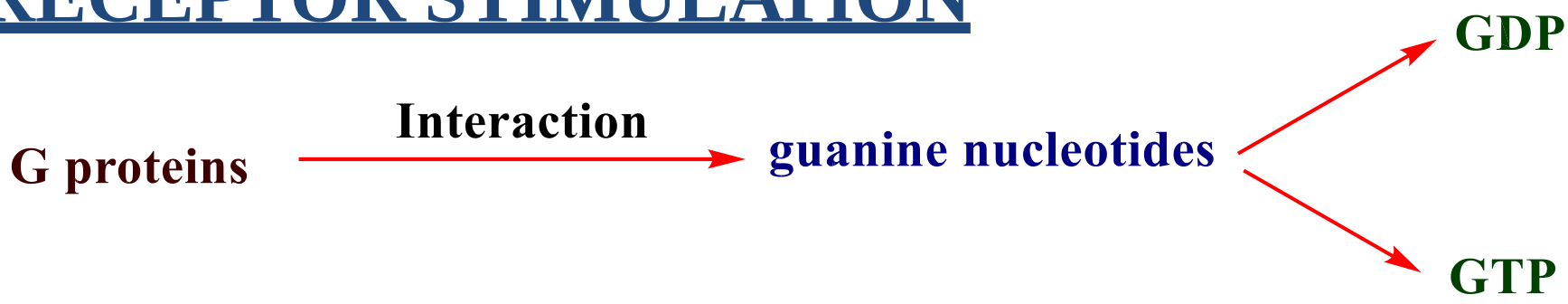
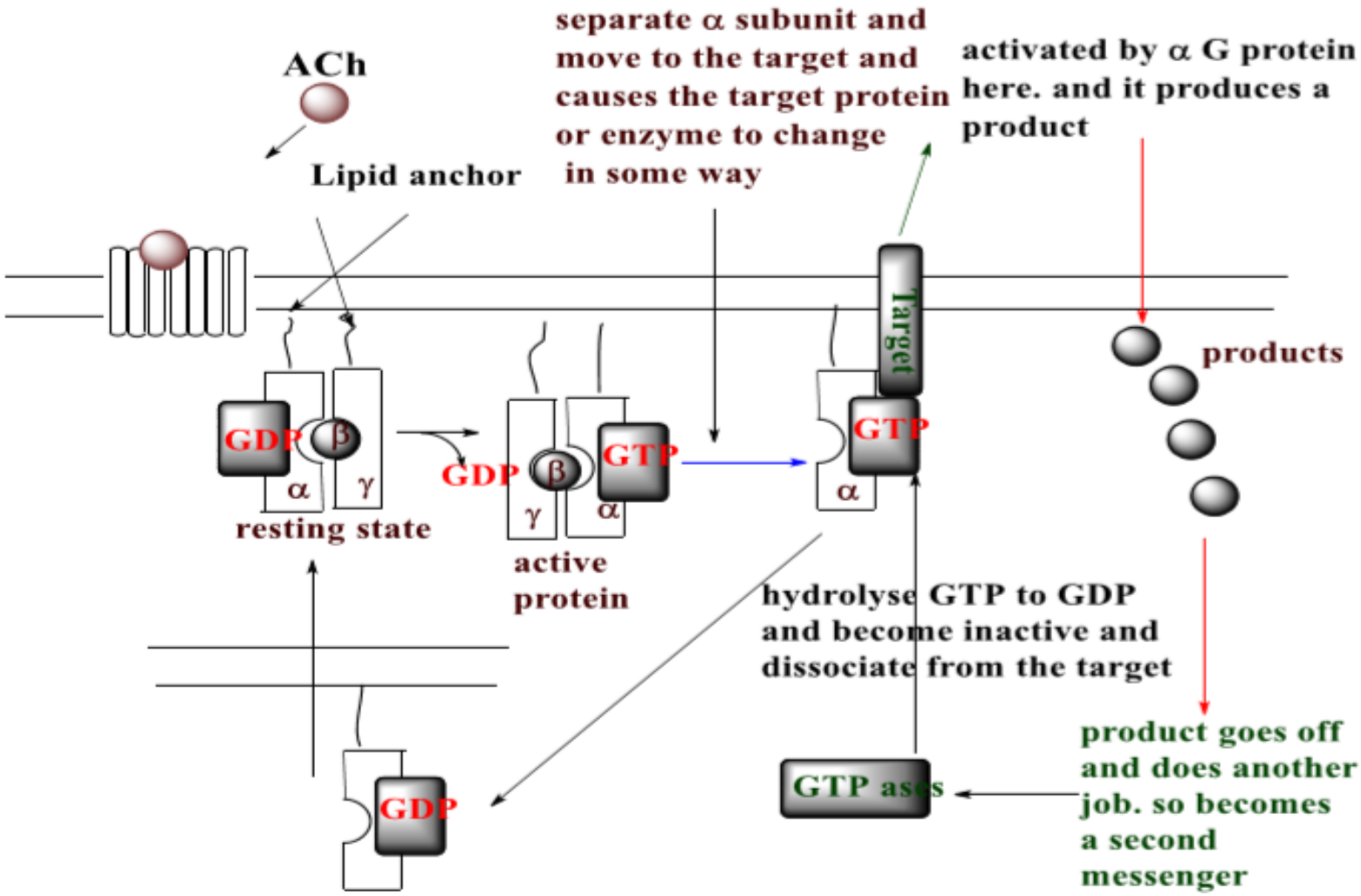
BIOCHEMICAL EFFECTS OF MUSCARINIC RECEPTOR STIMULATION

