoiding the problems 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 the eye muscles to contract and allow drainage, thus relieving the pressure.

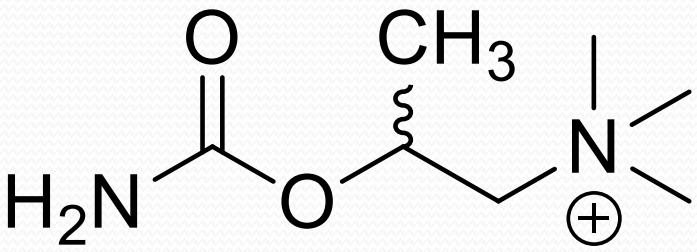
Acetylcholine analogues



Bethanechol

- More stable.
- More selective on muscarinic receptor.
- Used to stimulate GIT and urinary bladder after surgery.

Acetylcholine analogues



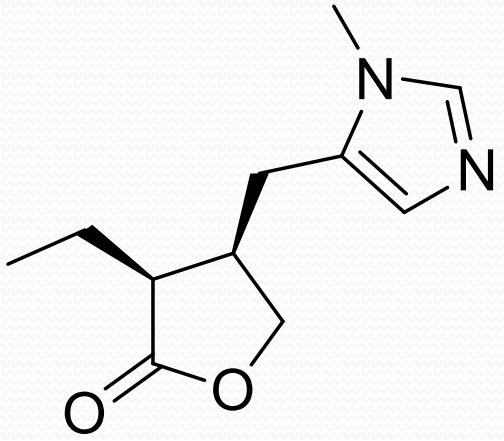
Bethanechol

- is inactivated more slowly by AChE in vivo than is methacholine.
- It is a carbamyl ester and is expected to have stability in aqueous solutions similar to that of carbachol.

Clinical uses for cholinergic agonists: Muscarine Agonists

- Clinical uses:
  - Treatment of glaucoma.
  - Stimulate GIT and UT after surgery.
  - In some heart defects.

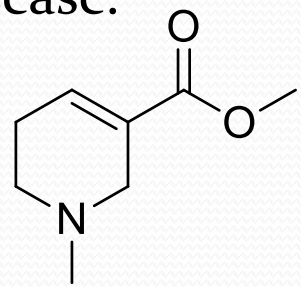
Pilocarpine



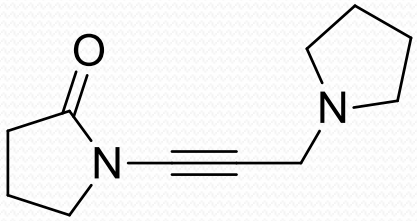
- An alkaloids from Pilocarpus shrubs.
- Used in glaucoma.
- Topically only
- It is a plant derived alkaloid whose structure does not match the established SAR but still acts like an cholinomimetic
- adopt a conformation having the correct pharmacophore for the muscarine receptor; i.e. a separation between nitrogen and oxygen of 4.4 A.
- It is not selective to muscarine.
- It can penetrate the eye well following topical application

Agonists

- Pilocarpine is also being considered for the treatment of Alzheimer's disease, as are other muscarinic agonists such as oxotremorine and various arecoline analogues .
- At present, anticholinesterases are used clinically for the treatment of this disease
- Oxotremorine and Arecoline
  - Act on the muscarinic receptors in brain.
  - Used in Alzheimer's disease.



Arecoline



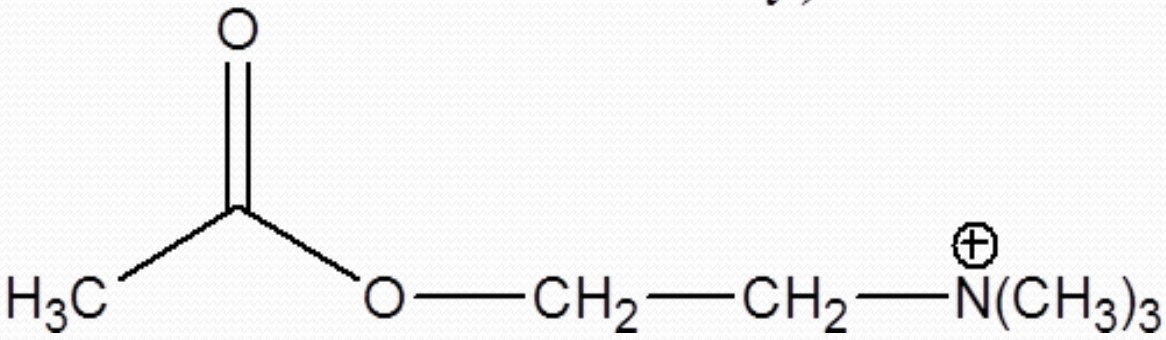
Oxotremorine

Acetylcholine

Acyloxy group (modify stability)

Ethylene group (controls selectivity)

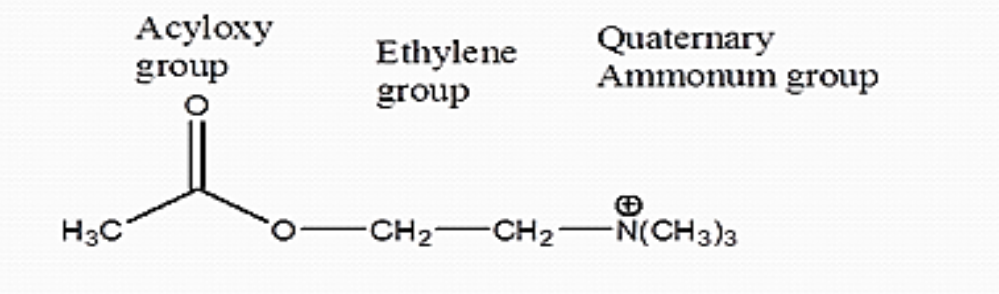
Quaternary Ammonum group (maintains activity)



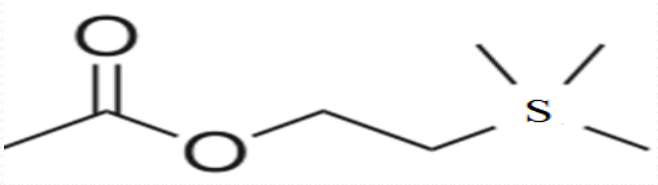
SAR of cholinergics as Muscarinic agonist

Simple changes to Ach hopefully design derivatives to improve stability and cell penetration.

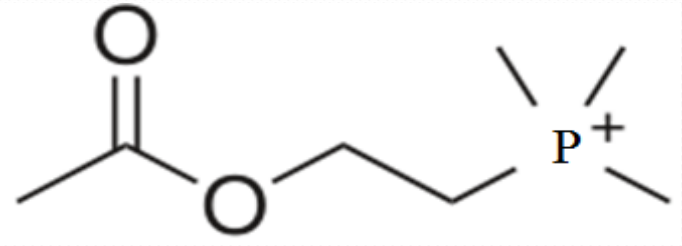
1. Modification of quaternary Ammonium group

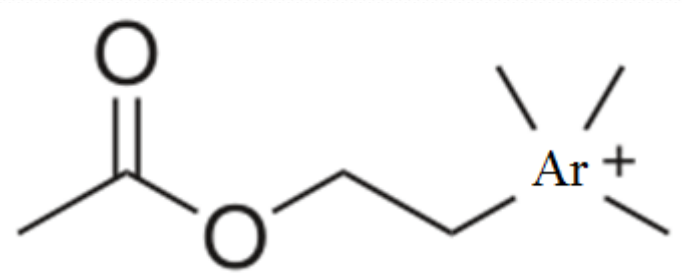


Replacement with sulphur removes the +ve charge and causes reduced activity



Replacement with Arsenic or Phosphorous maintains + charge but still reduces activity



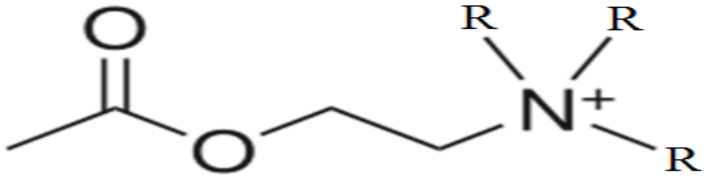


Conclusion

1) positive charge needed

2) Positive charge should be on Nitrogen only

3) Charge on Nitrogen only possible when it bonded to 4 atoms ie quaternary form needed



If R = methyl,(CH3) --> active

If R = ethyl (C2H5) --> antagonist!

If R = propyl (C3H9) and higher alkyls --> inactive

If only one of the R = ethyl or propyl ⟶ active but less potent than Ach

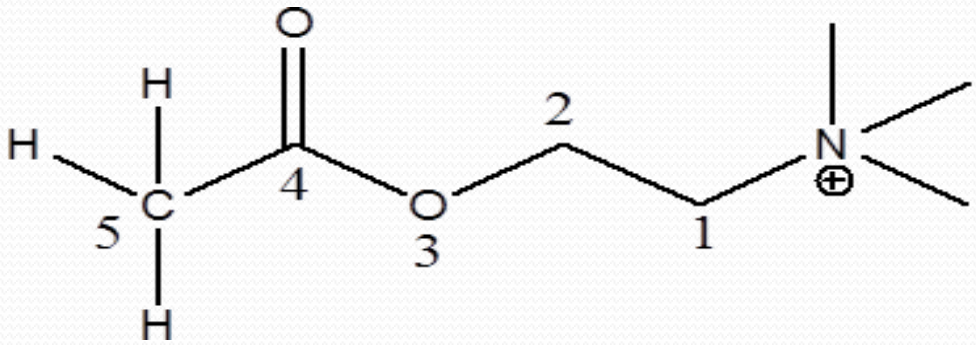
If any or all R = H ⟶ activity goes on decreasing

2. Change in the ethylene group

A “rule of five” idea states that there should be no more than 5 atoms between the Nitrogen and the terminal Hydrogen

As the chain length increased from two, activity

is rapidly lost. This tells us about the relative size of binding site

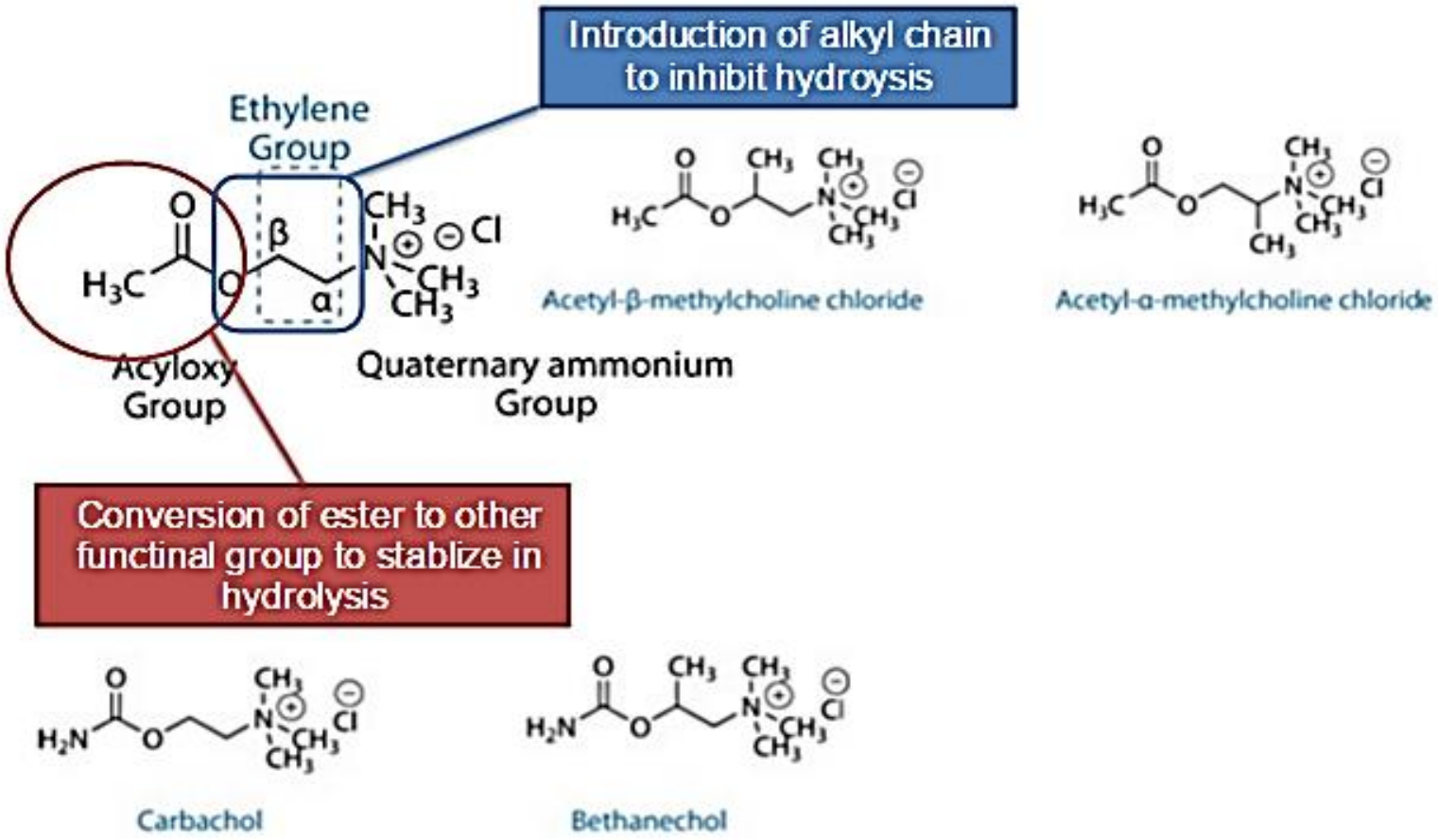


*For maximal muscarinic activity, the quaternary ammonium group should be followed by a chain of five atoms; this has been referred to as the *five-atom rule.*

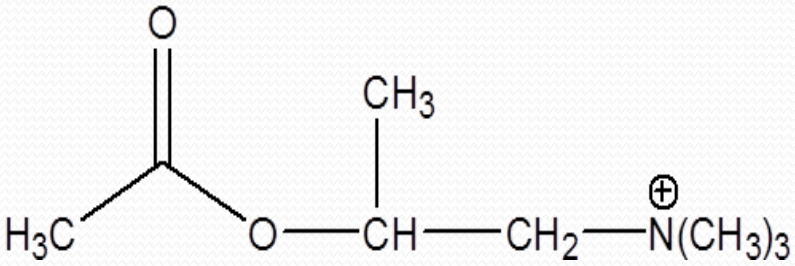
*Shortening or lengthening the chain of atoms that separates the ester group from the onium moiety reduces muscarinic activity.

