***Structure-Activity Relationships***

**Cholinergic receptor Agonists**

1. **Direct acting agonists:- bind and activate cholinergic receptors.**
2. **Indirect-acting agonists:- increase synaptic [ACh] by either inhibiting AChE or increasing the release of ACh from terminals,**

**Design of Cholinergic Agonists: Structural Modification of Acetylcholine. Alterations on the molecule may be divided into four categories:**

1. **The onium group.**
2. **The ester function,**
3. **The choline moiety.**
4. **Alkyl group (ethylene bridge).**



1. **Ammonium Group(-N+(CH3)3.** The onium group is essential for intrinsic activity and

contributes to the affinity of the molecule for the receptors, because its important to the binding of the compound to the negatively charged aspartic acid residue in the muscarinic receptor.

****

 **a- The replacement of the ammonium moiety with either a sulfonium(-S+(CH3)2 or phosphonium(-P+(CH3)3 and arsenonium isosters results in a complete loss of activity.**

1. **Increasing one methyl group to a larger alkyl (e.g., ethyl) results in 25% activity. Increase two methyl groups in size -> lose all activity.**

**Because increase the size of the onium moiety, produce diffusion of the positive charge, and interfere sterically with proper drug—receptor interaction, resulting in a decrease in activity.**



1. **Ethylene bridge. Acts as a "perfect spacer", the result show that for muscarinic activity, Should be no more than four atoms between the ammonium and the terminal methyl group, otherwise a loss of activity. (i.e.ammonium group should be followed by a chain of five atom, this has been referred to as the five atoms rules.**
2. **Shortening or lengthening the chain of atoms that separates the ester group from the onium moiety reduces muscarinic activity.**
3. **An α substitution on the choline moiety decreases both nicotinic and muscarinic activity, but muscarinic activity is decreased to a greater extent.**
4. **An β substitution on the choline moiety decreases both nicotinic and muscarinic activity, but nicotinic activity is decreased to a greater extent.**



1. **Hydrolysis by AChE is more affected by substitutions on the β than the α carbon. The hydrolysis rate of racemic acetyl β-methylcholine is about 50% of that of Ach ; racemic acetyl α- methylcholine is hydrolyzed about 90% as fast.**
2. **Ester Group. The ester group in ACh contributes to the binding of the compound to the muscarinic receptor because of hydrogen bond formation with threonine and asparagine residues at the receptor site.**

A comparison of the cholinergic activity of a series of alkyltrimethylammonium compounds {

R-N+(CH3), R = C1—C9} shows n-amyltrimnelhylammonium, which may be considered to have a size and mass similar to those of ACh and to be one magnitude weaker as a muscarinic agonist. The presence of the acetyl group in ACh is not as critical as the size of the molecule. Studying a series of n-alkyltrimeihylammonium salts revealed that 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.

***Oxotremorine***

 **Oxotremorine is a specific muscarinic agonist equipotent to Ach. Oxotremorine is relatively selective as M1-agonist probably due to a favorable distribution to the brain. Oxotremorine and its analogues are potentially used in the treatment of Alzheimer disease.**

**oxotremonne's *trans* conformation shows that distances between possible active centers correspond with (+ )- muscarine as shown below.**



**Arecoline**



**Arecoline act mainly at muscarinic receptors, but has some activity at nicotinic receptors. Arecoline is relatively selective as M1-agonist.**

***Cholinergic receptor antagonists***

Antagonists with high affinity for one receptor and a low affinity for the other four receptor

types are very few, however, and many antagonists bind to several subtypes with equal affinity.

M1 receptors have been identified as those with high affinity for pirenzepine and low

affinity for a compound such as AF-DX 116. Pirenzepine can distinguish between M1 and M2. or M5 hut has significant affinity for M4 receptors. Himbacine can distinguish between M1 and M4 receptors. Methoctramine.apolymethylenetetramine. not only discriminates between

M1 and M2 receptors but also has good selectivity for M2 muscarinic receptors. M2 receptors bind to AF-DX 116 and gallamine. a neuromuscular blocking agent. M3 Receptors have a high affinity for 4-diphenylacetoxy-N-methylpiperidine (4-DAMP) and hexahydrosiladifenidol (HHSiD) also exhibit affinity for M1, and M2.