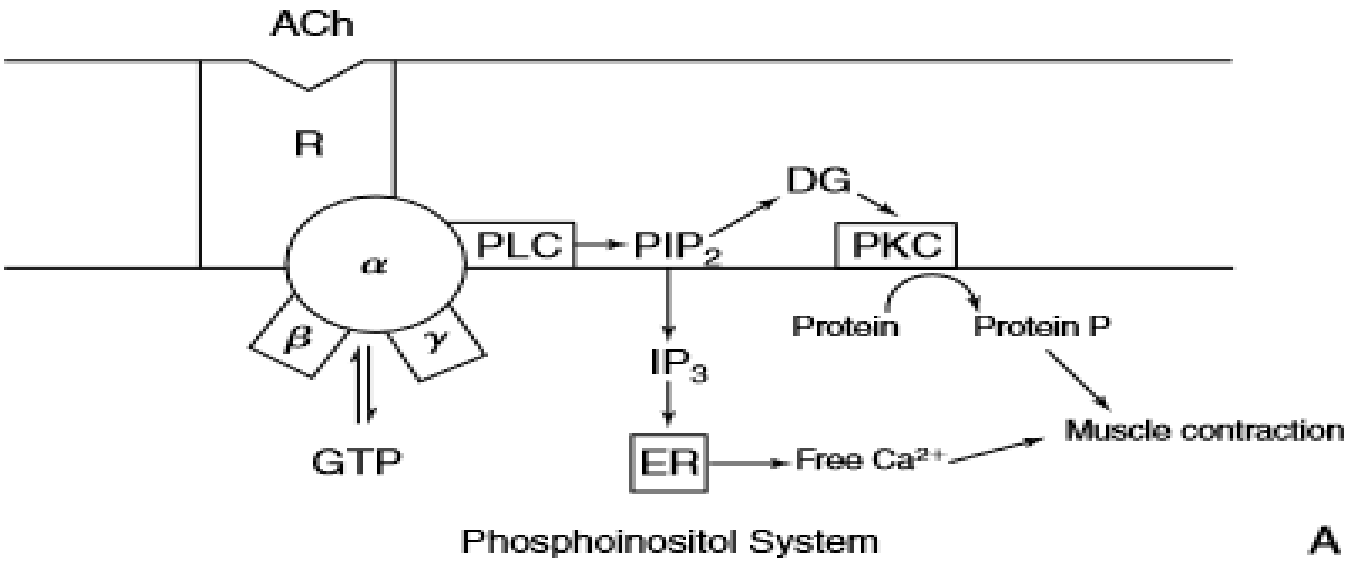


Transmission at the synapse involving second messengers is much slower, about 100 ms, compared with the few milliseconds at synapses where ion channels are activated directly. The delayed reaction to receptor stimulation is caused by a cascade of biochemical events that must occur to cause the pharmacological response. The sequence of events in these second-messenger systems begins with activation of the receptors by an agonist and involves the activation of G proteins that are bound to a portion of the intracellular domain of the muscarinic receptor. G proteins are so called because of their interaction with the guanine nucleotides GTP and guanosine diphosphate (GDP). They translate drug–receptor interactions at the surface of the cell to components inside the cell to create the biological response. G proteins consist of three subunits, α, β, and γ. When the receptor is occupied, the α subunit, which has enzymatic activity, catalyzes the conversion of GTP to GDP. The 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+ and Ca+2). G proteins are varied, and the α subunit may cause activation (Gs) or inactivation (Gi) of the enzymes or channels. Recent studies suggest that β and γ subunits also contribute to pharmacological effects.



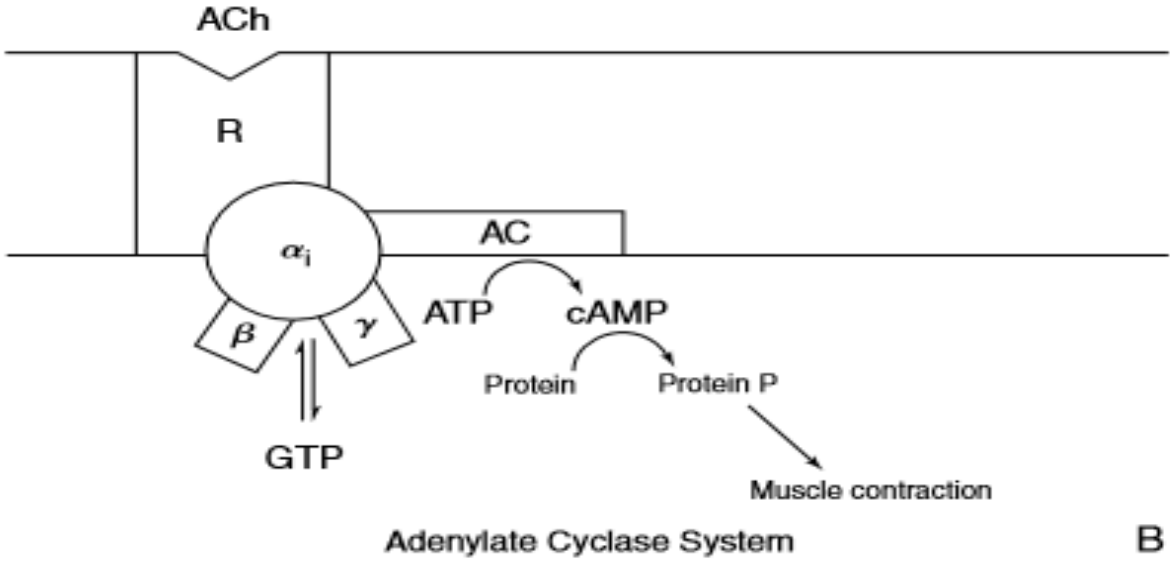
A single drug–receptor complex can activate several G protein molecules, and each in turn can remain associated with a target molecule (e.g., an enzyme) and cause the production of many molecules, amplifying the result of the
initial drug–receptor combination. M1, M3, and M5 receptors activate PLC, causing the release of IP3 and DAG, which in turn release intracellular Ca2 and activate protein kinases, respectively. M2 and M4 receptors produce inhibition of adenylate cyclase.