- An α- substitution on the choline moiety decreases both nicotinic and muscarinic activity, but muscarinic activity is decreased to a greater extent.
- Substitution on the β-carbon nicotinic activity is decreased to a greater degree

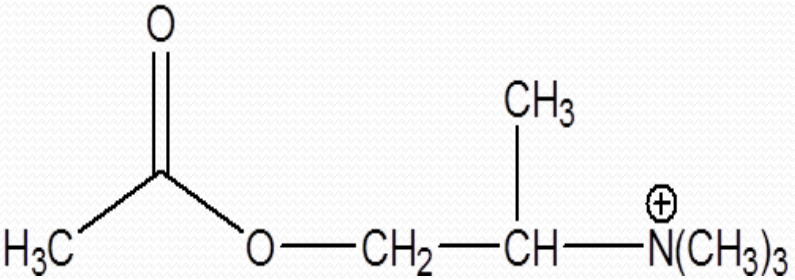
Ach Derivatives



Methacholine



Methyl group in Beta carbon
As potent as Ach
Selective to Muscarinic receptor
Used clinically

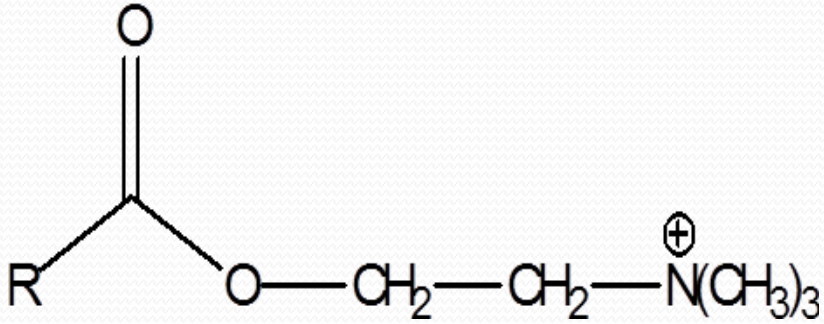


Methyl group in alpha carbon
Not As potent as Ach
Selective to Nicotinic receptor
Not Used clinically

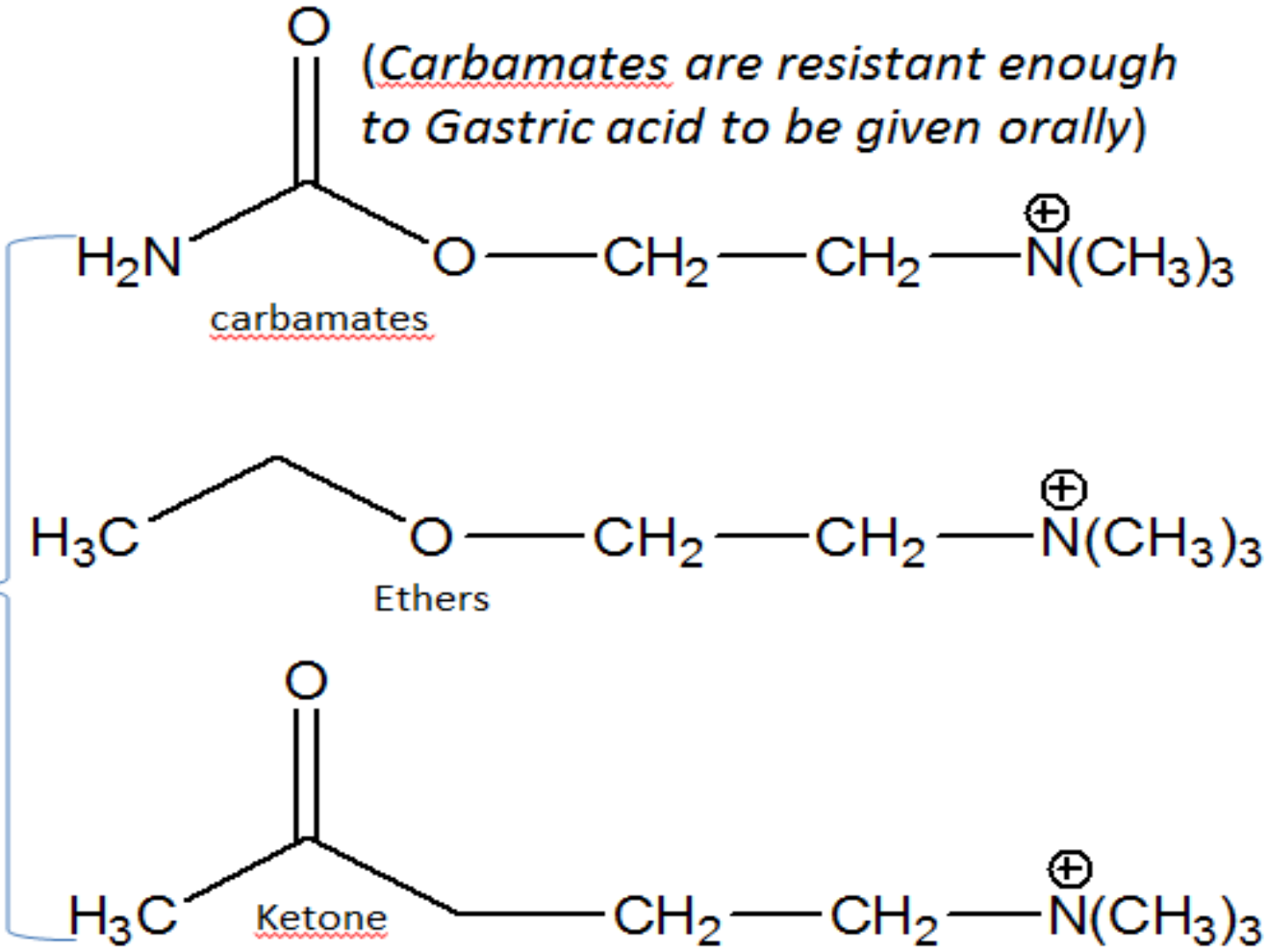
3. Modifications to the Acetoxy group

Substituting the Acetyl with higher homologous group such as propionyl or butyryl reduces activity. The ester group isn't mandatory as quanternary amine group but an oxygen atom is required in this region

Since ester group makes it liable to hydrolysis, alternate groups were included and found that replacing the ester with carbamate, ether or ketone function resists hydrolysis while maintaining activity.



Modification to reduce hydrolysis



Structure Activity Relationships

The structure of ACh divided into three groups;

1. The onium group
2. The ester function
3. Choline moiety

Structure Activity Relationships

1. The onium group:

- The onium group is essential for intrinsic activity and contributes to the affinity of the molecule for the receptors.
- Its action as a detecting and directing group.
- there are some exceptions are known (e.g, pilocarpine, arecoline, nicotine and oxotremorene).

Structure Activity Relationships

2. The ester group:

- The ester group in ACh contributes to the binding of the compound to the muscarinic receptor because of hydrogen bond formation with amino acid residues at the receptor site.

3. Choline moiety:

- An α substitution on the choline moiety decreases both nicotinic and muscarinic activity, but muscarinic activity is decreased to a greater extent.

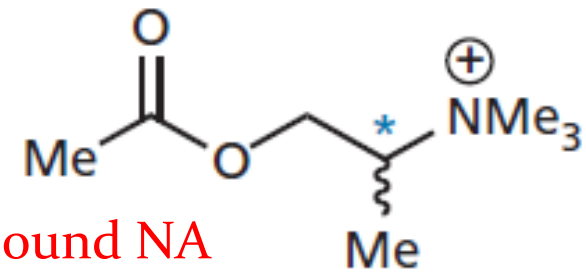
- Nicotinic activity is decreased to a greater degree by substitution on the β carbon→ Therefore, acetyl α-methylcholine, although less potent than ACh, has more nicotinic than muscarinic activity, while acetyl-β-methylcholine (methacholine) exhibits more muscarinic than nicotinic activity.
- Hydrolysis by AChE is more affected by substitutions on the β than the α carbon.

3. Choline moiety:

- For maximal muscarinic activity, the quaternary ammonium group should be followed by a chain of five atoms; this has been referred to as the five-atom rule. Shortening or lengthening the chain of atoms that separates the ester group from the onium moiety reduces muscarinic activity.

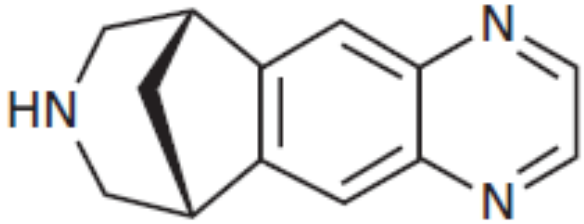
Nicotinic agonists

- Nicotinic agonists are used in the treatment of myasthenia gravis. (an autoimmune disease)
- Selective nicotinic agonist is compound NA (not used clinically)
- Varenicline: partial agonist at nicotinic receptors.



Compound NA

* Asymmetric centre



Varenicline

Examples of selective nicotinic agonists.

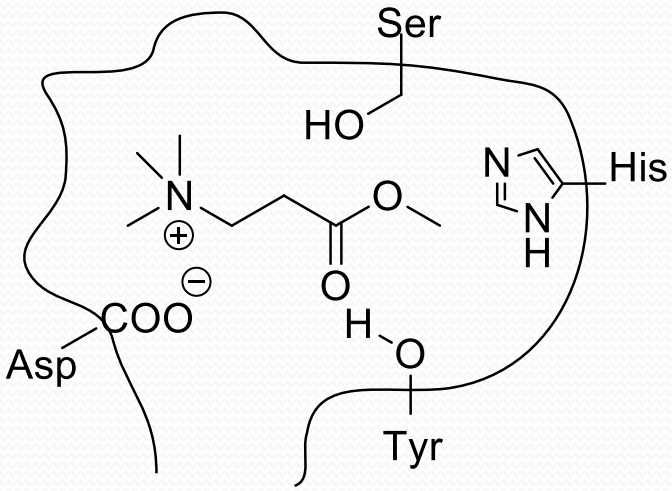
Anticholinesterases and acetylcholinesterase

Anticholinesterase

- Acetyl cholinesterase (AchE) is a enzyme that hydrolyzes Ach into Acetic acid and Choline.
- Anticholinesterase drugs work by inhibiting the enzyme Acetyl cholinesterase which prevents hydrolysis of Ach thus increasing their concentration in the synapse which promotes more Ach action.
- Since they promote Ach activity without binding to any receptor they are also called **indirectly acting cholinergic agonists.**

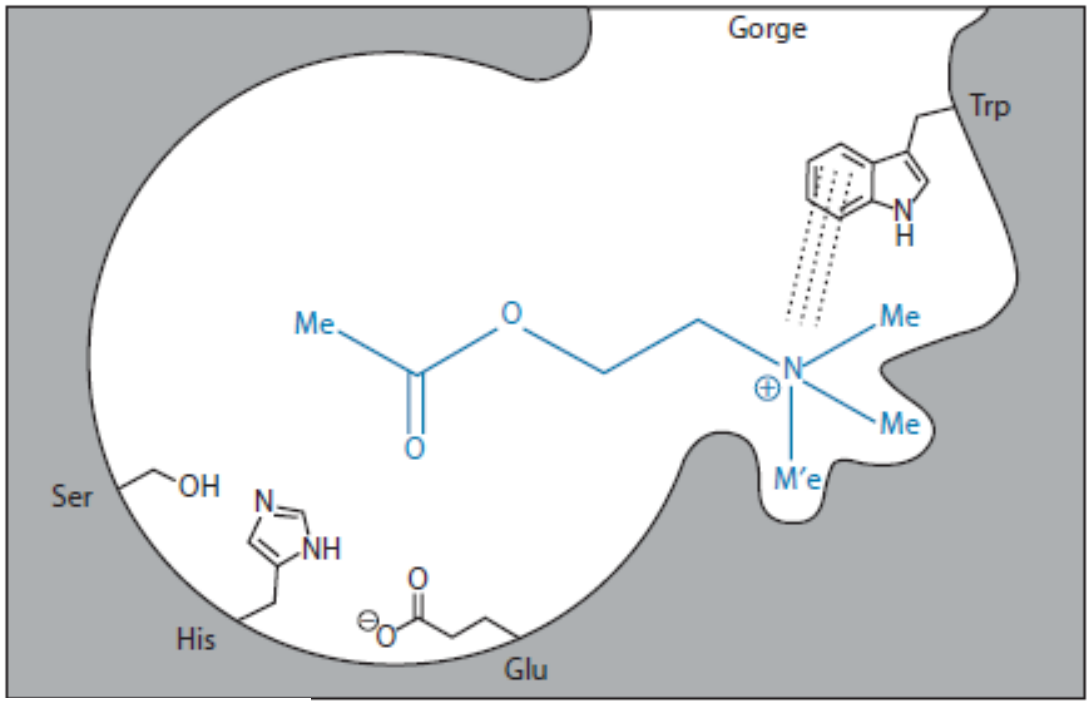
Anticholinesterase agents

- Lead to Ach accumulation… have cholinergic effect.
- The active site for acetylcholinesterase is similar to the binding site for the cholinergic receptor, but also includes a catalytic triad of amino acids—histidine, serine, and glutamate.



Anticholinesterase agents

- Histidine acts as an acid–base catalyst.
- serine acts as a nucleophile during the hydrolytic mechanism.
- Glutamate orientates and activates histidine.



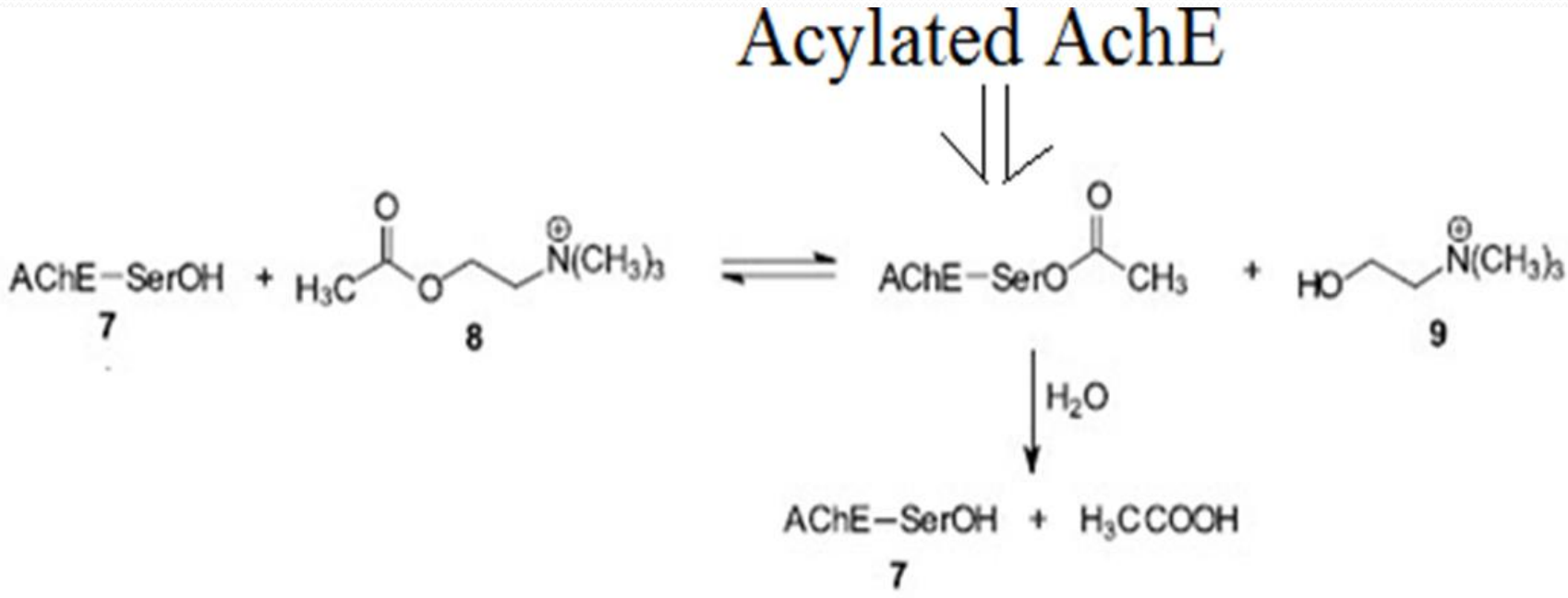
Key amino acid residues within the active site.