**Cholinergic Drugs and Related Agents**

**Direct acting cholinergic agents ( Agonist)**

**Products**

1. **Acetylcholine Chloride**



**ACh chloride exerts a powerful stimulant effect on the parasympathetic nervous system. But it's not very useful as cholinergic agent because of:-**

1. **Nonselective(when given systemically)**
2. **Short half life due to rapid hydrolysis by AChE and other cholinesterases.**

**Can be useful when directly injected into the eye to produce miosis in surgery.** **When applied topically to the eye, it has little therapeutic value because of poor corneal penetration and rapid hydrolysis by AChE.**

**Action of ACh**

1. **Stimulation of the vagus and the parasympathetic nervous system produces tonic action on smooth muscle and induces a flow from the salivary and lacrimal glands.**
2. **It is a cardio depressant and an effective vasodilator.**

**Its cardiac-depressant results from :-**

1. **A negative chronotropic effect that decrease in heart rate .**
2. **a negative inotropic action on heart muscle that produces a decrease in the force of myocardial contractions**

**Atropine is one of the most effective antagonists to the action of Ach (a nonselective muscarinic antagonist). Atropine blocks the depressant effect of ACh on cardiac muscle and its production of peripheral vasodilation (i.e., muscarinic effects) but does not affect the skeletal muscle contraction (i.e., nicotinic effect) produced.**

**Synthesis of ACh**



1. **Methacholine Chloride**



1. **Methacholine has sufficient stability in the body to give sustained parasympathetic stimulation. So methacholine has longer duration of action than ACh due to steric effect of β- methyl group on the rate of hydrolysis by AChE.**
2. **Methacholine has little or no nicotinic activity (i.e., nicotinic activity of methacholine is about 1/ 1000 of Ach)**
3. **Methacholine can exist as (S) and (R) enantiomers. Since the chemical is used as the racemic mixture, its muscarinic activity resides principally in the (S) isomer. The (S) / (R) ratio of muscarinic potency for these enantiomers is 240:1.**
4. **( + )-Acetyl-(S)-β-methylcholine is hydrolyzed by AChE, whereas the (R) (-) isomer is not. The hydrolysis rate of the (S)( + ) isomer is about 54% that of Ach . This rate probably compensates any decreased association (affinity) owing to the β- methyl group with the muscarinic receptor site and may account for the fact that ACh and (+)-acetyl-β-methylcholine have equimolar muscarinic potencies in vivo.**
5. **(—)-Acetyl-(R)-β-methylcholine weakly inhibits AChE and slightly reinforces the muscarinic activity of the (S)( +) isomer in the racemic mixture of aectyl-β-methylcholine.**
6. **Carbachol chloride**



1. **Carbachol differs chemically from ACh in its stability to hydrolysis. The carbamyl group of carbachol decreases the electrophilicity of the carbonyl and, thus, can form resonance structures more easily than can ACh. The result is that carbachol is less susceptible to hydrolysis and, therefore, more stable in aqueous solutions.**

****

1. **Unlike methacholine, carbachol has both nicotinic and muscarinic activity.**
2. **It can also act indirectly by promoting release of ACh and by its weak anticholinesterase activity (semireversible inhibitor of AChE) . Carbachol forms a carbamyl ester in the active site of AChE, which is hydrolyzed more slowly than an acetyl ester. This slower hydrolysis rate reduces the amount of free enzyme and prolongs the duration of ACh in the synapse.**



**Uses**

**Carbachol is a miotic and has been used to reduce the intraocular tension of glaucoma when a response cannot be obtained with pilocarpinc or neostigmine.**

1. **Bethanechol chloride**



1. **It has pharmacological properties similar to those of methacholine. Both are esters of β- methylcholine and have feeble nicotinic activity.**
2. **Bethanechol is inactivated more slowly by AChE in vivo than is methacholinc. It is a carbamyl ester and is expected to have stability in aqueous solutions similar to that of carbachol.**
3. **The main use of bethanechol chloride is in the relief of urinary retention and abdominal distention after surgery.**
4. **Bethancchol chloride should be used with caution in asthmatic patients; when used for glaucoma, it produces frontal headaches from the constriction of the sphincter muscle in the eye and from ciliary muscle spasms.**
5. **Pilocarpine Hydrochloride.**

****

**Pilocarpine is a nonselective agonist on the muscarinic receptors. Despite it acts on M3 receptors in smooth muscle to cause contractions in the gut, trachea and eye.**

 **In the eye, it produces pupillary constriction(miosis) and a spasm of accommodation. These effects are valuable in the treatment of glaucoma. Systemic effects include copious sweating, salivation, and gastric secretion**

**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. An anionic site was believed to have been formed by the γ-carboxylate**

 **group of a glutamic acid.**

1. **An esteratic Site, which causes hydrolysis of the ester portion of Ach.**
2. **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:-**

****

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

****

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**

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.**





**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 generate the free enzyme.**