• Phosphoinositol system



The phosphoinositol system requires the breakdown of membrane-bound phosphatidylinositol 4,5-diphosphate (PIP2) by PLC to IP3 and DAG, which serve as second messengers in the cell. IP3 mobilizes Ca2 from intracellular stores in the endoplasmic reticulum to elevate cytosolic free Ca2. The Ca2 activates Ca2dependent kinases (e.g., troponin C in muscle) directly or binds to the Ca2-binding protein calmodulin, which activates calmodulin-dependent kinases. These kinases phosphorylate cell-specific enzymes to cause muscle contraction. DAG is lipidlike and acts in the plane of the membrane through activation of protein kinase C to cause the phosphorylation of cellular proteins, also leading to muscle contraction

Adenylate cyclase.

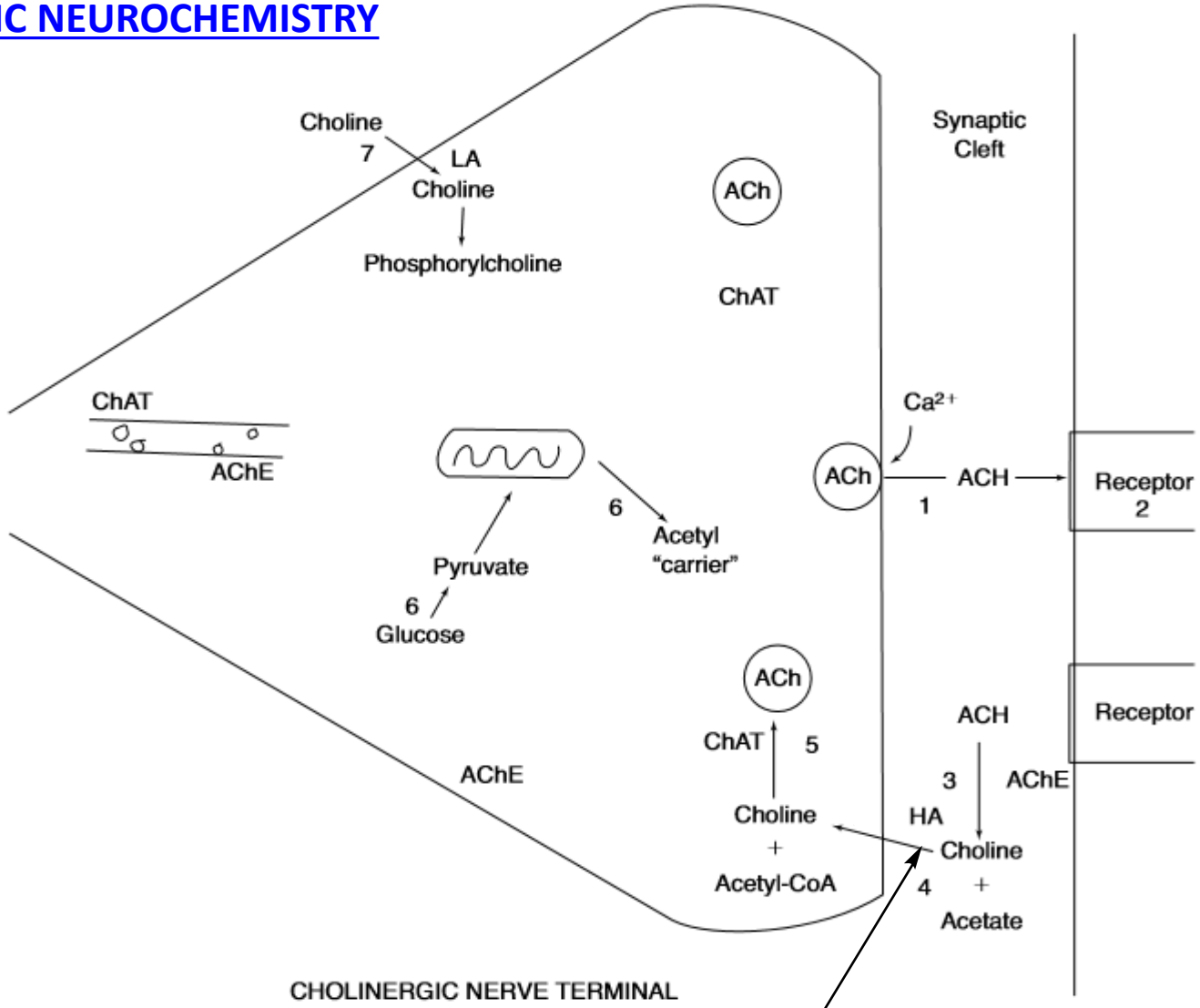


Adenylate Cyclase System B

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 can activate various 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-inhibitory protein (Gi) reduces the activity of adenylate cyclase, causing smooth muscle relaxation