Anticholinesterase

- **Applications:**
  - Improve muscle strength in Myasthenia gravis, Glaucoma ,Alzheimer's, Insecticides ,Chemical weapon (serine gas)
- There are two types:
  - ✓ **Reversible** and **Irreversible**
  - ✓ **carbamates** and **organophosphorus agents.**

Theory of AchE inhibitors

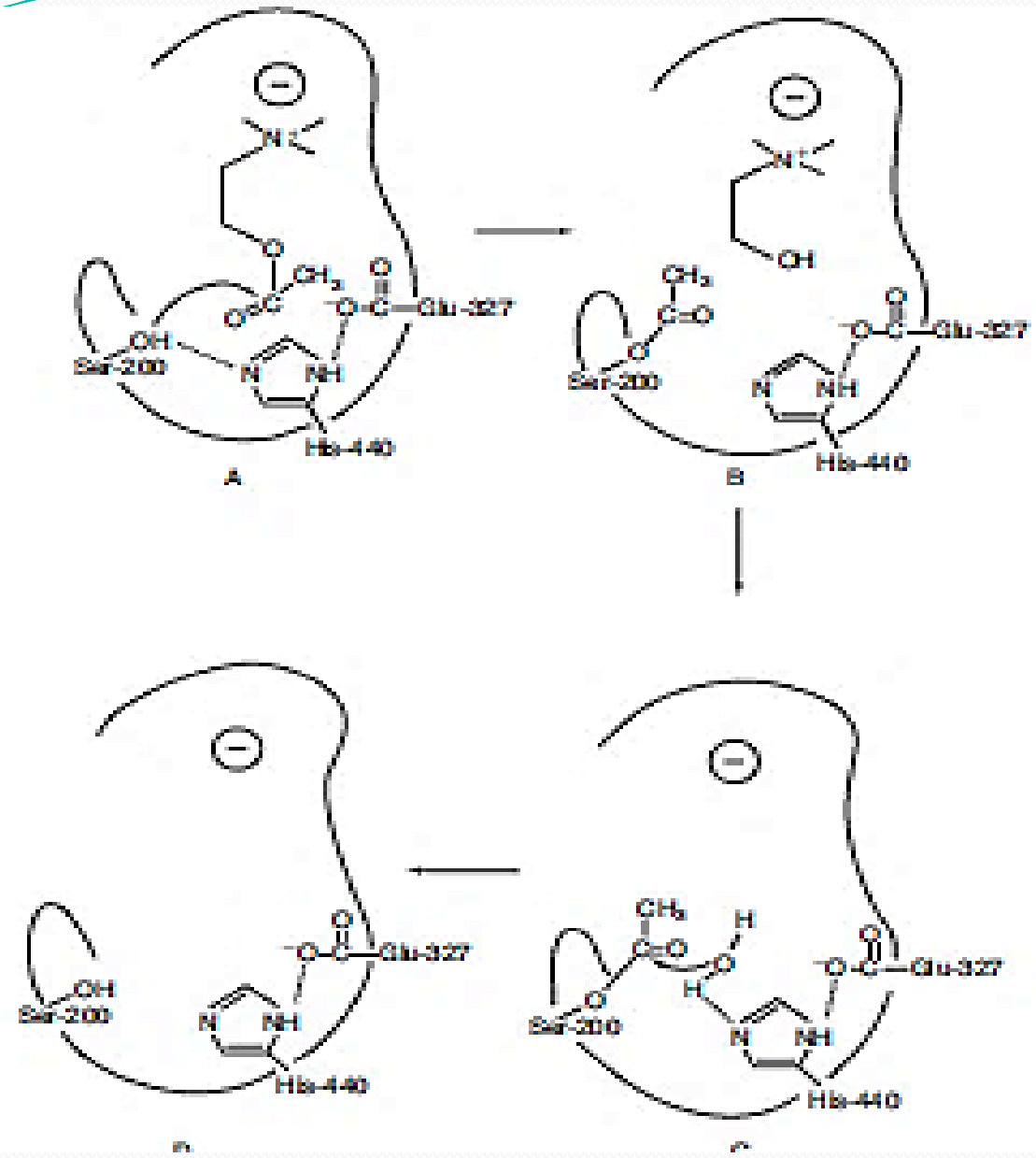
- During hydrolysis of Ach, the AchE gets acylated.
- It needs to be hydrolyzed by water to be regenerated in free from or else it can't function again.



- AChE attacks the ester substrate through a serine hydroxyl, forming a covalent acyl-enzyme complex.
- The serine is activated as a nucleophile to attack the carbonyl carbon of ACh.
- Choline is released, leaving the acetylated serine residue on the enzyme.

- intermediate is cleaved by a general base catalysis mechanism to regenerate the free enzyme.
- If instead of acetyl group there is carbamate group then hydrolysis will be resisted.
- The AchE which is not hydrolyzed cannot be used again.

  → Thus goal of AchE inhibitor is to provide such hydrolysis resistant functional group such **as carbamates or phosphate ester**.



Mechanism of hydrolysis of ACh by AChE.

A. ACh–AChE reversible complex.

B. Acetylation of esteratic site.

C. General base-catalyzed hydrolysis of acetylated enzyme.

D. Free enzyme.

Types of cholinesterase enzymes

- Acetylcholinesterase
  - Located in synapses
  - Substrate selectivity:
    - Ach
- Plasma cholinesterase (Butyrylcholineesterase, BuChE):
  - Located in plasma (non-neuronal)
  - Substrate selectivity:
    - Ach
    - Succinylcholine
    - Dietary esters and drug molecules in the blood.

Reversible Anticholinesterase

These are compounds can act by two ways:

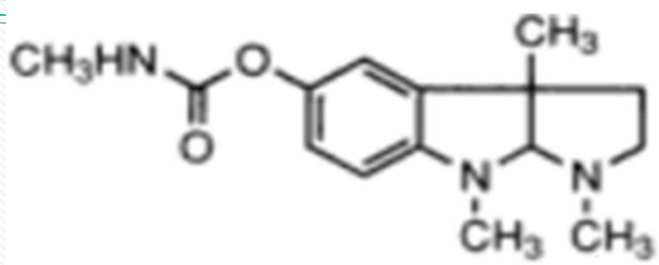
A) They bind but don't react with AchE with greater affinity than Ach like Ach does

B) These compounds that bind and react with AchE to form acylate AchE which is more stable form but still capable of being easily hydrolyzed

- Reversible means that they inhibit AchE for short time (only few mins)
- These compounds have more therapeutic uses than irreversible ones.

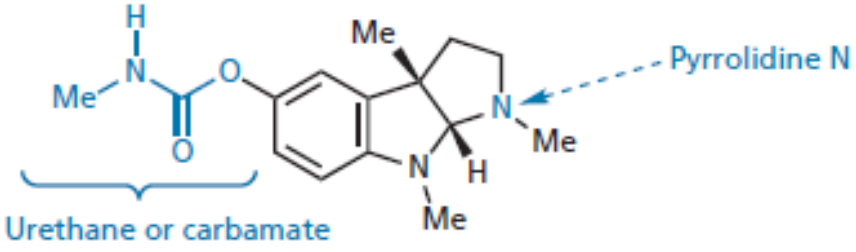
Physostigmine:

It is an alkaloid type anticholinesterase obtained from the seeds of calabar beans



- It has no charged amine and is more lipophillic and can thus penetrate the blood brain barrier.
- It has very great affinity for AchE but that can only in charged form. Thus there is pH limitation in it's activity. → Inhibition of cholinesterase is greater in acid media.
- Physostigmine is a relatively poor carbamylating agent of AChE and is often considered a reversible inhibitor of the enzyme.

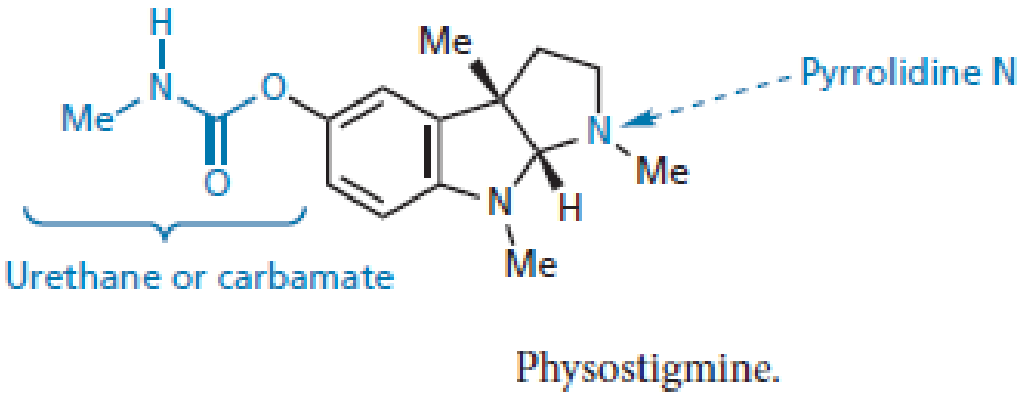
Physostigmine



Physostigmine.

- the positively charged pyrrolidine nitrogen is important because it binds to the anionic binding region of the enzyme.
- The benzene ring may be involved in some extra hydrophobic bonding (a good leaving group)
- Uses : Glaucoma, as an antidote for atropine poisoning (Counter CNS poisoning by atropine and tricyclic depressents)
- MOA – It inhibits AchE by binding and reacting to it and carbamylating it. This carbamylated enzyme is slow to hydrolysis

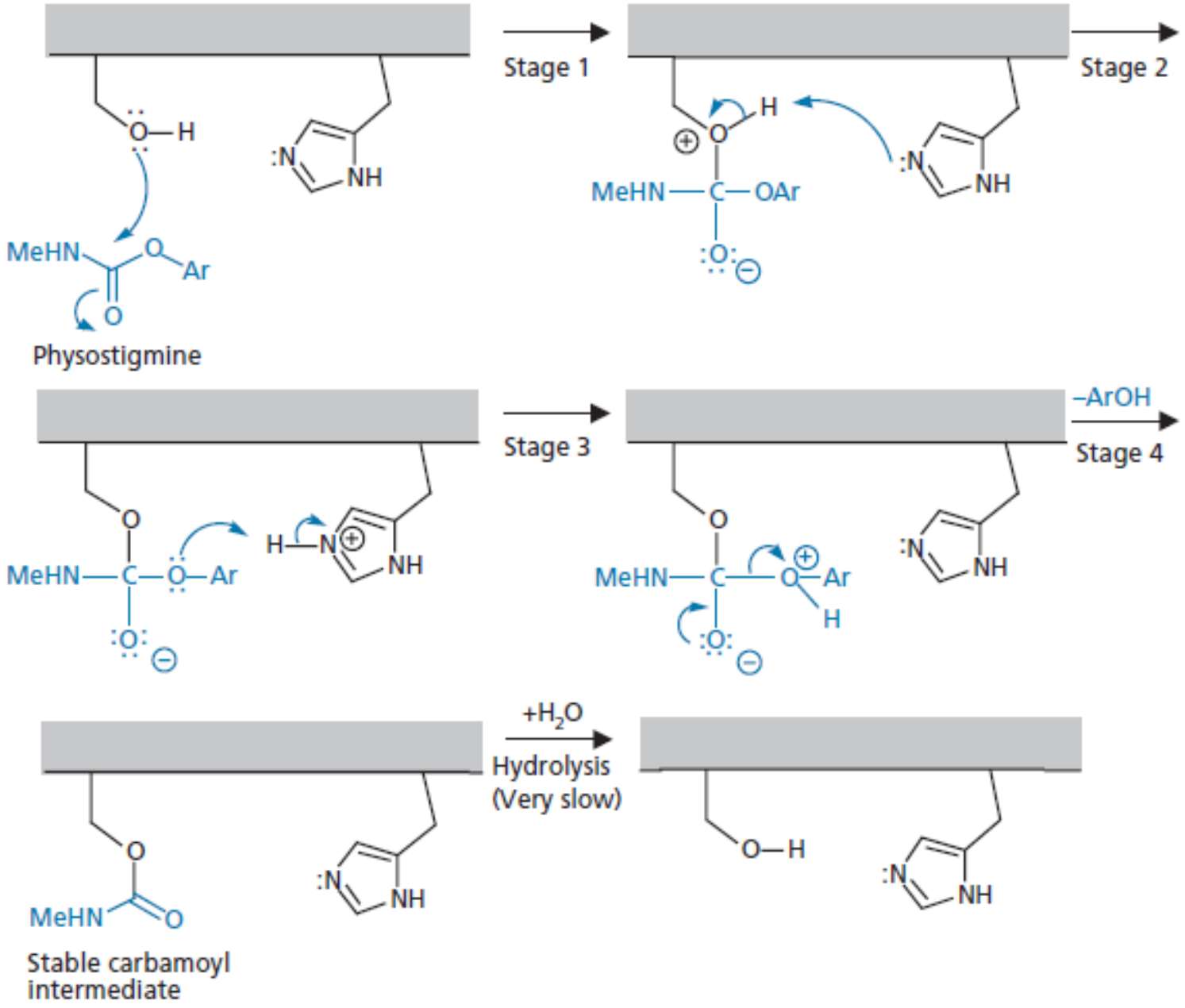
Physostigmine



Physostigmine.

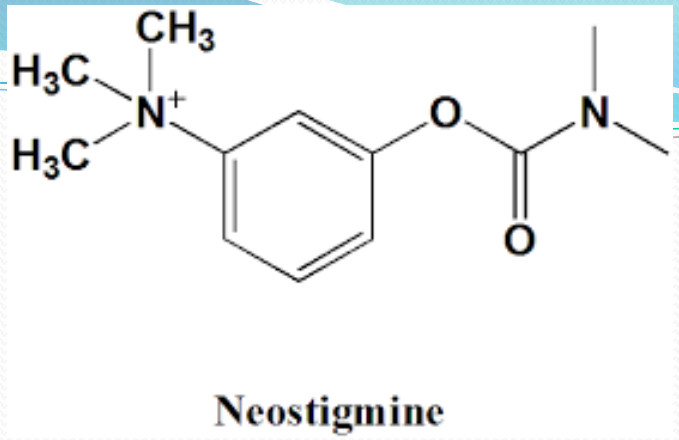
SAR studies of physostigmine demonstrate that:

- the carbamate group is essential to activity;
- the benzene ring is important;
- the pyrrolidine nitrogen is important and is ionized at blood pH.



Mechanism of inhibition by physostigmine (Ar represents the tricyclic system of physostigmine).

