**Indirect acting cholinergic agents (agonists)**

***Cholinesterase inhibitors***



**The active center of AChE consists of several major domains:-**

1. **an anionic site, to which the trimethylammonium group binds.**
2. **An esteratic Site, which causes hydrolysis of the ester portion of Ach.**
3. **Hydrophobic sites, which bind aryl substrates, other uncharged ligands, and the alkyl portion of the acyl moiety of ACh.**

**Three different chemical groupings may react with the esteratic site of AChE which includes:-**

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1. **Acetyl group ( ), reversible inhibitor.**
2. ** Carbamyl group ( ), semireversible inhibitor.**

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1. **Phosphoryl group ( ), irreversible inhibitor.**

**NOTE:- The chemical reactions of these groups are similar, the kinetic parameters for each type of substrate differ and result in differences between toxicity and usefulness.**

**The hydrolysis of Ach by AChE**

**Theory of AchE inhibitors**

During hydrolysis of Ach, the AchE gets acylated.

It needs to be hydrolyzed by water to be regenerated in free form or else it can’t function again.



1. **The initial step in the hydrolysis of ACh by AChE is a reversible enzyme—substrate complex formation. The association rate (k + 1) and dissociation rate (k-1 ) are relatively large.**
2. **The enzyme—substrate complex, EA—Ach, may also form an acetyl-enzyme intermediate at a rate (k2) that is slower than either the association or dissociation rates.**
3. **Choline is released from this complex with the formation of the acetyl-enzyme intermediate, EA.**
4. **This intermediate is then hydrolyzed to regenerate the free enzyme and acetic acid at a rate (K3).**

**Note:- The acetylation rate, K2, is the slowest step in this sequence and is rate-limiting step.**



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AChE attacks the ester substrate through a serine hydroxyl, forming a covalent acyl-enzyme complex. The serine is activated as a nucleophile by the glutamic acid and histidine residues that serve as the proton sink to attack the carbonyl carbon of ACh. Choline is released, leaving the acetylated serine residue on the enzyme. The acetyl-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.

**Semireversible inhibitors of AChE.**



**Carbamates such as carbachol are also able to serve as substrates for AChE, forming a carbamylated enzyme intermediate (E—C). The rate of carbamylation (k2) is slower than**

**the rate of acetylation. Hydrolysis (K3, decarbamylation of the carbamyl-enzyme intermediate is107 times slower than that of its acetyl counterpart. The slower hydrolysis rate limits the optimal functional capacity of AChE, allowing carbamate substrates to be semireversible inhibitors of AChE. In this mechanism, K3 is rate-limiting.**

**Note:- The rate K2 depends not only on the nature of the alcohol moiety of the ester but also on the type of carbamyl ester. Esters of carhamic acid are better carbamylating agents of AChE than the methylcarbamyl and dimethylcarbamyl.**



**Irreversible inhibitor of AChE**



**Organophosphate esters of selected compounds can also esterify the serine residue in the active site of AChE. The hydrolysis rate (k3) of the phosphorylated serine is extremely slow, and hydrolysis to the free enzyme and phosphoric acid derivative is so limited that the inhibition is considered irreversible. These organophosphorous compounds are used in the treatment of glaucoma, as agricultural insecticides, and, at times, as nerve gases in warfare and bioterrorism.**

***Reversible Inhibitors***

1. **Physostigmine**



**Physostigmine is an alkaloid obtained from the dried ripe seed of Physostigma venenosum.**

**The alkaloid, as the free base, is quite sensitive to heat, light, moisture, and bases, undergoing rapid decomposition. In solution it is hydrolyzed to methyl carbamic acid and eseroline, neither of which inhibits AChE. Eseroline is oxidized to a red compound, rubreserine, and then further decomposed to eserine blue and eserine brown. Addition of sulfite or ascorbic acid prevents oxidation of the phenol. Eseroline to rubreserine. Hydrolysis does take place, however, and the physostigmine is inactivated. Solutions are most stable at pH 6 and should never be sterilized by heat.**



SAR studies of physostigmine demonstrate that:



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

**Physostigmine is a relatively poor carbamylating agent of AChE and is often considered a reversible inhibitor of the enzyme. Its cholinesterase-inhibiting properties vary with the pH of the medium. The conjugate acid of physostigmine has a pKa of about 8, and as the pH of the solution is lowered, more is present in the protonated form. Inhibition of cholinesterase is greater in acid media, suggesting that the protonated form makes a contribution to the inhibitory activity well as its carbamylation of the enzyme.**



**Uses of physostigmine**

1. **Physostigmine was used first as a topical application in the treatment of glaucoma. Its lipid solubility properties permit adequate absorption from ointment bases.**
2. **It is used systemically as an antidote for atropine poisoning and other anticholinergic drugs by increasing the duration of action of Ach at cholinergic sites through inhibition of AChE.**
3. **Physostigmine, along with other cholinomimetic drugs acting in the CNS, and has been use in the treatment of Alzheimer's disease because it has no charged amine and is more lipophillic and can thus penetrate the blood brain barrier.**
4. **Neostigmine Bromide**



**Uses of neostigmine**

1. **The most frequent application of neostigmine is to prevent atony of the intestinal, skeletal, and bladder musculature.**
2. **The important use is in the treatment of myasthenia gravis.**

**Synthesis of Neostigmine bromide**



Uses:

Myasthenia gravis