Ion Channels. In addition to the action of protein kinases that phosphorylate ion channels and modify ion conductance, G proteins are coupled directly to ion channels to regulate their action. The Ca2 channel on the cell membrane is activated by G proteins without the need of a second messenger to allow Ca2 to enter the cell. The subunit of the G protein in heart tissue acts directly to open the K channel, producing hyperpolarization of the membrane and slowing the heart rate.

CHOLINERGIC NEUROCHEMISTRY



quatemary ammonium bases act as competitive inhibitors of choline uptake

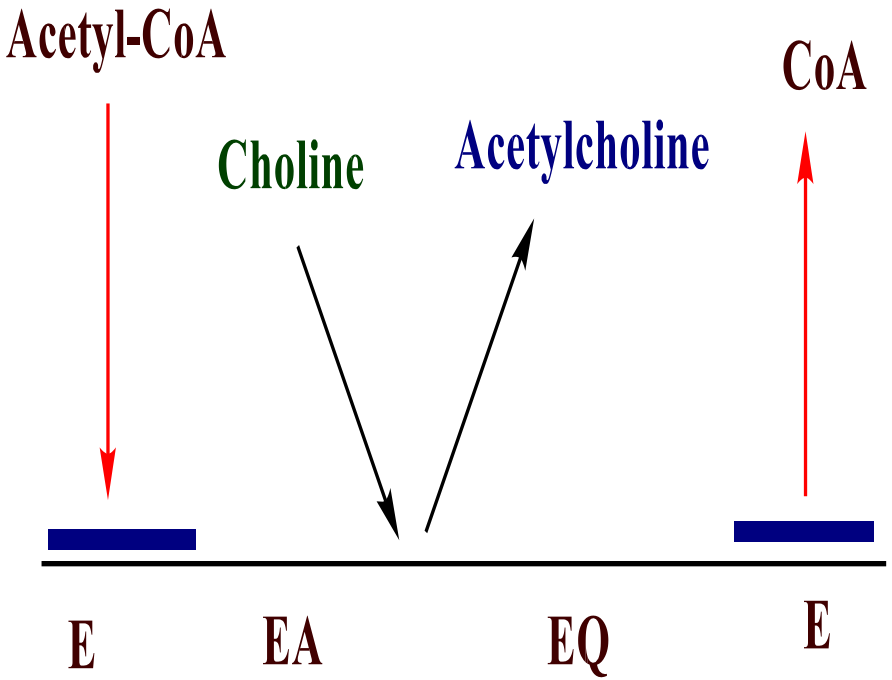
Hemicholinium (HC-3),

2-hydroxyethyltriethyl ammonium

Cholinergic neurons synthesize, store, and release ACh. The neurons also form choline acetyltransferase (ChAT) and AChE. These enzymes are synthesized in the soma of the neuron and distributed throughout the neuron by axoplasmic flow. 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. Cell fractionation studies show that 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 ions to synthesize ACh.

Several quaternary ammonium bases act as competitive inhibitors of choline uptake. Hemicholinium (HC-3), a bisquaternary cyclic hemiacetal, and the triethyl analog of choline, 2-hydroxyethyltriethylammonium, 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 caused by 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 acetylCoA. 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. Transfer of the acetyl group from acetylCoA to choline may be by a random or an ordered reaction of the Theorell-Chance type. In the ordered sequence, acetylCoA first binds to the enzyme, forming a complex (EA) that then binds to choline. The acetyl group is transferred, and the ACh formed dissociates from the enzyme active site. The CoA is then released from the enzyme complex, EQ, to regenerate the free enzyme. The scheme is diagrammed in Figure below. ChAT is inhibited in vitro by trans-N-methyl4-(1-naphthylvinyl)pyridinium iodide; Newly formed ACh is released from the presynaptic membrane when a nerve action potential invades a presynaptic nerve terminal.



Order synthesis of acetylcholine (ACh) by choline acetyltransferase (ChAT)

The release of ACh results from depolarization of the nerve terminal by the action potential, which alters membrane permeability to Ca2. Calcium enters the nerve terminal and causes release of the contents of several synaptic vesicles containing ACh into the synaptic cleft. This burst, or quantal release, of ACh causes depolarization of the postsynaptic membrane. The number of quanta of ACh released may be as high as several hundred at a neuromuscular junction, with each quantum containing between 12,000 and 60,000 molecules. ACh is also released spontaneously in small amounts from presynaptic membranes. This small amount of neurotransmitter maintains muscle tone by acting on the cholinergic receptors on the postsynaptic membrane.