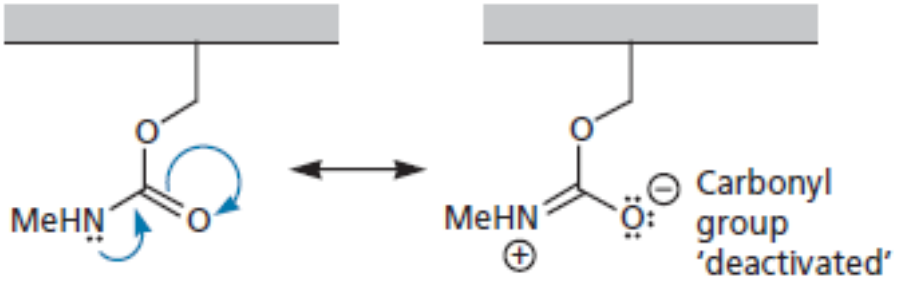
Neostigmine



Neostigmine

- It is a synthetic anticholinesterase based on Physostigmine.
- It resembles the aromatic features of physostigmine and also the distance between the ester and ammonium is same but since it has charge on Nitrogen, it cannot cross the CNS like physostigmine does
- Also its half life is shorter than physostigmine

Neostigmine



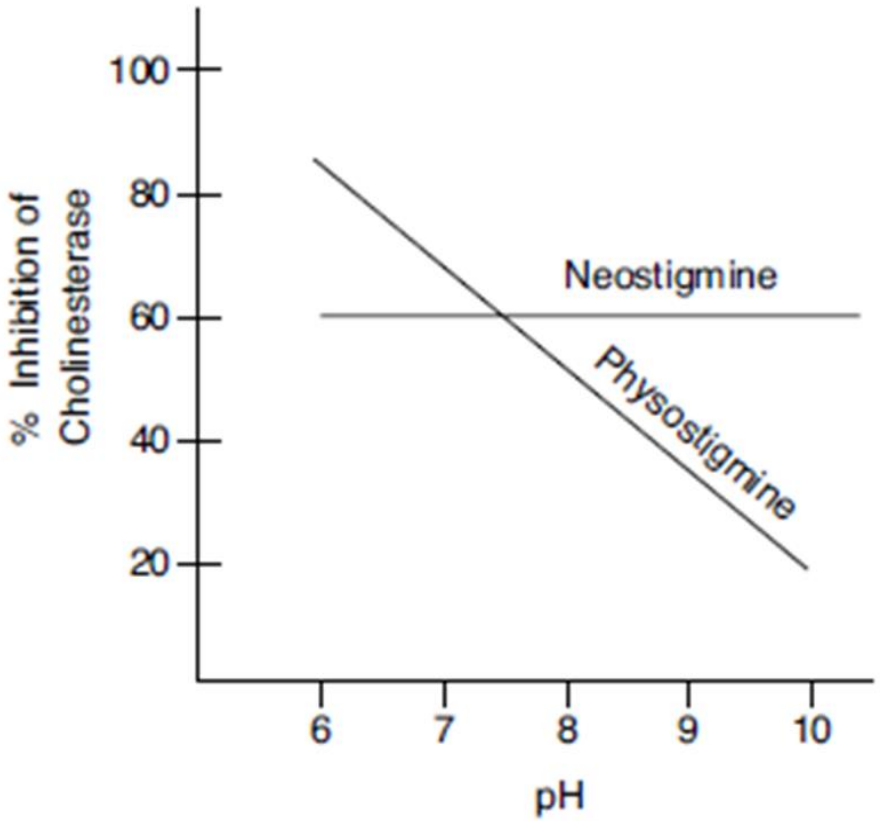
Stabilization of the carbamoyl–enzyme intermediate.

- dimethylcarbamate group

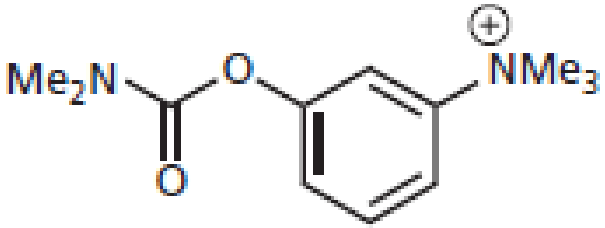
Uses:

- given intravenously to reverse the actions of neuromuscular blockers
- Myasthenia gravis

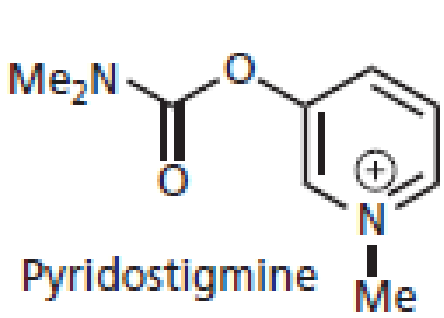
MOA – It inhibits AchE by binding and reacting to it and carbamylating it. This carbamylated enzyme is slow to hydrolysis



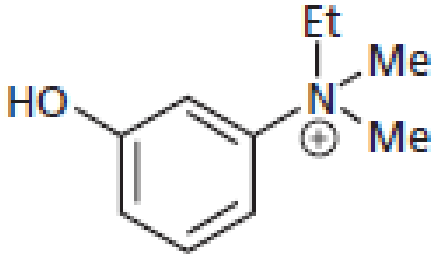
Effect of pH on inhibition of cholinesterase by physostigmine and neostigmine.



Neostigmine



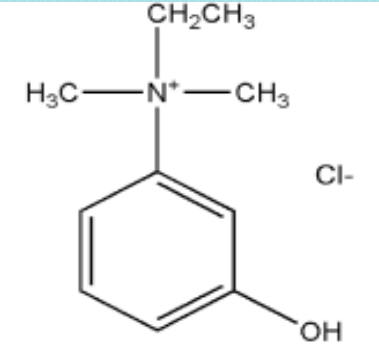
Pyridostigmine



Edrophonium

- Pyridostigmine was one of the drugs used in the chemical cocktail provided to allied troops in Iraq during Operation Desert Shield .
  - Uses: to reverse the actions of neuromuscular blockers, Myasthenia gravis
  - was present to help protect against possible exposure to organophosphate nerve gases.

Edrophonium Chloride



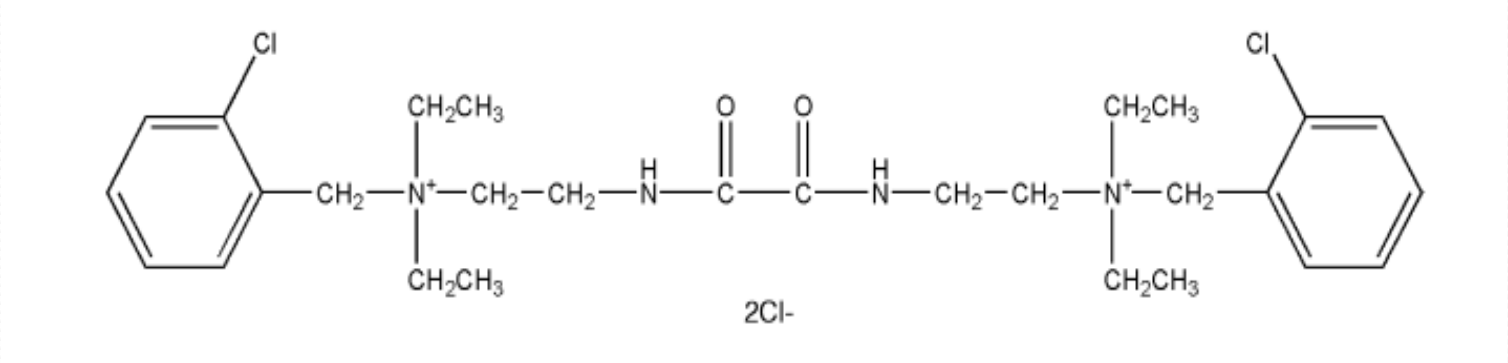
Edrophonium Chloride

- used to reverse neuromuscular blocking
- also used as a treatment of myasthenia gravis.
- rapid onset and shorter duration of action than neostigmine, pyridostigmine, or ambenonium.
- It is a specific anticurare agent and acts within 1 minute to alleviate overdose of d-tubocurarine, dimethyl d - tubocurarine, or gallamine triethiodide.

Ambenonium Chloride.

- treatment of myasthenia gravis in patients who do not respond satisfactorily to neostigmine or pyridostigmine.
- prolonged duration of action.
- Because of its quaternary ammonium structure, ambenonium chloride is absorbed poorly from the GI tract. (fewer side effects)

Ambenonium Chloride.



Ambenonium chloride

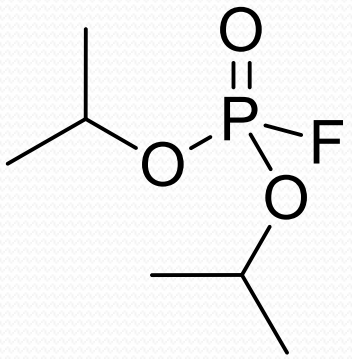
Irreversible Anticholinesterase

- These compounds act by only one way: that bind and react with AchE to AchE with greater affinity than Ach to form acylated enzyme which **strongly resist hydrolysis**
- Irreversible means that they inhibit AchE for very long time(many hours)
- These compounds have **less therapeutic** uses than reversible ones.

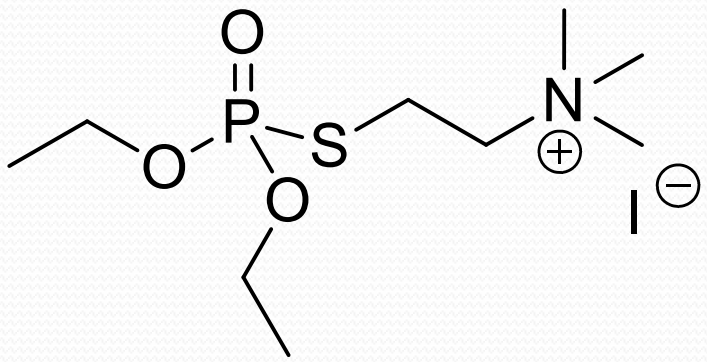
Irreversible Anticholinesterase

- Sarin gas and organophosphate insecticides are based on this concept.
- They cause cholinergic crisis
- Their common structural feature is the presence of **Phosphate ester** bond which strongly resist hydrolysis

Irreversible Acetylcholinesterase inhibitors



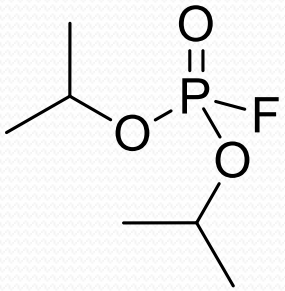
Diisofluorophate
(insecticide)



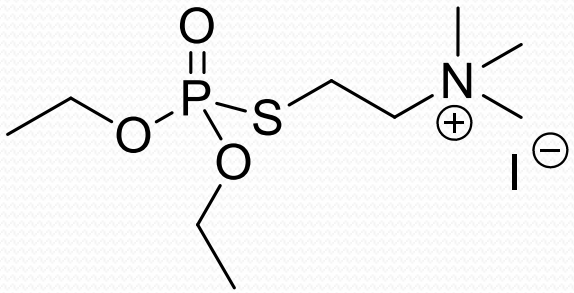
Ecothiophate
(Anti-glaucoma)

- Organophosphates
- Irreversible binding to Cholinesterase active site
- Ecothiopate is used medicinally in the form of eye drops for the treatment of glaucoma
- Ecothiopate slowly hydrolyses from the enzyme over a matter of days.

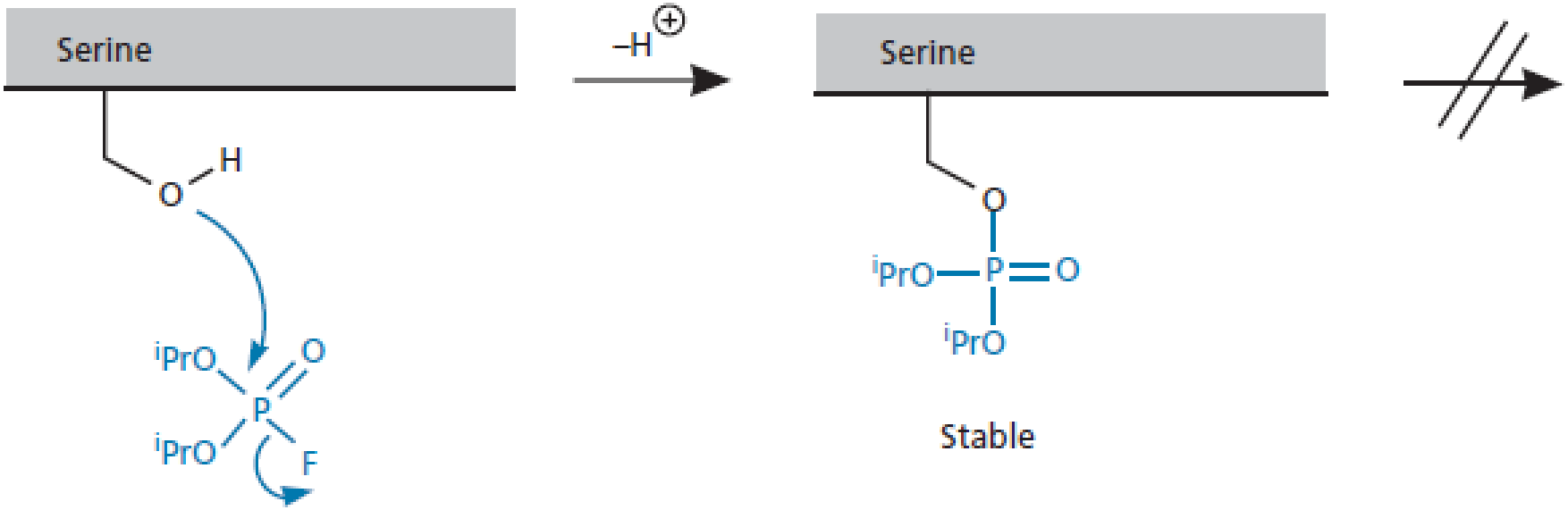
Irreversible Acetylcholinesterase inhibitors



dyflos Diisofluorophate
(insecticide)

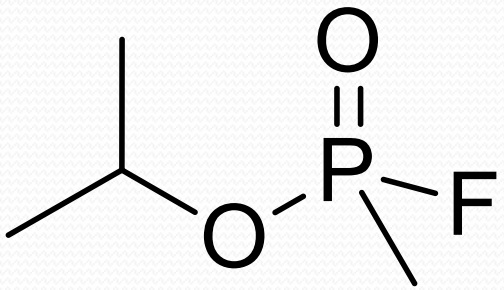


Ecothiophate
(Anti-glaucoma)

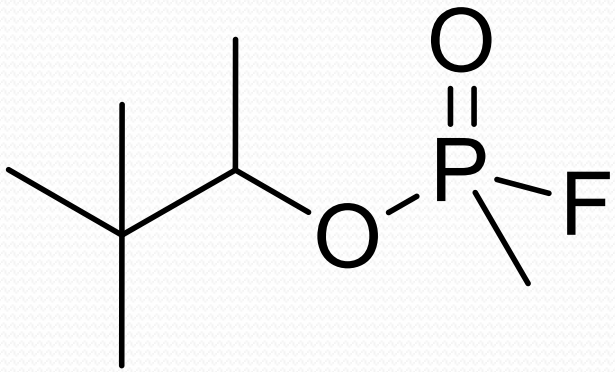


Simplified mechanism of action of dyflos at the active site of acetylcholinesterase.

