Chapter 12

Drugs Affecting Cholinergic Neurotransmission

E. Kim Fifer

Introduction

It is likely that no other mammalian system or chemical neurotransmitter has been studied as exhaustively as the parasympathetic nervous system and acetylcholine. Acetylcholine functions as the neurotransmitter for many different neurons (Fig. 12.1) (1). In the autonomic nervous system, it is released by pre- and postganglionic fibers of the parasympathetic division, preganglionic fibers of the sympathetic division, and a few postganglionic fibers of the sympathetic division (e.g., sweat and salivary glands). It is also released by neurons of the somatic (voluntary) nervous system and by some neurons in the central nervous system (CNS). Neurons that release acetylcholine are referred to as cholinergic, as are the receptors on which these neurons synapse. These receptors are further classified as either muscarinic or nicotinic, depending on their ability to bind the naturally occurring alkaloid muscarine or nicotine, respectively. Parasympathetic nerve impulses stimulate contraction of smooth muscle in the gastrointestinal tract and urinary tract, contraction of the ciliary muscle of the eye, relaxation of smooth muscle of the blood vessels, and decreased heart contractility and rate.

Chemical compounds that cause stimulation of the parasympathetic nervous system are called cholinomimetic or, more specifically, parasympathomimetic agents. Cholinomimetic agents might be agonists that act directly on cholinergic receptors or function as inhibitors of acetylcholinesterase (AChE), the enzyme responsible for hydrolysis of acetylcholine. Those compounds that possess affinity for cholinergic receptors but exhibit no intrinsic activity are called cholinergic antagonists, cholinolytic, or parasympatholytic agents. This chapter is devoted to the discussion of cholinergic agonists and antagonists and to the biochemistry of cholinergic neurotransmission.
Studies of the parasympathetic nervous system and cholinergic agents led to the concept of neurochemical transmission and were instrumental in the development of early drug receptor hypotheses and our understanding of the stereochemical influence on drug action. An excellent summary of this history is presented by Holmstedt (2).

In 1914, Dale defined two subdivisions of the parasympathetic nervous system when he observed that ethers and esters (including acetylcholine) of choline produced effects similar to those of muscarine (muscarinic effects) or nicotine (nicotinic effects) (2). The initial experiments were performed using an ergot extract contaminated with acetylcholine, although Dale was unaware of this contamination. Ewins, a chemist who collaborated with Dale, isolated acetylcholine from the ergot extract and subsequently synthesized acetylcholine, thus allowing Dale to show that the unexpected muscarinic effects observed with the ergot preparation were the result of acetylcholine. He proposed the term “parasympathomimetic” to describe the ability of acetylcholine to produce the same effects as electrical stimulation of parasympathetic nerves, and he suggested that acetylcholine was a chemical neurotransmitter in the parasympathetic nervous system. Dale also observed that the action of acetylcholine was short-lived, and he proposed that tissues contained an esterase that hydrolyzed acetylcholine. In 1921, Loewi (3) demonstrated that a chemical compound mediated impulses between nerves; he referred to the chemical substance in his preparation as vagusstoff. In 1926, Loewi and Navratil (4) provided experimental evidence suggesting that vagusstoff was acetylcholine.

Clinical Significance

Agents affecting cholinergic neurotransmission are some of the most widely studied agents to date. It also is one of the most intriguing classes of study in that the clinical utility of these compounds runs the gamut from the life-saving potential of atropine given to patients undergoing cardiac life support to the life-threatening capacity of chemical warfare agents, such as sarin. Advancements in our understanding of muscarinic and nicotinic receptor activity and compounds that modulate these effects have led to decreased morbidity and mortality and increased quality of life for millions of individuals throughout the world. Additionally, those agents employed as insecticides or pesticides have had tremendous economic impact as well. Agents affecting cholinergic neurotransmission are used to treat a variety of clinical conditions, including impaired or excessive gastric motility/secretion, glaucoma, bradycardia, Alzheimer’s disease, Parkinson’s disease, and myasthenia gravis. Nicotinic antagonists are used to facilitate surgical procedures by reducing the amount of anesthetic or sedative agent required, thus reducing risk to the patient.