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. For example, there is sufficient AChE in the nerve junction of rat intercostal muscle to hydrolyze about $2.7 * 10^8$ ACh molecules in 1 ms; this far exceeds the $3 * 10^6$ molecules released by one nerve impulse

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).

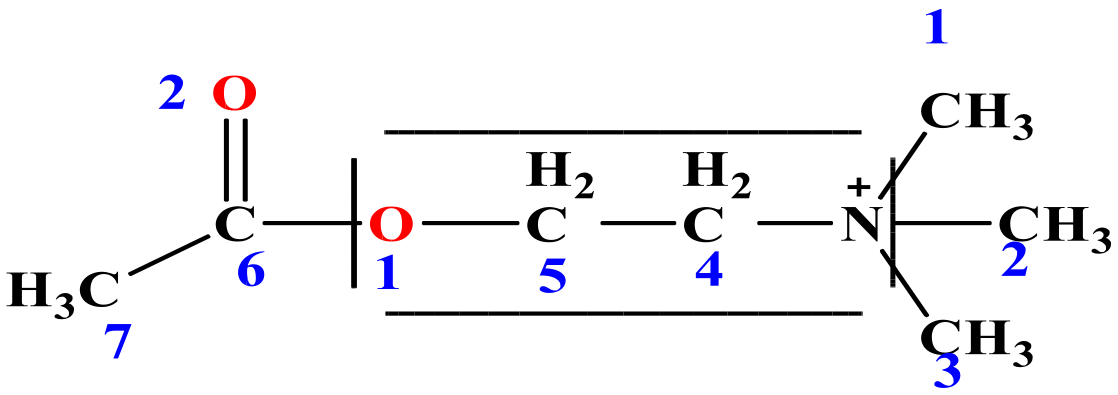
-3 Molecular modeling by computation.

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

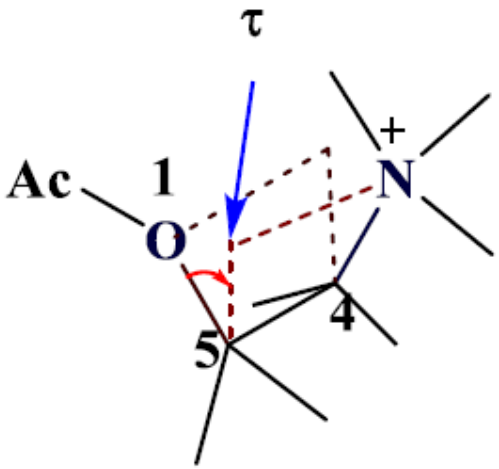
A torsion angle (τ_2):- is the angle formed between two planes.

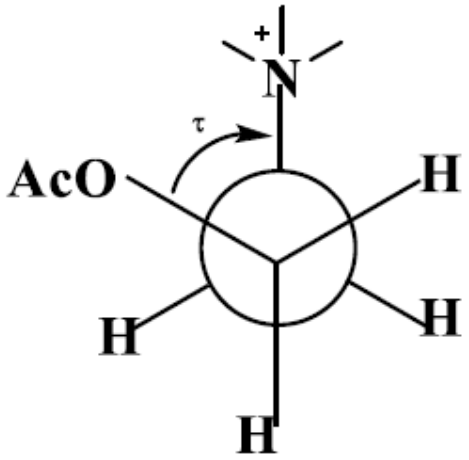
Example

O1—C5—C4—N atoms in ACh



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





Newman projection

A torsion angle has a positive sign when the bond of the front atom is rotated to the right to eclipse the bond of the rear atom.

The spatial orientation of ACh is described by four torsion angles (Fig. 17.8).

τ_1 C5—C4—N—C3
τ_2 O1—C5—C4—N
τ_3 C6—O1—C5—C4
τ_4 C7—C6—O1—C5

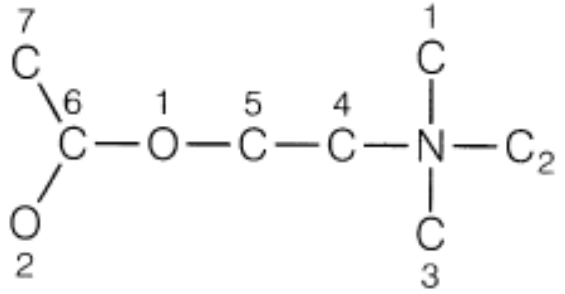
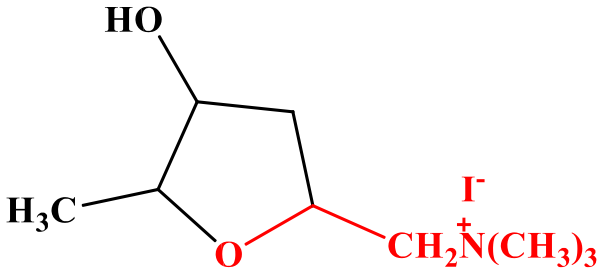


Figure 17.8 • ACh torsion angles.