Irreversible Acetylcholinesterase inhibitors



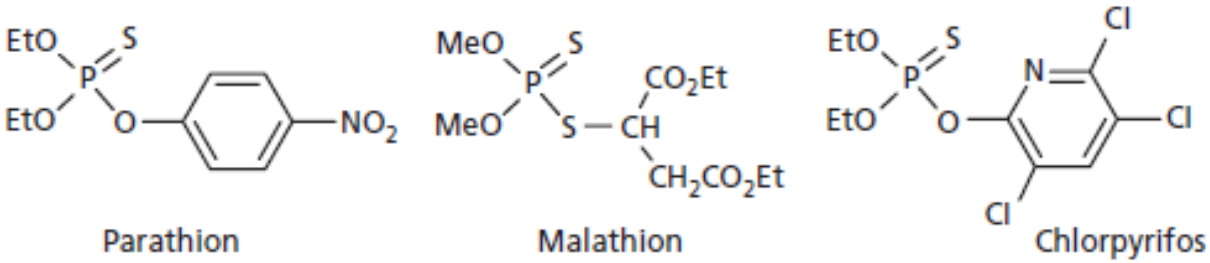
Sarin



Soman

- Organophosphates Nerve gases
- Irreversible binding to AchE

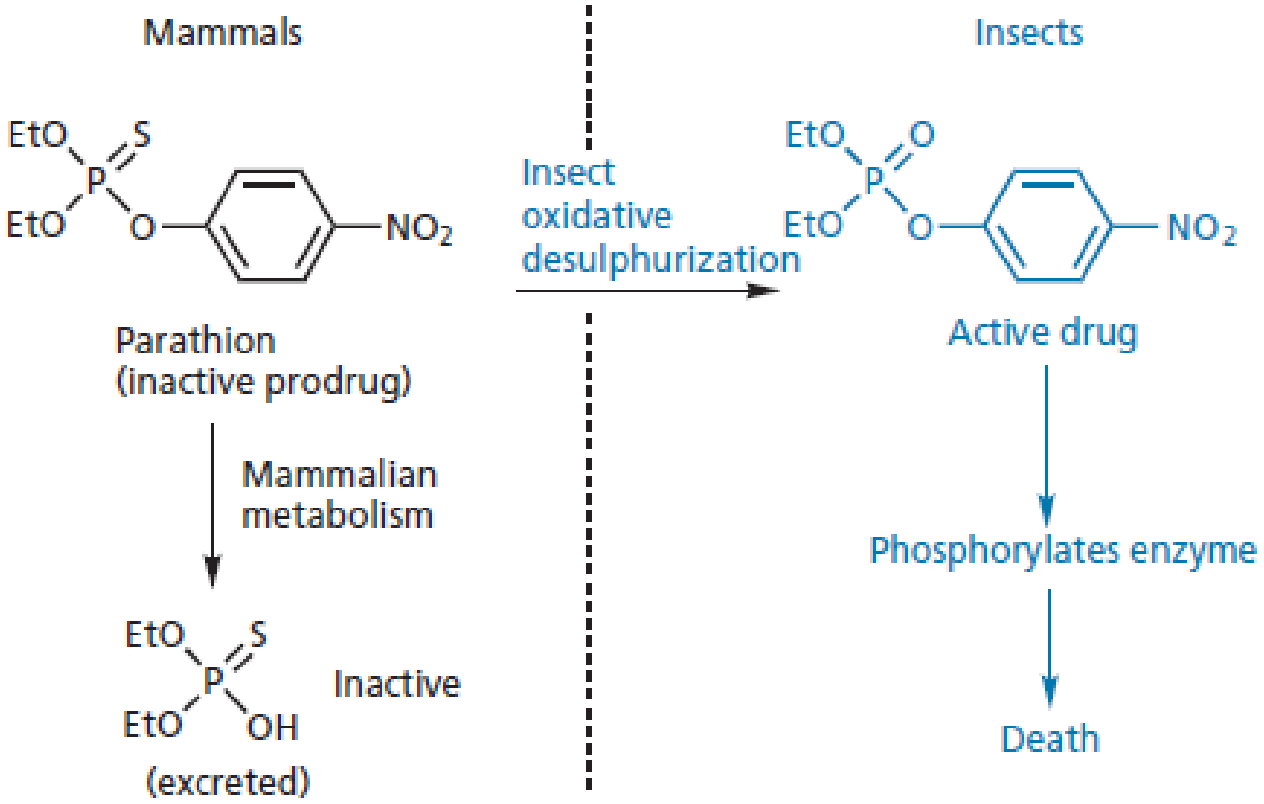
Insecticides



Organophosphates used as medicines and insecticides.

- insecticides parathion , malathion , and chlorpyrifos
- These agents are relatively non-toxic compared with nerve gases because the P = S double bond inhibition of the acetylcholinesterase enzymes.
- In contrast, the equivalent compounds containing a P = O double bond are highly lethal.
- Fortunately, there are no metabolic pathways in mammals which can convert the P = S double bond to a P = O double bond. In insects, however, the insecticides act as prodrugs and are metabolized by oxidative desulphurization.
- The resulting anticholinesterases prove lethal.

- In mammals, the same compounds are metabolized in a different way to give inactive compounds which are then excreted

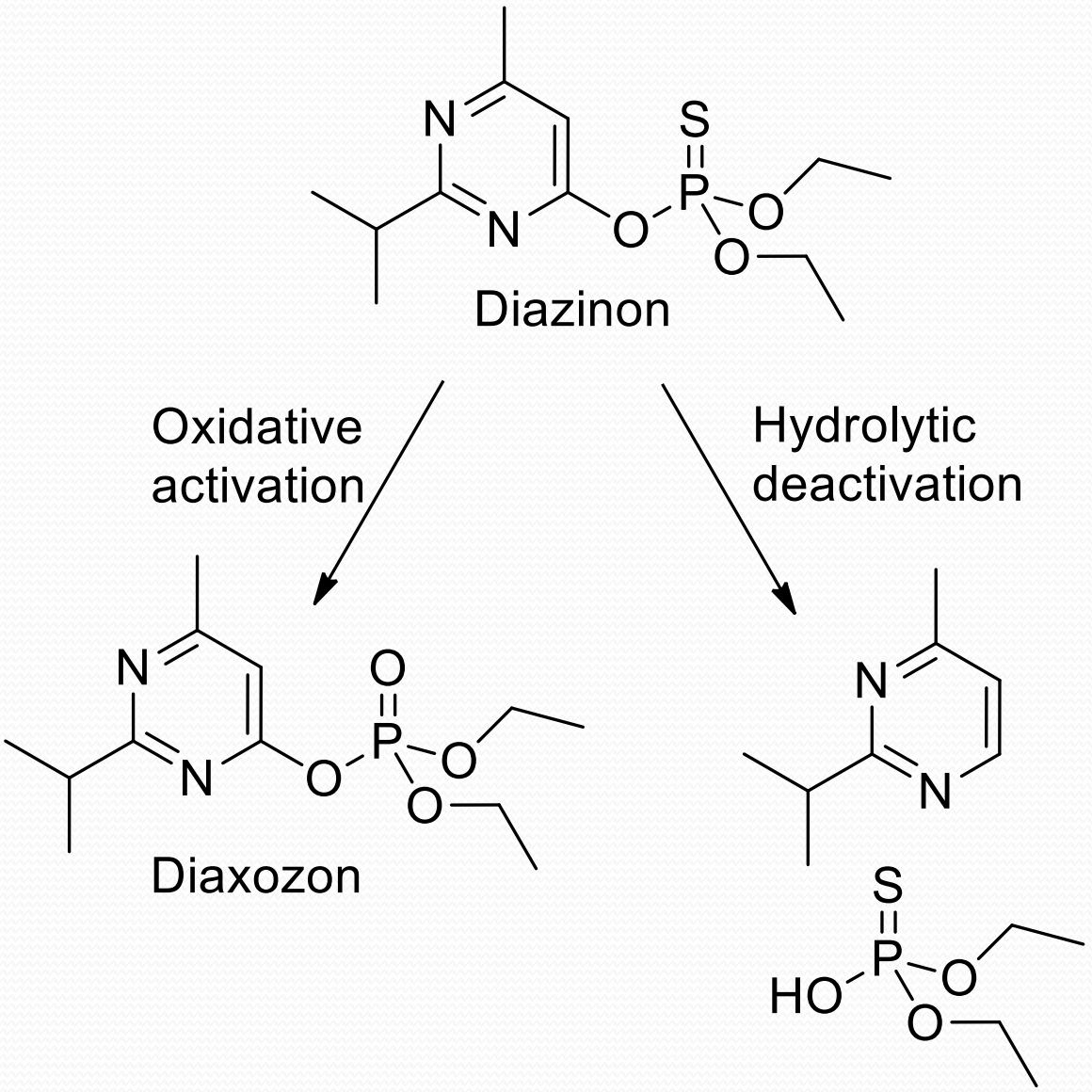


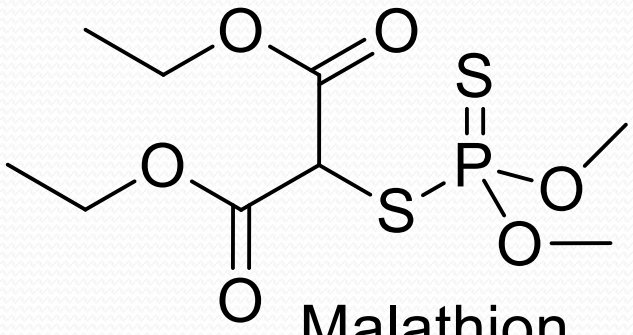
Metabolism of insecticides in mammals and insects.

Irreversible Acetylcholinesterase inhibitors

- Despite this, organophosphate insecticides are not totally safe and prolonged exposure to them can cause serious side effects if they are not handled with care.
- Parathion has high lipid solubility and is absorbed easily through mucous membranes, and can also be absorbed through the skin.
- Preparations of malathion are used medicinally for the treatment of head lice, crab lice, and scabies, but should not be used too frequently or over prolonged periods.

Irreversible Acetylcholinesterase inhibitors



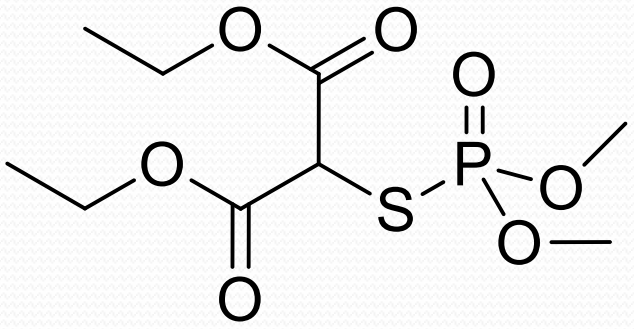


Malathion

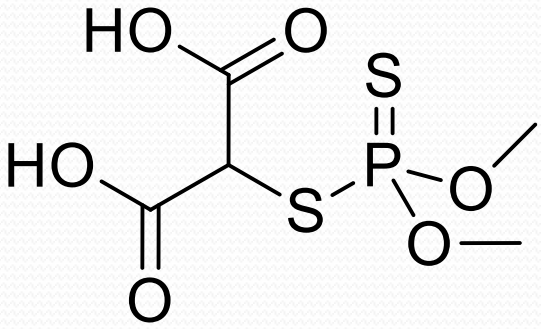
CYP450

Carboxyesterase

Carboxyesterase
Mammals, Birds



Malaoxon
(more active)



Inactive, non
toxic

Clinical Uses of acetylcholinesterase inhibitors

Drug	Type of inhibition	Route of administration	Clinical Use
Edrophonium	Rev	IM or IV	Diagnostic for Myasthenia Gravis
Neostigmine	Rev	IM, IV, or oral	Myasthenia Gravis, post-operative ileus and bladder distention, surgical adjunct
Physostigmine	Rev	IM, IV, or local	Glaucoma, Alzheimer's disease, antidote to anticholinergic overdose
Tacrine	Rev	Oral	Alzheimer's disease
Donepezil	Rev	Oral	Alzheimer's disease
Isofluorophate	Irrev	Local	Glaucoma
Echothiophate	Irrev	Local	Glaucoma

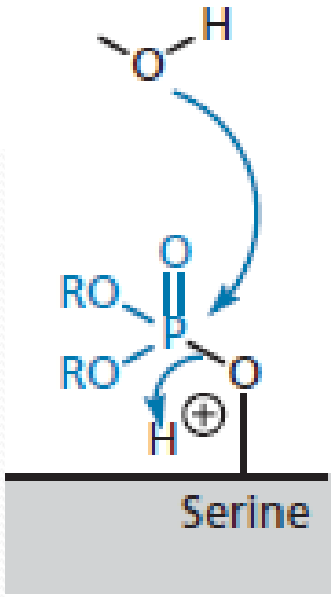
Cholinergic crisis

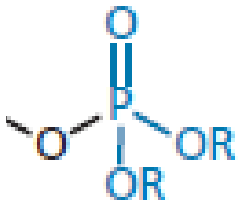
- A cholinergic crisis is an over-stimulation at a neuromuscular junction due to an excess of acetylcholine (ACh).
- This happens due to inactivity (perhaps even inhibition) of the AChE enzyme, which normally breaks down acetylcholine.
- This is a consequence of some types of nerve gas, (e.g. sarin gas) or insecticides.
- It causes muscle paralysis and respiratory failure

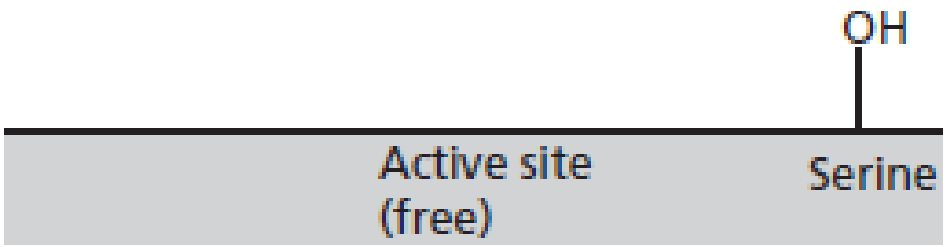
Aging

- Irreversible Anticholinesterase (Phosphate ester→ resist hydrolysis
- undergo a feature called aging → increases this resistance more.
- AchE becomes acylated → **one of the ester bond is broken**. → a negative charge that oppose nucleophillic attack on phosphorous and in this state it resists hydrolysis even more.
- At this stage antidotes such as PAM doesn't work.
- regeneration of AchE is blocked for longer periods → cholinergic crisis


AChE—Ser·O·P·OR
O
OR
Aging
AChE—Ser·O·P·O
O
OR
⊖
+ ROH


H
O
RO
RO
P
O
O
H
⊕
Serine

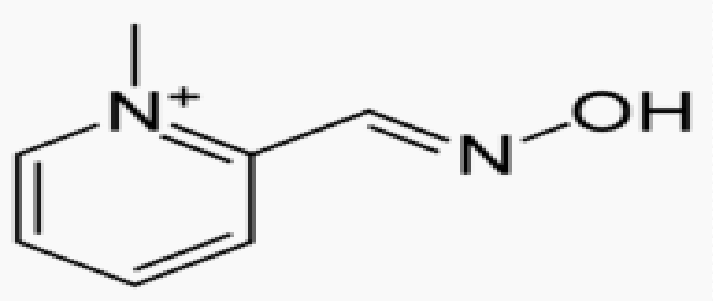

O
P
O
OR
OR


OH
Active site
(free)
Serine

Antidote to insecticides (Pralidoxime)