A thorough understanding of the structure–activity relationships of these compounds has led to an ability to enhance their desired pharmacodynamic effects while minimizing unwanted or harmful adverse effects. The impact of the application of this knowledge is multifaceted for the clinician. Examples include the synthesis of newer chemical compounds used to treat Alzheimer’s disease that provide greater affinity for acetylcholinesterase in the brain than in the periphery, decrease the frequency of dosing required, and alleviate the risk of hepatotoxicity that was associated with the first Alzheimer’s agent, tacrine. These advances have increased the utility of these agents and given hope...
to countless individuals and families affected by this devastating disease. Modifications to the neuromuscular blocking agents have resulted in differences in onset and length of activity, reduction in adverse effects (e.g., hypotension), and alternate routes of elimination, which increase their utility for patients with certain comorbid conditions (e.g., cardiac disease or renal dysfunction). Finally, it also is important for the clinician to recognize the capacity of certain chemical configurations to be allergenic or more prone to producing adverse effects so that the best agent for a particular patient can be selected.

**Kathryn Neill, Pharm.D.**

*Assistant Professor*

*Critical Care Specialist*

*Department of Pharmacy Practice*

*College of Pharmacy*

*University of Arkansas for Medical Sciences*

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**Fig. 12.1.** Schematic representation of autonomic and somatic motor nerves. The sites of action of acetylcholine (ACh), norepinephrine (NE), epinephrine (Epi), and dopamine (D) are indicated. Cholinergic receptors are designated as nicotinic (N) or muscarinic (M). (From Katzung BG. Introduction to autonomic pharmacology. In: Katzung BG, ed. Basic and Clinical Pharmacology, 9th Ed. New York: McGraw-Hill, 2004, pp. 75–93; with permission.)

These classic studies are the foundation of our current understanding about the role of acetylcholine in cholinergic nerve transmission and our recognition of muscarinic and nicotinic cholinergic receptors. They provided the stimulus for subsequent studies of acetylcholine biochemistry, synthesis of new organic compounds (e.g., cholinergic and anticholinergic drugs), and purification of cholinergic receptors.

The concept that muscarinic and nicotinic receptors may explain the different physiologic responses...
produced by acetylcholine was derived from this early research of Dale and Loewi. Although it currently is recognized that there are multiple subclasses of both muscarinic and nicotinic receptors, the general classification of these two types of cholinergic receptors continues to
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effectively explain the different physiologic responses produced by acetylcholine.

Because of the important role of acetylcholine as a chemical neurotransmitter in the autonomic nervous system, an imbalance in parasympathetic tone can lead to serious consequences. Conceptually, deficiencies in acetylcholine could be treated by administering the neurotransmitter itself, but acetylcholine is a poor therapeutic agent. Its actions are nonselective, producing effects at all cholinergic receptor sites, which could result in serious consequences for the patient. Because acetylcholine is a quaternary ammonium salt, it is poorly absorbed across biological membranes, resulting in poor bioavailability regardless of the route of administration. Furthermore, its ester functional group is rapidly hydrolyzed in the acidic conditions of the gastrointestinal tract and by esterases in plasma.

Muscarinic cholinergic agents are used postsurgically to reestablish smooth muscle tone of the gastrointestinal and urinary tracts to relieve abdominal distention and urinary retention. They also are used to treat some forms of glaucoma by enhancing the outflow of aqueous humor, thereby reducing intraocular pressure. Cholinomimetic compounds with CNS activity are being evaluated for the treatment of cognitive disorders (e.g., Alzheimer’s disease). Those that exhibit nicotinic effects are commonly used to treat myasthenia gravis.

Cholinergic muscarinic antagonists (anticholinergic drugs) are sometimes referred to as antispasmodics because of their ability to reduce smooth muscle spasms resulting from overstimulation of the gastrointestinal smooth muscles. Newer drugs in this class have found use in the treatment of overactive bladder.

Many synthetic cholinergic agonists have been designed using structure–activity relationships (SARs) based on the structure of acetylcholine. To design cholinergic agents that are selective for specific cholinergic receptors, it is necessary to have a thorough understanding of acetylcholine neurochemistry as well as the chemical nature and role of cholinergic receptors.

**Cholinergic Receptors**

**History**

Much effort has gone into understanding how cholinergic receptors carry out the two primary functions of all receptors—molecular recognition and signal transduction. A thorough understanding of these phenomena is essential to achieving rational, efficient, and selective drug therapy.