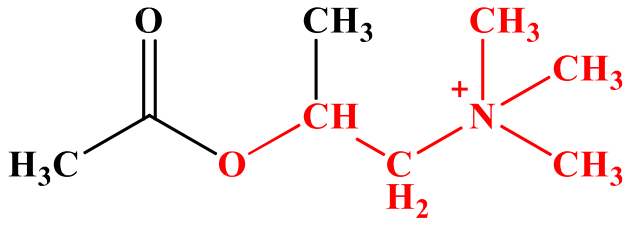
The conformation of the choline moiety of ACh has drawn the most attention in studies relating structure and pharmacological activity. The torsion angle (τ_2) determines the spatial orientation of the cationic head of ACh to the ester group.

X-ray diffraction studies have shown that the torsion angle (τ_2) on ACh has a value of + 77°.

Many compounds that are muscarinic receptor agonists containing a choline component—e.g., O-C-C-$N^+(CH_3)_3$—have a preferred synclinal (gauche) conformation, with values ranging from 68 to 89° (table 17-2, p555.



(+)-2S,3R,5S-muscarine iodide
muscarinic rec. agonist
preferred synclinal (gauche)conformation
τ_2=73°



(+)-Acetyl-(S)-β-methylcholineiodide
muscarinic rec. agonist
preferred synclinal (gauche)conformation
τ_2=85°

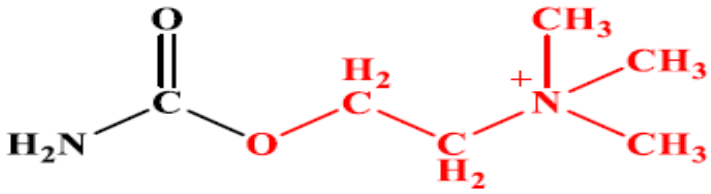
There are two dominant factors that lead to a preference the synclinal conformation in the crystal state:-

1-Intermolecular packing forces in the crystal.

2-Electrostatic interactions between the nitrogen group and the ether oxygen of the ester group.

Some choline esters display an antiperiplanar (*trans*) conformation between the onium and ester groups. For example, carbamoyl choline chloride (T2, 178°) is stabilized in this *trans* conformation by several hydrogen bonds.

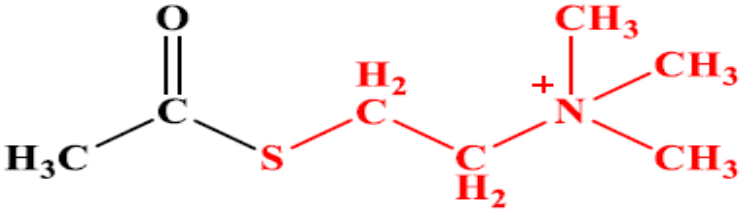
Antiperiplanar (trans) conformation between the onium and ester groups, occurs in some choline esters.



cabamoly choline iodide

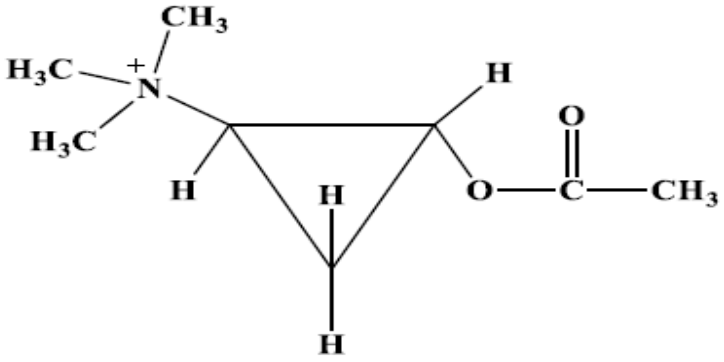
$\tau_2 = 178°$

in this conformation because of the presence of the bulkier and less electronegative sulfur atom,



acetyl thiocholinechloride

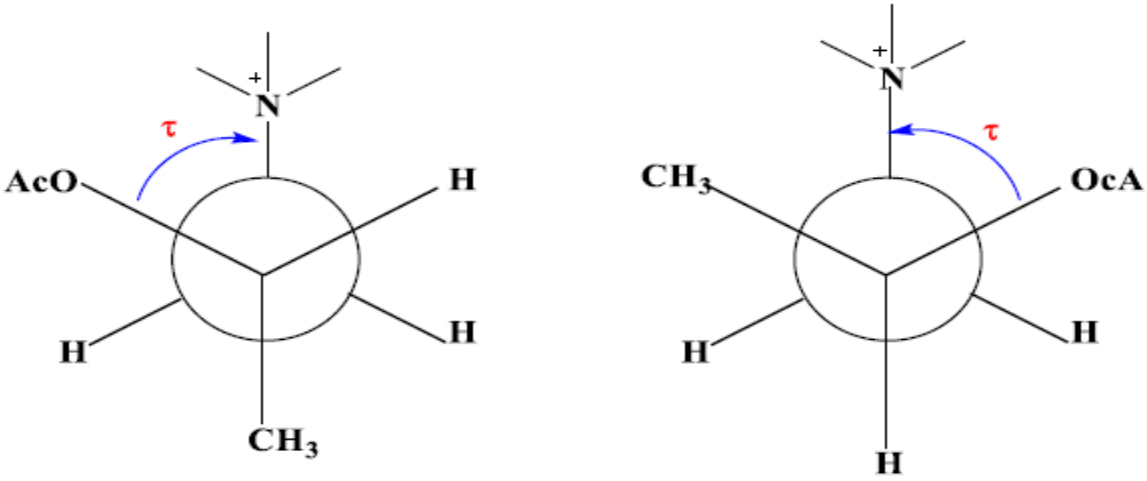
$\tau_2 = 171°$



***(+) -trans*-2-acetoxycyclopropyltrimethylammonium**

$\tau_2 = 137°$

NMR spectroscopy of cholinergic molecules in solution



Gauch conformers of methacholine

NMR spectroscopy of cholinergic molecules in solution is more limited than crystallography in delineating the conformation of compounds and is restricted to determining the torsion angle O1-C5-C4-N.