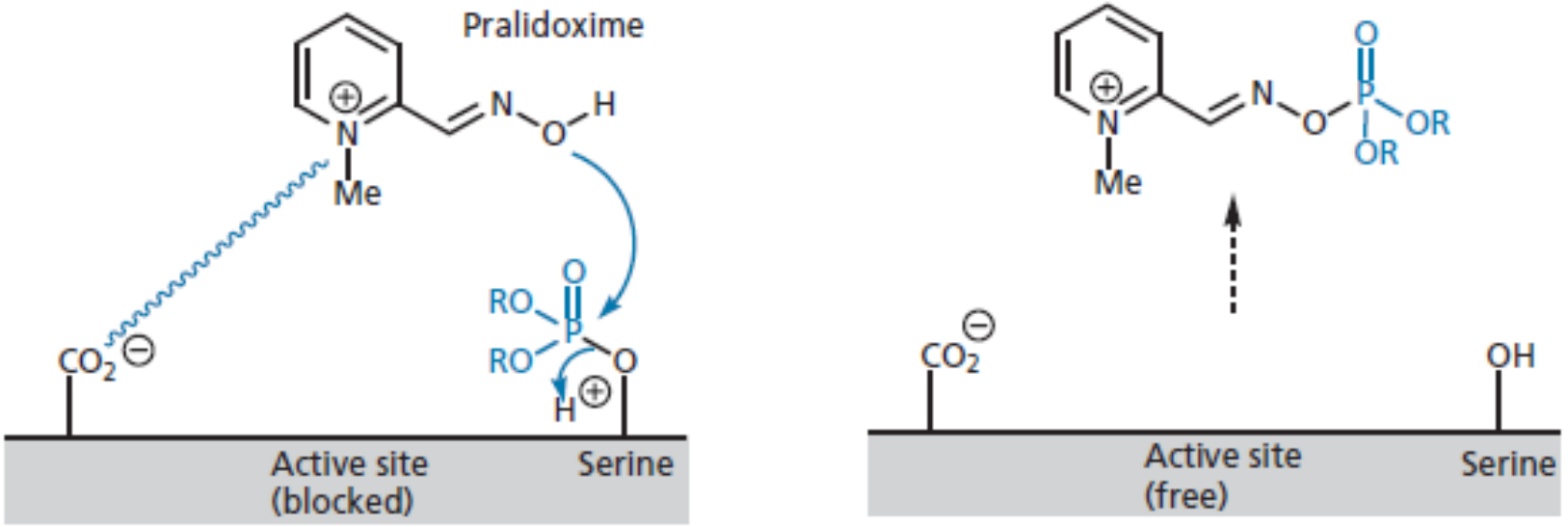
- Because insecticides can lead to cholinergic crisis, antidotes were designed to hydrolyze the acylated AchE.
- A successful antidote was PAM
- It has a quaternary ammonium and a very strong nucleophile called oxime group and both work together to free AchE from the phosphate ester compounds

Antidote PAM



Antidote to insecticides (Pralidoxime)

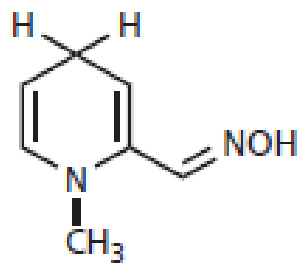
- phosphates can be hydrolysed with hydroxylamine
- This proved too toxic a compound to be used on humans
- anionic binding region is vacant → less chance of the antidote taking part in toxic side reactions.
- The ortho position (correct position to react with the phosphate ester.)



Pralidoxime as an antidote for organophosphate poisoning.

Antidote to insecticides (Pralidoxime)

- Pralidoxime :
  - has a quaternary nitrogen → fully charged and cannot pass through the blood–brain barrier into the CNS.
  - antidote cannot work on any enzymes that have been inhibited in the brain.
- Pro-2-PAM
  - is a prodrug of pralidoxime which avoids this problem
  - is oxidized to pralidoxime once it has entered the CNS.



ProPAM.

Organophosphate poisoning

- Most insecticides use concept of irreversible AchE
- contain phosphate ester bond → resist hydrolysis.
- They are very lipophillic and volatile also.
- quickly enter the blood stream and inhibit AchE (hours).
- This promotes Ach activity in the synapse which leads to 'cholinergic crisis'.
- When this happens muscles stop responding to Ach causing paralysis and respiratory failure (death)

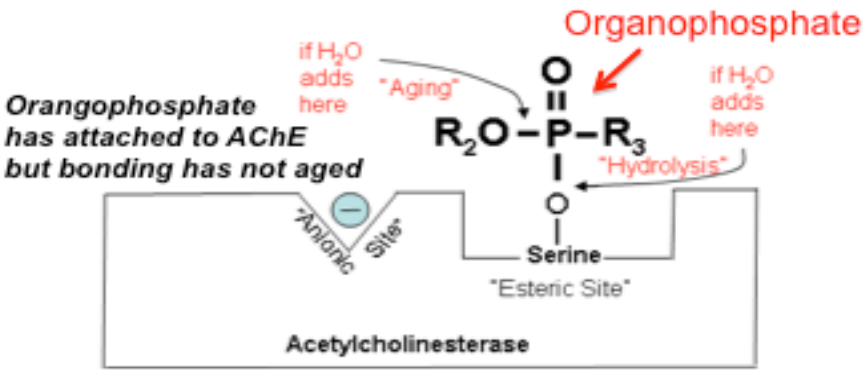
How PAM reverses poisoning by organophosphate or sarin gas ?

- There are two sites in the binding pocket of AchE. One is a anionic site which is empty and other is the Esteric site where the phosphate compound sits. 3 event follows:
- First : the charged ammonium of PAM binds to the anionic site. From there PAM's strong nucleophillic oxime group can be in close distance to attack the ester bond between Phosphorous and serine amino acid of AchE.

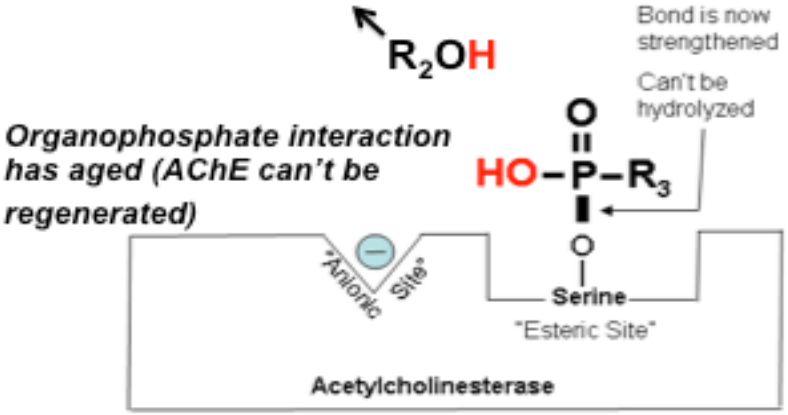
- Second: 2-PAM bound to organophosphate
- Third : organophosphate bond to serine is broken
- This form free AchE and phosphorylated PAM
- Limitation: For PAM to work, it must be used immediately following exposure to insecticides or serine gas or no more than 36 hrs of exposure or else aging will occur and it wont be able to dislocate the phosphate form the receptor.

How PAM reverses poisoning by organophosphate or sarin gas ?

Organophosphate Aging – chemical stabilization of phosphate bond to AChE occurs over time

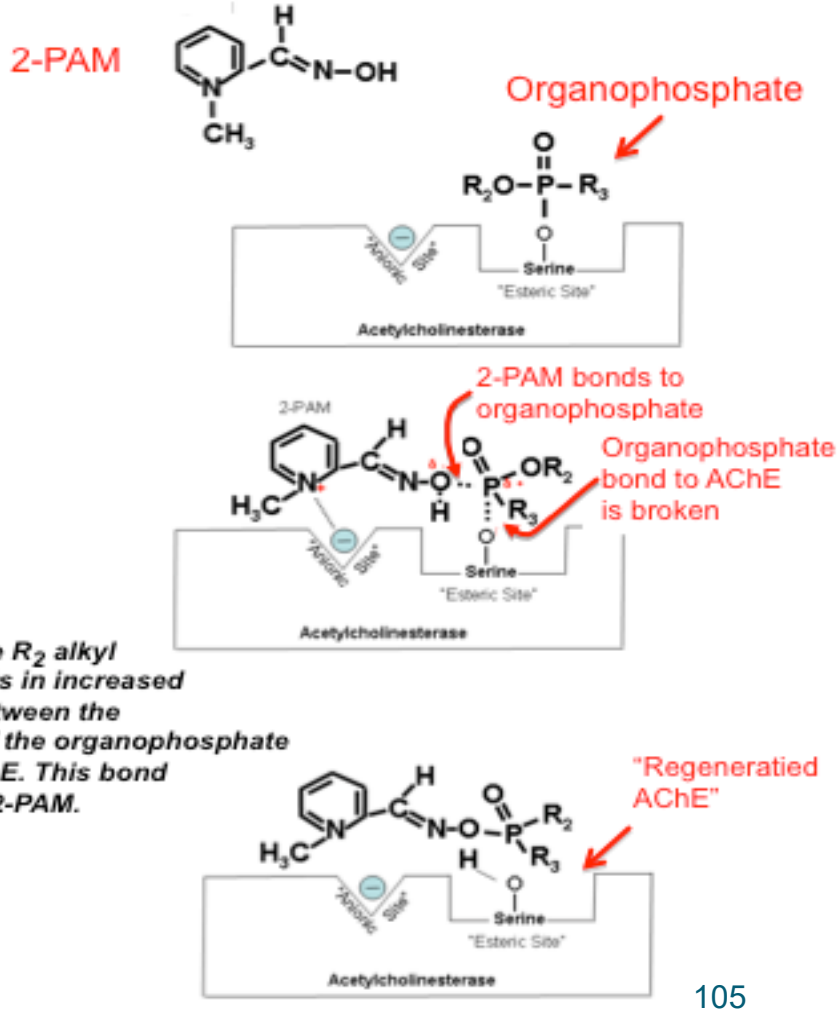


The rate of aging is unique for each organophosphate compound, and can occur over minutes to days depending on the agent



The departure of the R_2 alkyl group (aging) results in increased electron sharing between the phosphate group of the organophosphate & the serine on AChE. This bond can't be broken by 2-PAM.

Pralidoxime (2-PAM) prevents aging & regenerates AChE

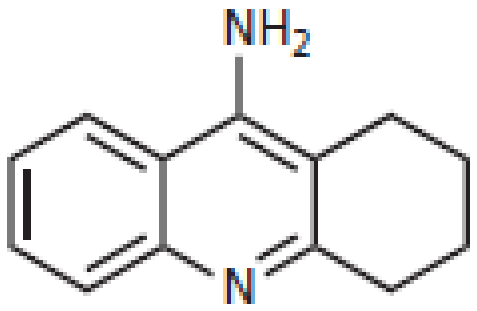


Anticholinesterases as ‘smart drugs’

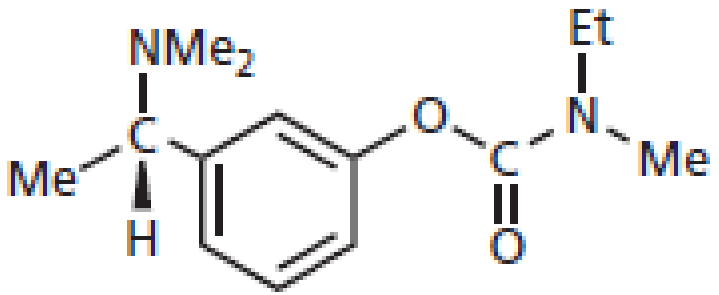
- anticholinesterases for the treatment of Alzheimer’s disease—the so-called smart drugs .
- have to cross the blood–brain barrier
- Limitations:
  - beneficial in the early stages of Alzheimer's disease, but are of less benefit when the disease has become advanced.(alleviate the symptoms by increasing the duration of action of acetylcholine such that activation of the cholinergic receptors remaining is prolonged)
  - Side effects (GIT)

Anticholinesterases as 'smart drugs'

- Tacrine: extremely toxic drug
- Rivastigmine (an analogue of physostigmine) was the first drug to be approved in all countries of the European Union.



Tacrine (Cognex, Parke-Davis)



Rivastigmine (Exelon, Novartis)

Cholinergic Antagonists

Cholinergic Antagonists

CHOLINERGIC ANTAGONISTS

Cholinergic Agonists	Cholinergic Antagonists
1. Miosis Constriction of Pupil	1. Mydriasis-dilation of pupil Cycloplagia (paralysis of the ciliary structure of the eye
2. ↑ GI Motility	2. ↓ GI Motility-Antispasmodic
3. ↑ Secretion (Saliva)	3. ↓ Secretion, i.e. antisialagogue, ↓ Saliva flow or formation
4. In CNS ↑ Cognitive function Stimulate motor function	4. In CNS ↑ Confusion/disorientation Blocks stimulation of motor function
5. Cardiac Function Slows heart rate	5. Cardiac Function ↑ Heart rate; used to treat Bradyarrhythmias

Parkinson's Disease

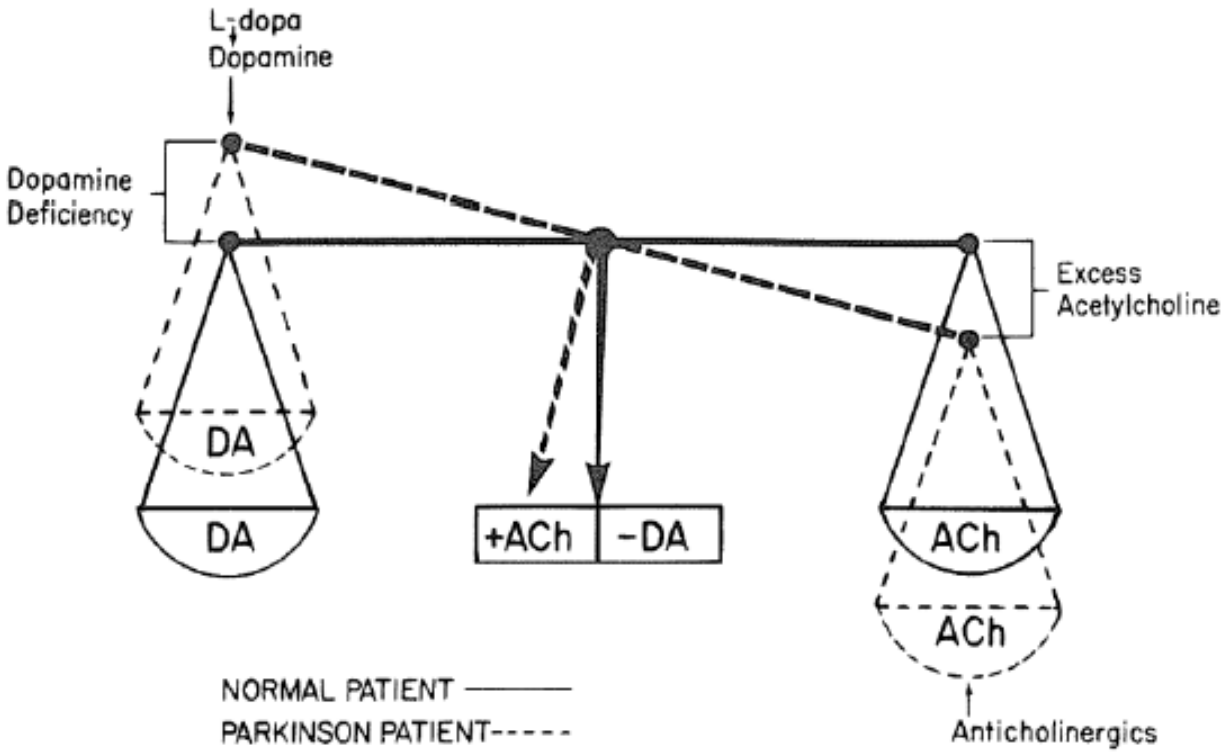
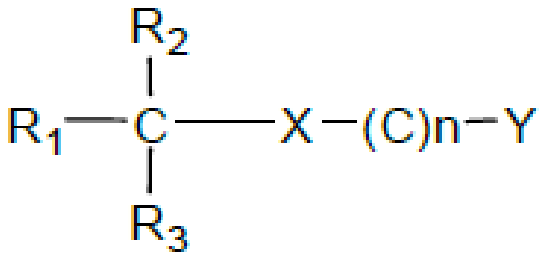


Fig. 13–2. Schematic representation of the imbalance between the excitatory neurotransmitter acetylcholine and the inhibitory neurotransmitter dopamine in the basal ganglia of parkinsonian patients.

