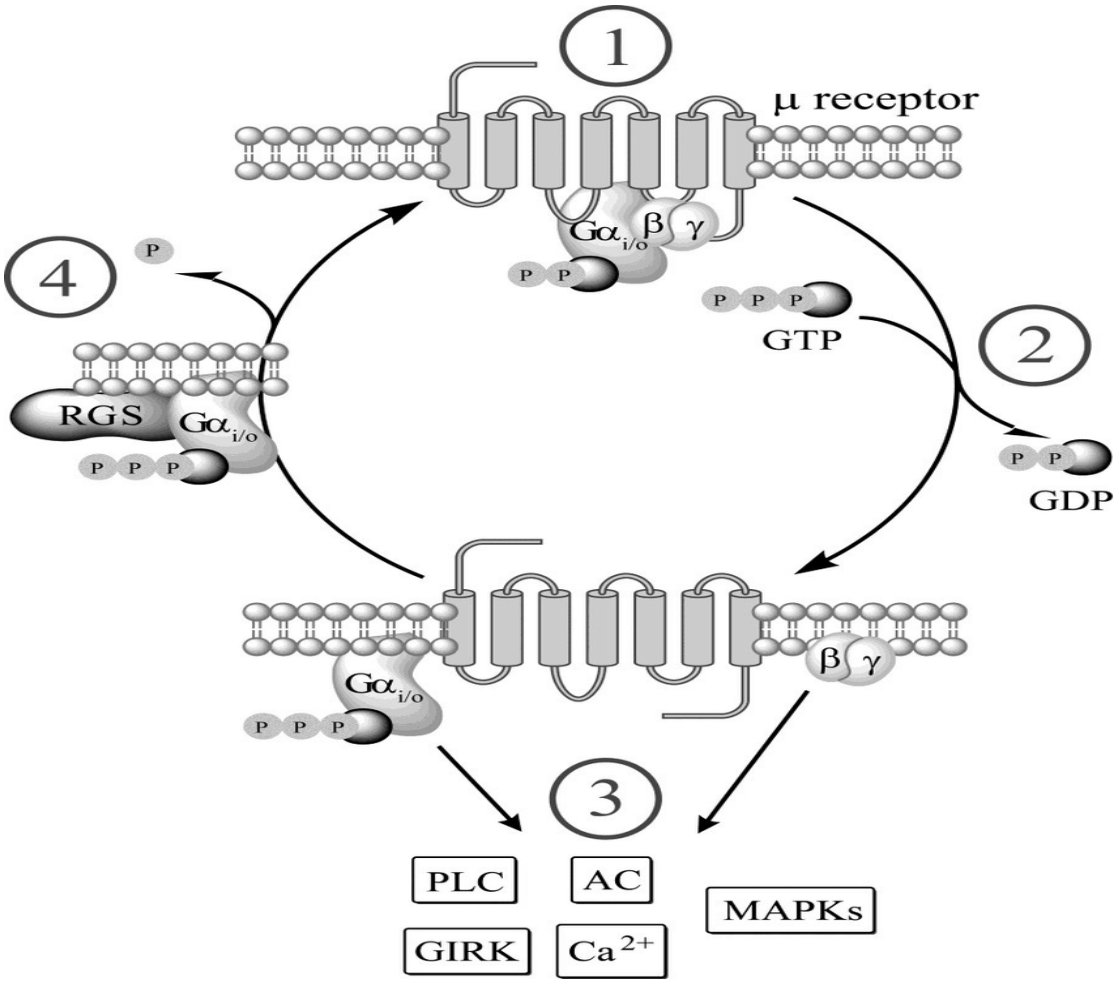
BIOCHEMICAL EFFECTS OF MUSCARINIC RECEPTOR STIMULATION

G proteins are so called because of their interaction with the guanine nucleotides 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}).