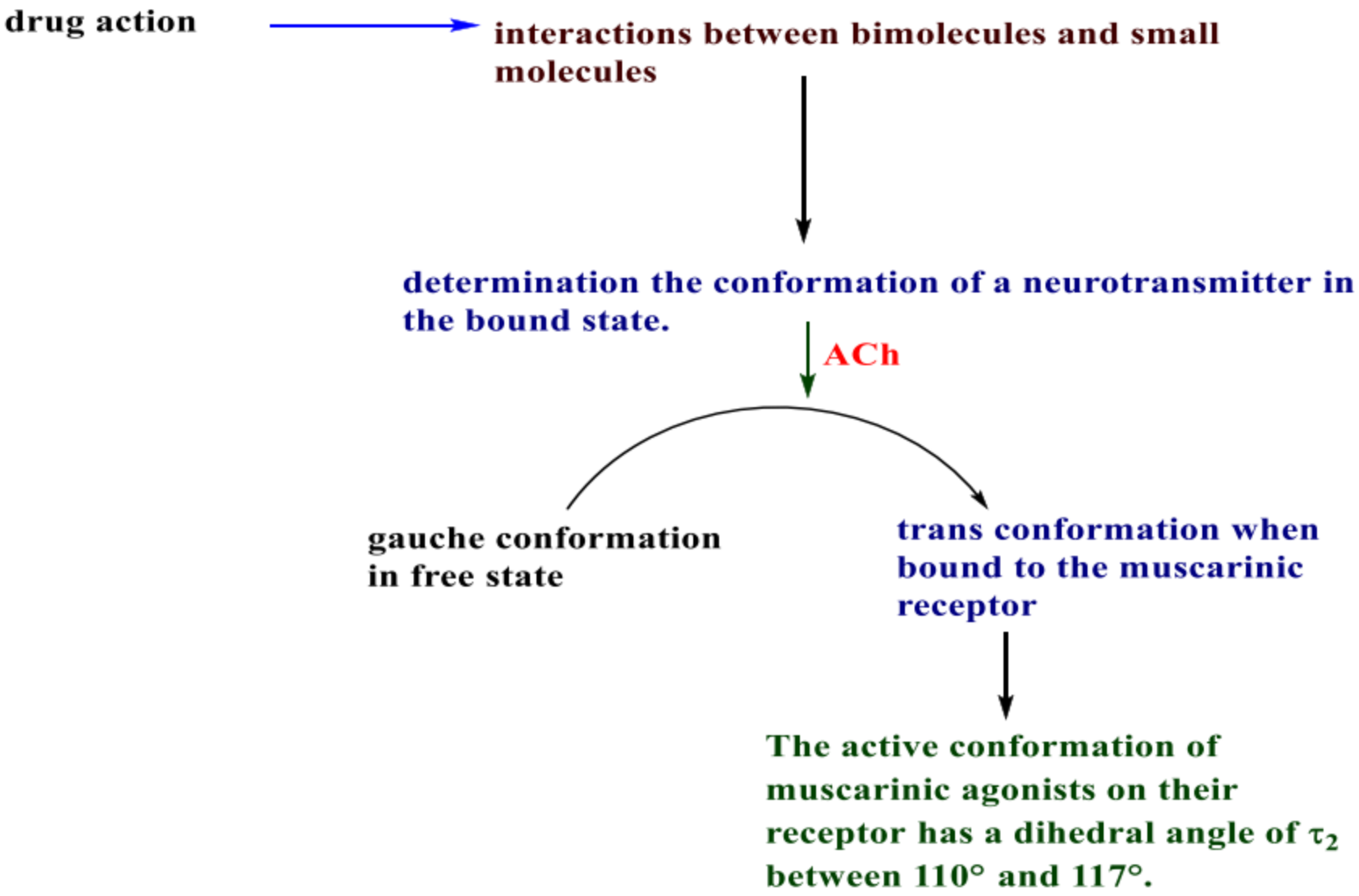
Most NMR data are in agreement with the results of x-ray diffraction studies. NMR studies indicate that ACh and methacholine apparently are not in their most stable trans conformation but exist in one of two gauche conformers . This may result from strong intramolecular interactions that stabilize the conformation of these molecules in solution.

Molecular orbital calculations based on the principles of quantum mechanics :-may be used to determine energy minima of rotating bonds and to predict preferred conformations for the molecule.