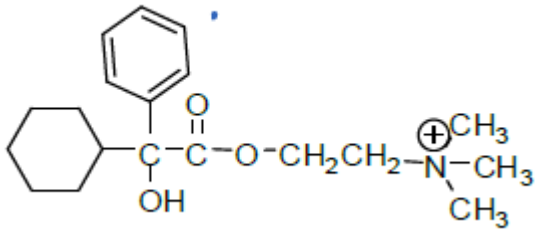
Cholinergic Antagonist

- Also referred to as Cholinergic Blocking Agents or Antimuscarinic Agents
- General Structure of Cholinergic Antagonists



Where R_1 or R_2 or both are cyclic
R_3 = H, OH, CH_2OH, CH_3 or $CONH_2$
Y = is a 3° amine or quaternary ammonium group
n = 2 or 3
X = COO, an ester
= C-O, an ether
= CON, an amide or X can be absent

A Cholinergic Antagonist



- structural requirements for an antagonist are less rigid than those for a Direct-acting Agonist.

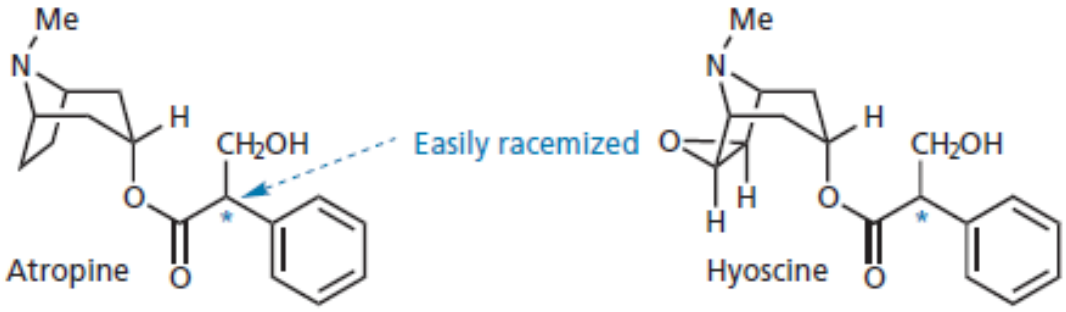
Atropine and hyoscyamine

- asymmetric center
- hyoscyamine (Single enantiomer)
- Racemization.
- Clinically, atropine has been used to decrease gastrointestinal motility and to counteract anticholinesterase poisoning.

Hyoscine (or scopolamine)

- similar in structure to atropine.
- treatment of motion sickness

❖ All cause CNS effects



Atropine and hyoscine.

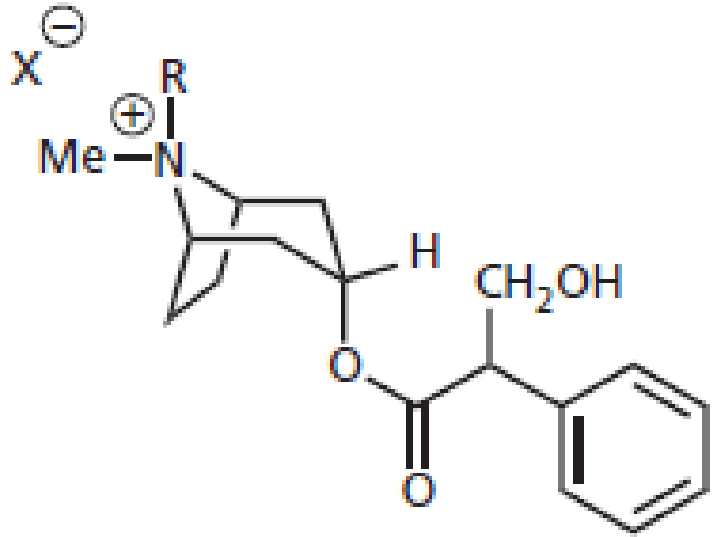
Atropine methonitrate

- acts at the intestine to relieve spasm.

ipratropium

- used as a bronchodilator in chronic obstructive pulmonary disease.

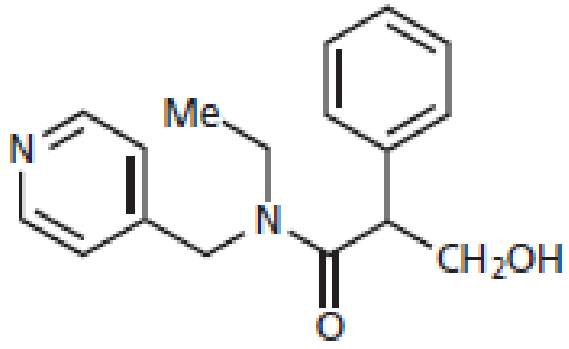
❖ reduce CNS side effects



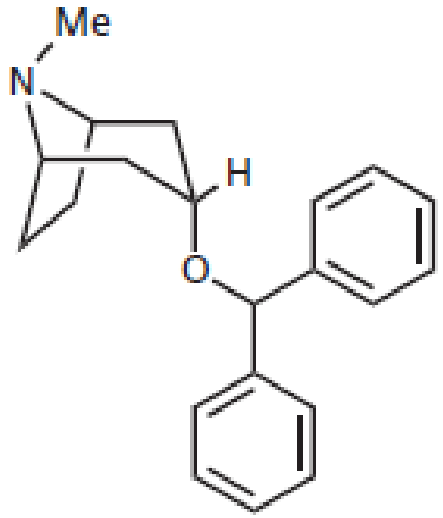
R = Me, X = NO_3^-; Atropine methonitrate
R = ^{i}Pr, X = Br^-; Ipratropium

Structural analogues of atropine.

- tropicamide used in eye drops to dilate pupils for ophthalmic examination
- Benzatropine → Treatment of Parkinsonism
- Selectivity of action owes more to their distribution properties than to receptor selectivity. (parts of the body more easily than others)



Tropicamide



Benzatropine

Antagonists of the nicotinic cholinergic receptor

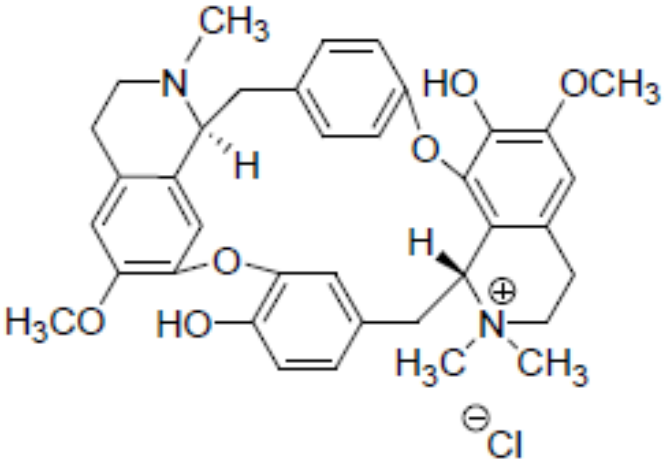
- Antagonists of ganglionic nicotinic receptor sites are not therapeutically useful because they cannot distinguish between the ganglia of the sympathetic nervous system and the ganglia of the parasympathetic nervous system (both use nicotinic receptors)
- Consequently, they have many side effects.
- antagonists of the neuromuscular junction are therapeutically useful and are known as neuromuscular blocking agents .

Nicotinic antagonists (Curare and tubocurarine)

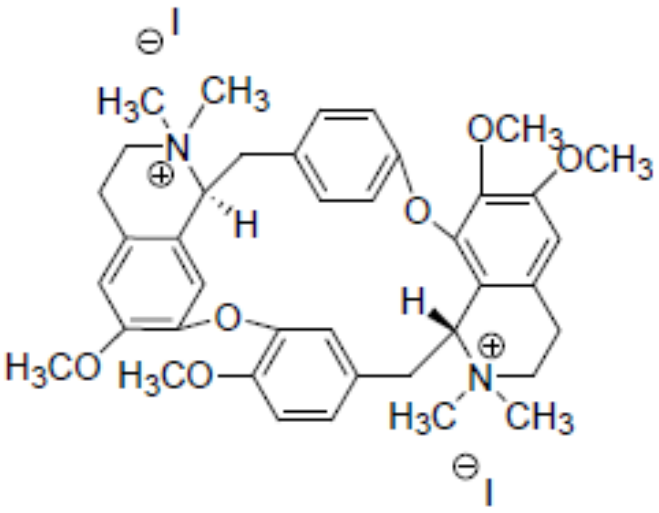
Non-Depolarizing Competitive Blocking Agents:

- used in surgery to **promote skeletal muscle relaxation** needed to set bone fractures, to **facilitate endotracheal intubation** or to allow endotracheal procedures such as laryngoscopy.
- are **antagonists** that compete with acetylcholine for binding to the receptor.
- do not activate or stimulate the receptors at the neuromuscular junction.
- The principal structural difference between Non-depolarizing Agents and Depolarizing Agents is in the size of the onium or charged groups on the molecule.
- Nondepolarizing agents have larger or bulkier onium groups within their structures.

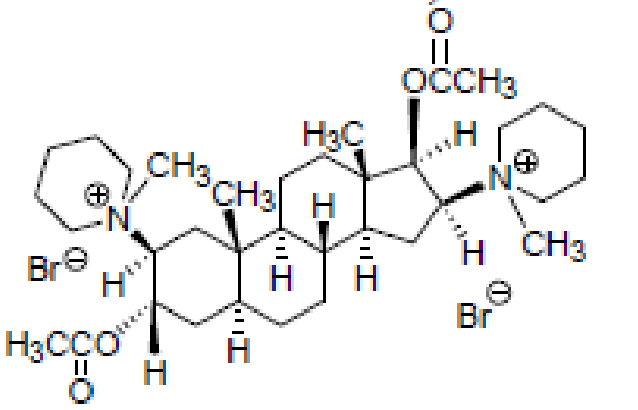
Tubocurarine Chloride Hydrochloride (withdrawn)



Metocurine Iodide, Metubine®
(withdrawn)



Pancuronium Bromide, Pavulon®



Nicotinic antagonists (Curare and tubocurarine)

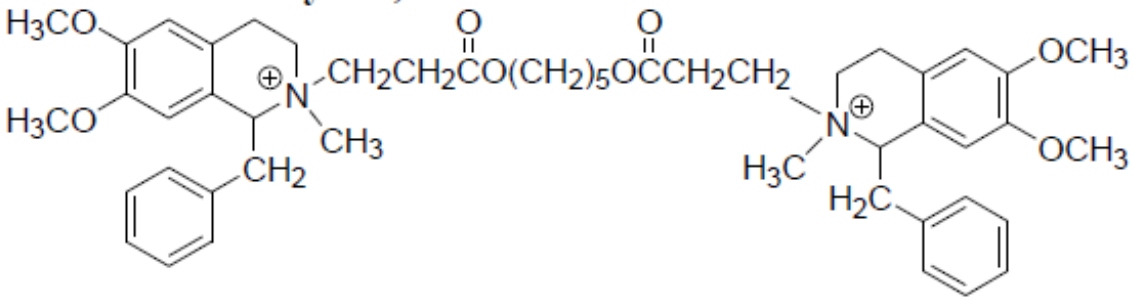
The second series of non-depolarizing neuromuscular blocking agents are the Bis-Benzylisoquinolines.

- They are structurally-related to tubocurarine, with the exception that the **isoquinoline** moieties are connected to one another at a single point which originates from the nitrogen atom within the tetrahydro isoquinoline ring.
- There number of atoms between the onium groups in this series ranges from 12-16 atoms.
- In all instances, these compounds have two onium groups both of which consist of quaternary ammonium groups.

Nicotinic antagonists (Curare and tubocurarine)

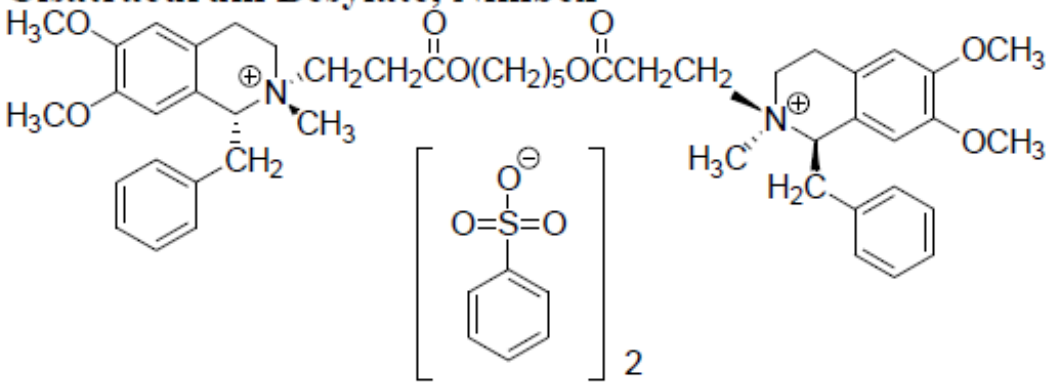
The second series of non-depolarizing neuromuscular blocking agents are the Bis-Benzylisoquinolines.

Atracurium Besylate, Tracrium®



Atracurium has a duration of action of between 30-40 minutes.

