**BIOCHEMICAL EFFECTS OF MUSCARINIC RECEPTOR STIMULATION**

**G proteins are so called because of their interaction with the guanosine triphosphate (GTP) and guanosine diphosphate (GDP). G proteins consist of three subunits, α, and β, and γ. When the receptor is occupied, the α subunit, which has enzymatic activity, catalyzes the conversion of GTP to GDP. The α subunit bound with GTP is the active form of the G protein that can associate with various enzymes (i.e., PLC and adenylate cyclase) and ion channels (K+andCa+2).**

**The binding of a signal molecule by the extracellular part of the G-protein linked receptor causes the cytosolic tail of the receptor to interact with, and alter the conformation of, a G-protein. This has two consequences:**

1. **The alpha subunit of the G- protein loses its GDP and binds a GTP instead**
2. **the G-protein breaks up into two components: the GTP-bound α part and the ß,γ dimer part.**



1. ***Phosphoinositol system .***

**The phosphoinositol requires the breakdown of membrane-bound inositol 4,5-diphosphate (PIP2) by PLC to 1P3(inositol triphosphate) and DAG(diacylglycerol) which serve as second messengers in the cell. IP3 mobilizes Ca+2 from intracellular stores in the endoplasmic reticulum to elevate cytosolic free Ca+2. The Ca+2 activates Ca+2 -dependent kinases (e.g., troponin C in muscle) directly or binds to the Ca+2-binding protein calmodulin, which activates calmodulin-dependent kinases. These kinases phosphorylate cell-specific enzymes to cause muscle contraction. DAG is lipid-like and acts in the plane of the membrane through activation of protein kinase C to cause the phosphorylation of cellular proteins, also leading to the muscle contraction.**



**Adenylate cyclase.**

**Adenylate cyclase, a membrane enzyme, is another target of muscarinic receptor activation.**

**The second messenger cAMP is synthesized within the cell from adenosine triphosphate (ATP) by the action of adenylate cyclase. The regulatory effects of cAMP are many, as it activate a variety of protein kinases. Protein kinases catalyze the phosphorylation of enzymes and ion channels, altering the amount of calcium entering the cell and thus affecting muscle contraction. Muscarinic receptor activation causes lower levels of cAMP, reducing cAMP protein-dependent kinase activity, and a relaxation of muscle contraction. Some have suggested that a GTP-inhihitory protein (Gi) reduces the activity of adenylate cyclase. causing smooth muscle relaxation.**

 **Cholinergic Neurochemistry**

**Cholinergic neurons synthesize, store, and release Ach. The neurons also form choline acetyltransferase (ChAT) and AChE. AChE is also located outside the neuron and is associated with the neuroglial cells in the synaptic cleft. ACh is prepared in the nerve ending by the transfer of an acetyl group from acetyl-coenzyme A (CoA) to choline. The reaction is catalyzed by ChAT. Much of the ACh is contained in synaptic vesicles in the nerve ending but that some is also free in the cytosol. Choline is the limiting substrate for the synthesis of Ach. Most choline for ACh synthesis comes from the hydrolysis of ACh in the synapse. Choline is recaptured by the presynaptic terminal as part of a high affinity uptake system under the influence of sodium ion to synthesize ACh.**

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**Several quaternary ammonium bases act as competitive inhibitors of choline uptake. Hemicholinium (HC-3l, and the triethyl analogue of choline. 2-hydroxy ethyl triethyl**

**ammonium, act at the presynaptic membrane to inhibit the high-affinity uptake of choline into the neuron. These compounds cause a delayed paralysis at repetitively activated cholinergic synapses and can produce respiratory paralysis in test animals. The delayed block is due to the depletion of stored ACh which may be reversed by choline.**



The acetyl group used for the synthesis of ACh is obtained by conversion of glucose to pyruvate in the cytosol of the neuron and eventual formation of acetyl- CoA. Because of the impermeability of the mitochondrial membrane to acetyl-CoA, this substrate is brought into the cytosol by the aid of an acetyl "carrier." The synthesis of ACh from choline and acetyl-CoA is catalyzed by ChAT.

Newly formed ACh is released from the presynaptic membrane when a nerve action potential invades a presynaptic nerve terminal. The release of ACh results from depolarization of the nerve terminal by the action potential, which alters membrane permeability to Ca+2. Calcium enters the nerve terminal and causes release of the contents of several synaptic vesicles containing ACh into the synaptic cleft. After ACh has been released into the synaptic cleft, its concentration decreases rapidly. It is generally accepted that there is enough AChE at nerve endings to hydrolyze into choline and acetate any ACh that has been liberated.

**Cholinergic Agonists**

**Cholinergic Stereochemistry**

**Conformational of ACh and other cholinergic chemicals have been studied by using three techniques:-**

1. **X-ray crystallography.**
2. **Nuclear magnetic resonance (NMR).**
3. **Molecular modeling by computation.**

**Each of these methods describes the spatial distribution of atoms in a molecule in terms of torsion angles.**

**A torsion angle (τ2):- is the angle between two planes formed by the four atoms .**

**Example**

**O1—C5—C4—N atoms in ACh**



**The angle between the oxygen and nitrogen atoms is best depicted by Newman projections.**



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***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 it’s important to the binding of the compound to the negatively charged aspartic acid residue in the muscarinic receptor.

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**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.**

**Q/ Hydrolysis by AChE is more affected by substitutions on the β than the α**

**carbon. Why?**

1. **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.**