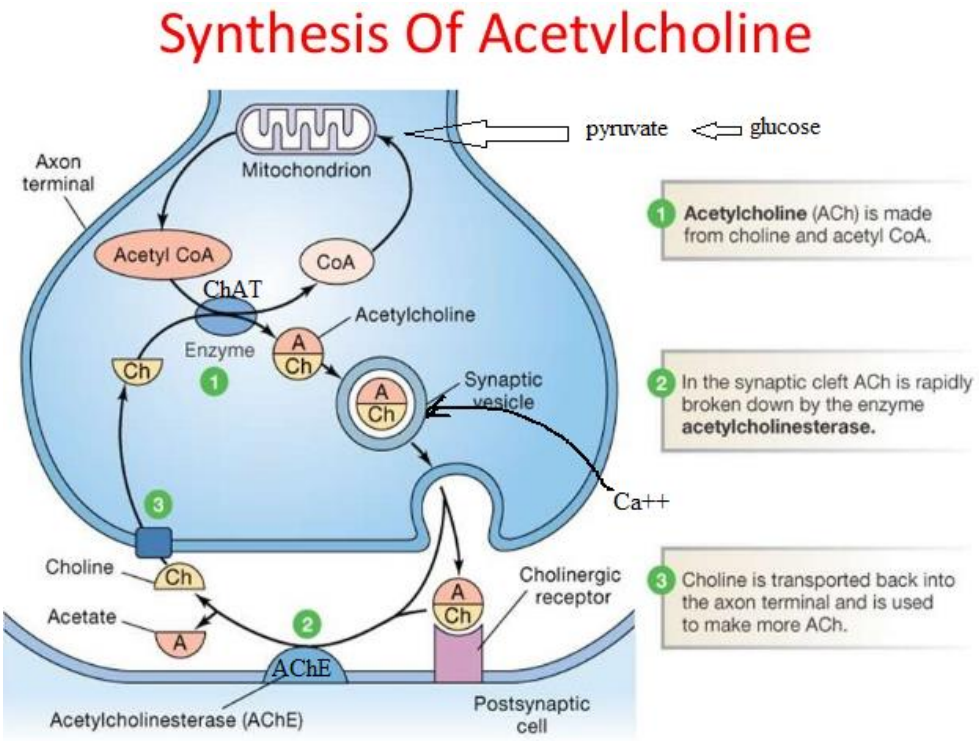
The binding of a signal molecule by the extracellular part of the G-protein linked receptor causes the cytosolic tail of the receptor to interact with, and alter the conformation of, a G-protein. This has two consequences:

- First, the alpha subunit of the G- protein loses its GDP and binds a GTP instead
- Second, the G-protein breaks up into the GTP-bound α part and the ß part.

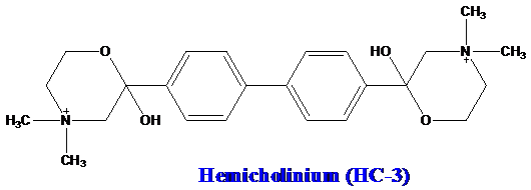


CHOLINERGIC NEUROCHEMISTRY

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 stored 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.



Several quatemary ammonium bases act as competitive inhibitors of choline uptake. Hemicholinium (HC-3) as example, 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.



Hemicholinium (HC-3)

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 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} .

Cholinergic Agonists

Cholinergic Stereochemistry

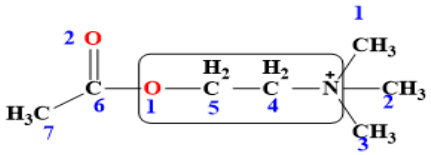
Conformational of ACh and other cholinergic chemicals have been studied by using different techniques:-

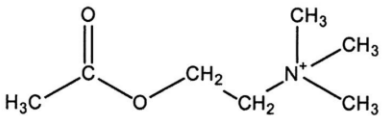
Each of these methods describes the spatial distribution of atoms in a molecule in terms of torsion angles.

A torsion angle (τ_2):- is the angle formed between two planes.

Example

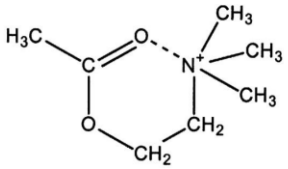
O1—C5—C4—N atoms in ACh





Extended

interact with muscarinic and AChE



Quasi-ring

with nicotinic receptors

Acetylcholine

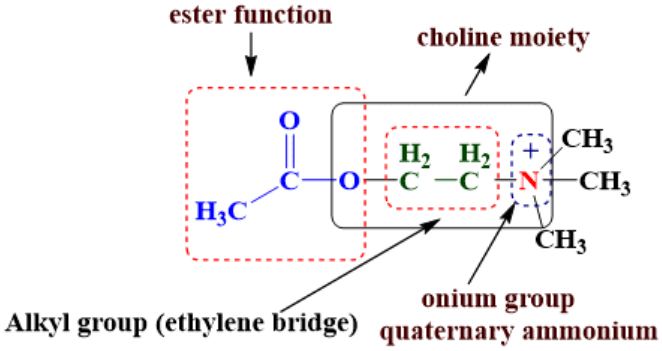
Structure-Activity Relationships

Cholinergic receptor Agonists

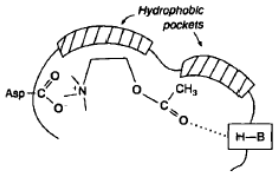
- Direct acting agonists:- bind and activate cholinergic receptors.
- Indirect-acting agonists:- increase synaptic [ACh] by either inhibiting AChE or increasing the release of ACh from terminals,

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

- The onium group.
- The ester function,
- The choline moiety.
- Alkyl group (ethylene bridge).