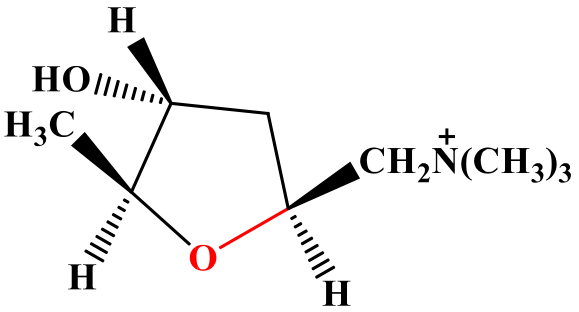
τ_2 torsion angle at about 84° for Ach

And that the preferred conformation of ACh corresponds closely in aqueous solution to that found in the crystal state

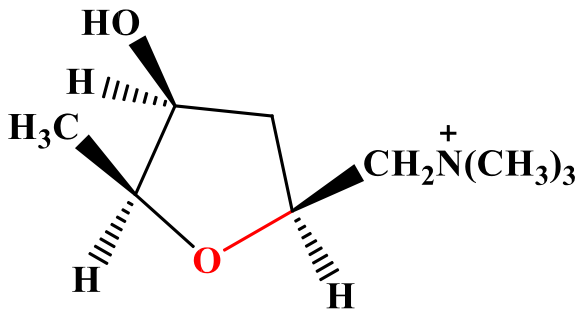
the nicotinic receptors are not considered as highly stereoselective as their muscarinic counterparts.


drug action
interactions between bimolecules and small molecules
determination the conformation of a neurotransmitter in the bound state.
ACh
gauche conformation in free state
trans conformation when bound to the muscarinic receptor
The active conformation of muscarinic agonists on their receptor has a dihedral angle of τ_2 between 110° and 117°.

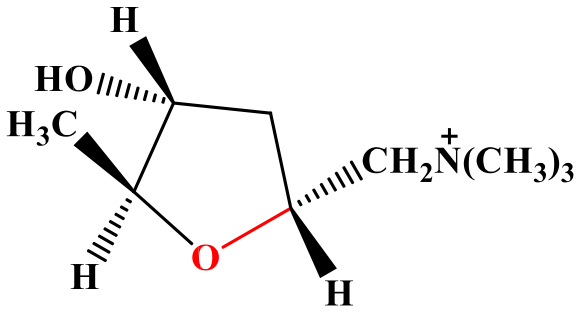
Muscarine has four geometric isomers: muscarine, epimuscarine, allomuscarine, and epiallomuscarine



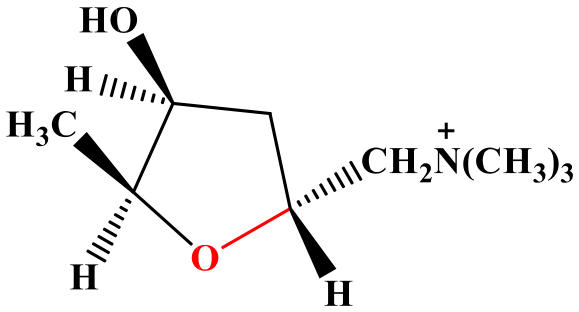
muscarine



epimuscarine



Allomuscarine



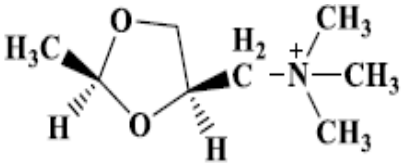
epiallomuscarine

None has a center or plane of symmetry. Each geometric isomer can exist as an enantiomeric pair. The activity of muscarine, a nonselective muscarinic receptor agonist, resides primarily in the naturally occurring (+)- muscarine enantiomer.

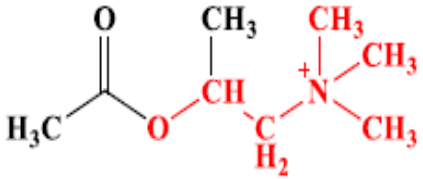
It is essentially free of nicotinic activity and apparently has the optimal stereochemistry to act on the muscarinic receptor subtypes.

Synthetic molecules with a substituent on the carbon atom that corresponds to the β carbon of ACh also show great differences in muscarinic activity between their isomers.

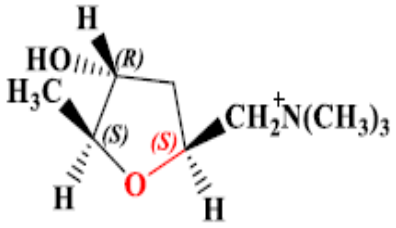
Their (S)/(R) ratios (Table 17.3) show the greatest stereoselectivity of the muscarinic receptor in guinea pig ileum for the configuration at the carbon adjacent to the ester group. In contrast, the nicotinic receptors are not considered as highly stereoselective as their muscarinic counterparts.



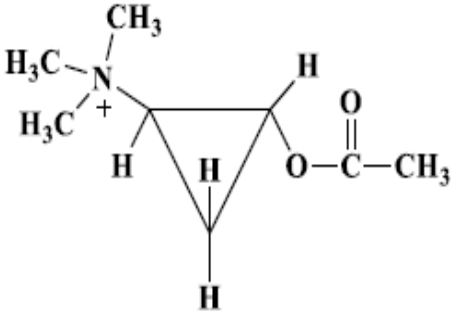
cis-2-methyl-4-trimethylammonium-1,3 dioxolane



(+)-Acetyl-(S)-β-methylcholineiodide



(+)-2S,3R,5S-muscarine



trans-2-acetoxycyclopropyltrimethylammonium

These are more potent than their enantiomers and have very high ratios of activity between the (S) and (R) isomers.