Cisatracurum Besylate, Nimbex®

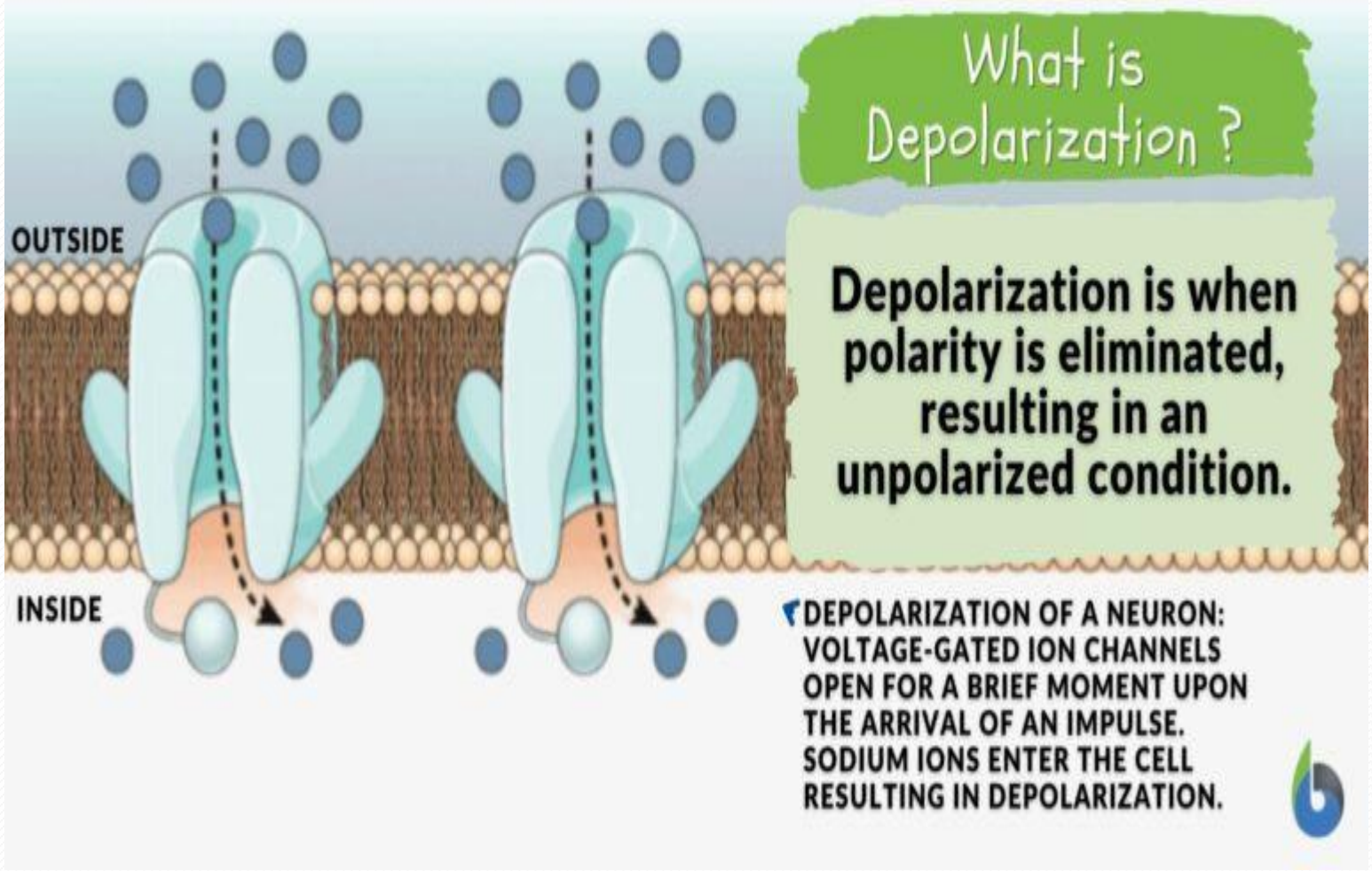


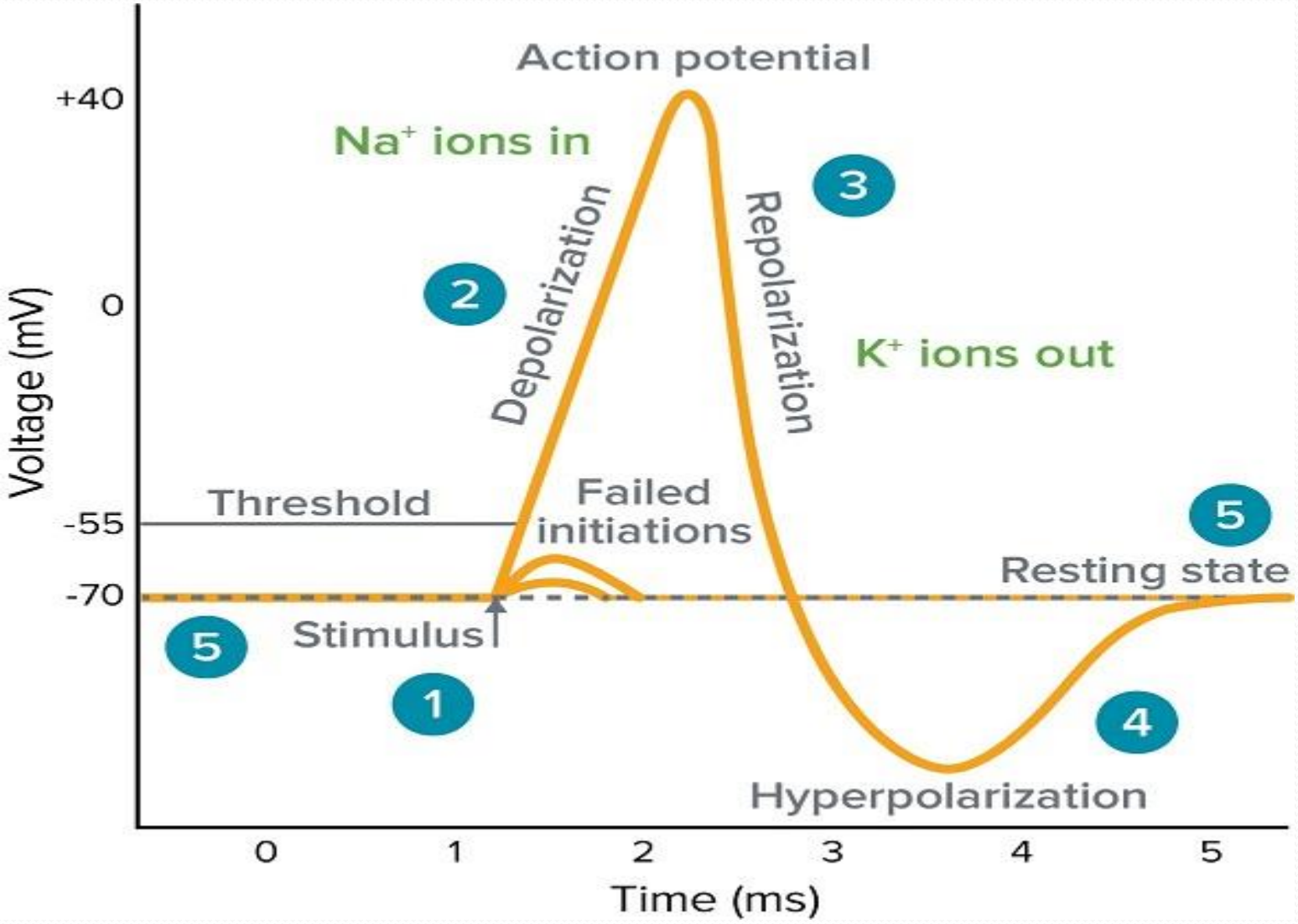
Depolarizing Neuromuscular Blocking Agents

Depolarizing Neuromuscular Blocking Agents

- binds initially to nicotinic receptors, it acts as an agonist rather than an antagonist.
- it **switches on receptors** such that sodium ion channels open up to **depolarize** muscle cell membranes and cause brief contractions of the muscle.
- Because the drug is not rapidly hydrolysed in the same way as acetylcholine, it remains bound to the receptor leading to persistent depolarization and subsequent desensitization of the end plate.
- At that stage, it can be viewed as an **antagonist** as it no longer stimulates muscle contraction and blocks access to acetylcholine.

Depolarization



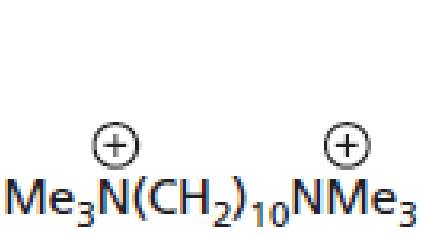

Action potential
+40
0
-55
-70
Voltage (mV)
Na+ ions in
Depolarization
Repolarization
K+ ions out
Threshold
Failed initiations
Resting state
Stimulus
Hyperpolarization
1
2
3
4
5
5
0
1
2
3
4
5
Time (ms)

Depolarizing Neuromuscular Blocking Agents

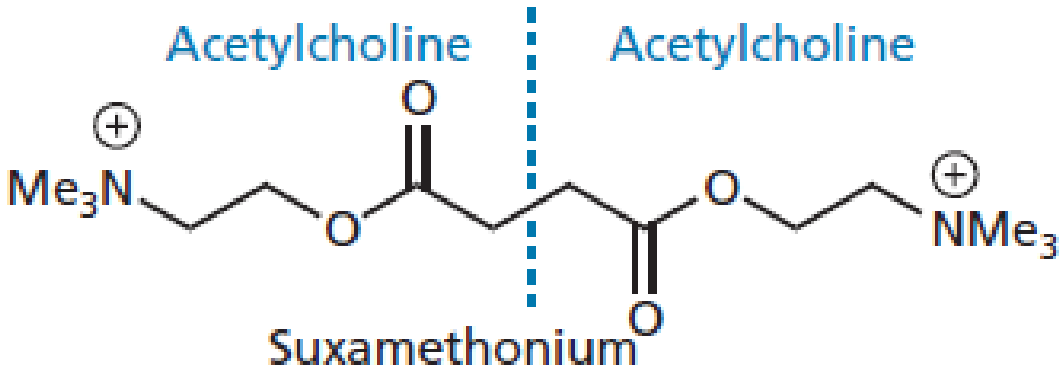
Decamethonium Bromide:

- the first Depolarizing Competitive Blocking Agent that was developed into the clinic.
- One major problem with decamethonium was that its duration of action was too long.

Suxamethonium: used clinically in short surgical procedures (insertion of tracheal tubes)



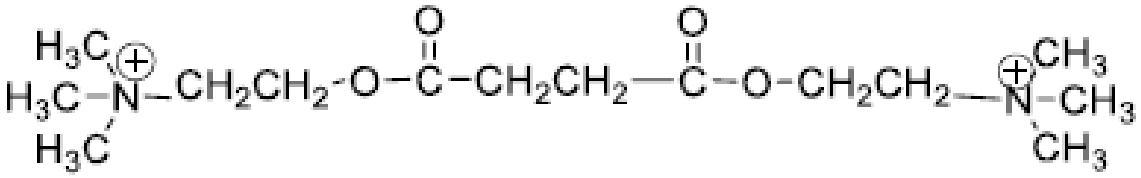
Decamethonium



Suxamethonium

Distance Between onium Groups is Critical

Activity at Neuromuscular Junctions



Succinylcholine

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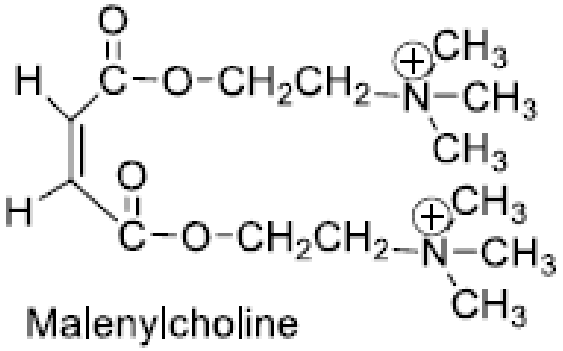

Malenylcholine

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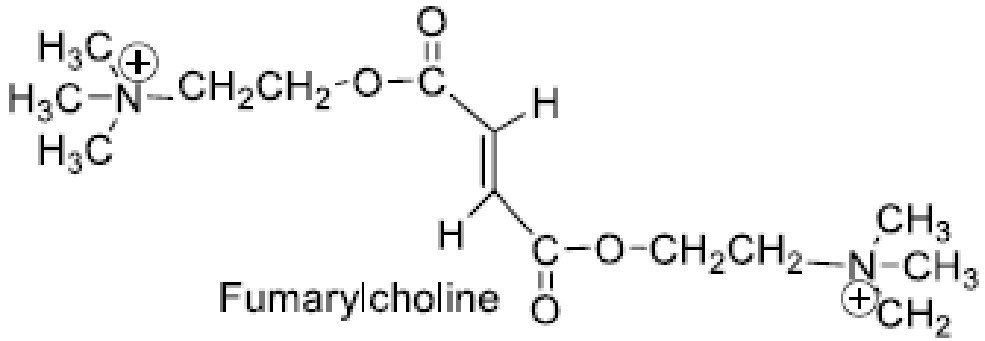

Fumarylcholine

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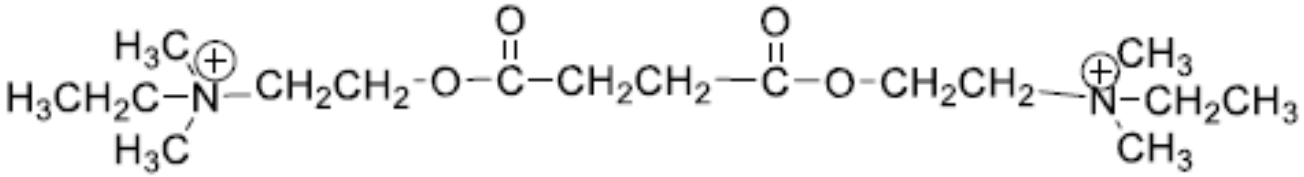
Activity at Neuromuscular Junction

The size of the onium group is critical



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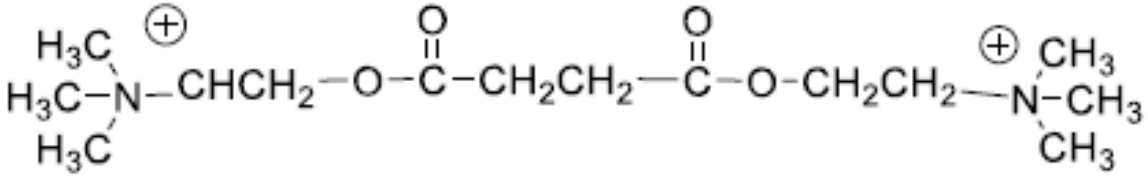

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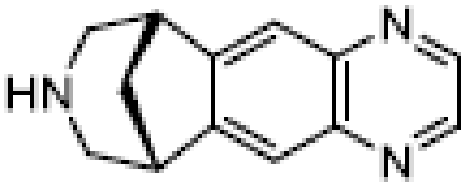
Selective Nicotine Partial Agonist

- Varenicline:
  - This is a agonist at α7 nicotinic receptors and a partial agonist at several nicotinic receptor subtypes.
  - As a nicotine partial agonist it stimulates nicotinic receptors to a lesser extent than nicotine itself.
  - It is used to assist some patients in stopping smoking.




Thank
you!