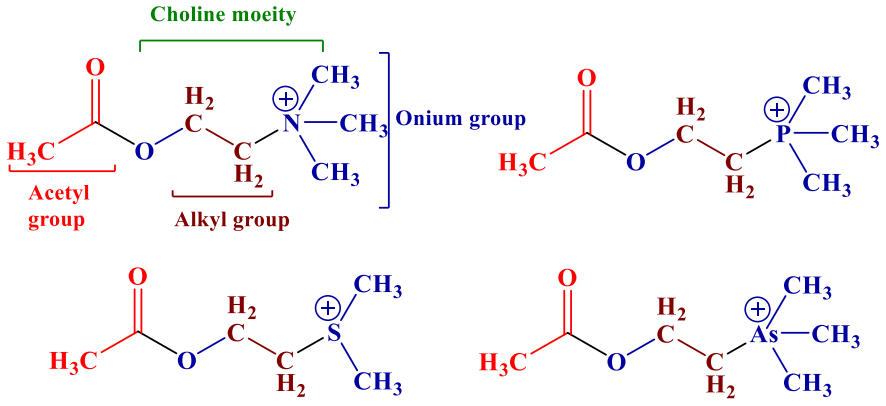


Ammonium Group($-N^+(CH_3)_3$). The onium group is essential for intrinsic activity and contributes to the affinity of the molecule for the receptors, because it's important to the binding of the compound to the negatively charged aspartic acid residue in the muscarinic receptor.



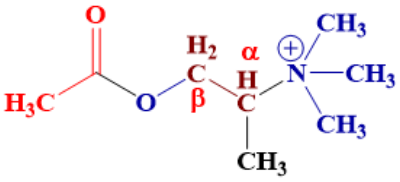
- The replacement of the ammonium moiety with either a sulfonium($-S^+(CH_3)_2$ or phosphonium($-P^+(CH_3)_3$ and arsenonium isosters results in a complete loss of activity.
- Increasing one methyl group to a larger alkyl (e.g., ethyl) results in 25% reduction in activity. Increase two methyl groups 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.



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.

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



Acetyl-α-methylcholine

Acetyl-β-methylcholine (Methacholine)

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

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.

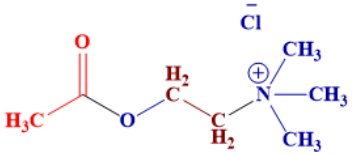
Arecoline act mainly at muscarinic receptors, but has some activity at nicotinic receptors. Arecoline is relatively selective as M_1-agonist.

Cholinergic Drugs and Related Agents

Direct acting cholinergic agents (Agonist)

Products

1-Acetylcholine Chloride



Acetylcholine chloride
Cholinechloride acetate

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

- Nonselective(when given systemically)
- 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

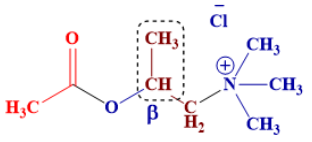
- Stimulation of the vagus and the parasympathetic nervous system produces tonic action on smooth muscle and induces a flow from the salivary and lacrimal glands.
- It is a cardio depressant and an effective vasodilator.

Its cardiac-depressant results from :-

- A negative chronotropic effect that decrease in heart rate .
- 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.

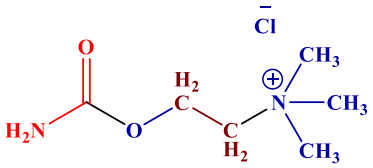
2-Methacholine Chloride



Methacholine chloride
Acetyl-β-methylcholine
(2-hydroxypropyl)trimethylammonium chloride acetate

- 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.
- Methacholine has little or no nicotinic activity

3-Carbachol chloride



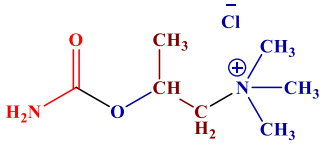
Carbacol chloride
Choline chloride carbamate

- 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.

- Unlike methacholine, carbachol has both nicotinic and muscarinic activity.
- 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.

4-Bethanechol chloride



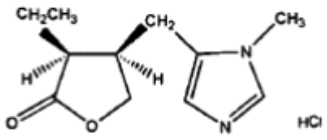
Bethanechol
β-methylcholinechloride carbamate
(2-hydroxypropyl)trimethylammonium chloride carhamate
carbamylmethylcholine chloride

- It has pharmacological properties similar to those of methacholine. Both are esters of β- methylcholine and have feeble nicotinic activity.
- 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.
- The main use of bethanechol chloride is in the relief of urinary retention and abdominal distention after surgery.
- 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



Pilocarpine hydrochloride