Knowledge regarding the structure and function of cholinergic receptors has increased substantially since the concept of distinct muscarinic and nicotinic receptors was first postulated. Early efforts to describe these receptors were hindered in that receptors were only a concept. Indeed, their existence was not established until 1973, when Pert and Snyder (5) provided demonstrable evidence for the existence of opiate receptors.

Early attempts to characterize cholinergic receptors were based on SAR and stereochemical studies of cholinergic agonists and antagonists. This led to synthesis of agonists and antagonists with exceptionally
high affinity and selectivity for cholinergic receptors as well as to synthesis of radiolabeled cholinergic ligands with high specific radioactivity. These advances were paralleled by advances in biochemistry, molecular pharmacology, and molecular biology that made possible the purification and sequencing of small quantities of protein, the measurement of ligand binding to cell membranes and subcellular components, and the cloning and sequencing of genes. This led to isolation, purification, and amino acid sequencing of one of the nicotinic cholinergic receptors—the first acetylcholine receptor and the first neurotransmitter receptor to be fully characterized (6,7). Subsequently, muscarinic receptors were isolated, purified, and sequenced using these techniques.

Current pharmacological and molecular biological research indicates that multiple muscarinic and nicotinic acetylcholine receptor subtypes exist (8,9). The traditional classification of muscarinic and nicotinic receptors, however, adequately describes the actions of most cholinergic medicinal agents and is used throughout this chapter. Furthermore, most of the current therapeutic agents acting at muscarinic receptors exhibit little selectivity for the receptor subtypes, with the exception of a few recently introduced anticholinergic agents for treatment of overactive bladder.

**Muscarinic Receptors**

The SAR regarding the affinity and efficacy of cholinergic agonists provided the basis for early models of muscarinic receptor structure. An early model of the muscarinic receptor, depicted in Fig. 12.2, illustrates the importance of muscarinic agonists having an ester functional group and a quaternary ammonium group separated by two carbons. This model depicts ionic bonding between the positively charged quaternary nitrogen of acetylcholine and a negative charge at the anionic site of the receptor. The negative charge was suggested to result from a carboxylate ion from the free carboxyl group of a dicarboxylic amino acid (e.g., aspartate or glutamate) at the binding site of the receptor protein. This model also involved a hydrogen bond between the ester oxygen of acetylcholine and a hydroxyl group contributed by the esteratic site of the receptor.

Although this early muscarinic receptor model accounted for two important SAR requirements for muscarinic agonists, it failed to explain the following: 1) At least two of the alkyl groups bonded to the quaternary nitrogen must be methyl groups, 2) the known stereochemical requirements for agonist binding to the receptor, and 3) the fact that all potent cholinergic agonists have only five atoms between the quaternary nitrogen and the terminal hydrogen atom. This last point is known as Ing's “Rule of Five” (10).
Subsequent models of the cholinergic muscarinic receptor depicted the receptor as a binding site on a protein molecule and explained more completely the structural and stereochemical requirements for cholinergic agonist activity. Some scientists proposed that the muscarinic receptor and AChE were the same entity, but this proposal was dispelled by experiments demonstrating that interaction of cholinergic ligands with the muscarinic receptor did not lead to hydrolysis of the ligand. None of these models, however, completely explained the diverse pharmacological effects produced by all muscarinic agonists and antagonists.

Subsequent developments suggested that the effects of muscarinic receptor stimulation are mediated by second messengers that are biosynthesized by at least two important events: 1) inhibition of adenylyl cyclase*, and 2) activation of phospholipase C. Both involve a guanosine triphosphate (GTP)–dependent mechanism. Two other important developments were the synthesis of radiolabeled muscarinic ligands and the utilization of molecular biology techniques in the study of muscarinic receptors.

Heterogeneity in the muscarinic receptor population was first suggested in the late 1970s during pharmacological studies using the muscarinic antagonist pirenzepine. At the time, pirenzepine was the only muscarinic antagonist to block gastric acid secretion at concentrations that did not block the effects of muscarinic agonists. This observation initiated research that ultimately led to discovery of muscarinic receptor subtypes, designated as M1, M2, and M3 based on their pharmacological responses to various ligands. Rapid advances in molecular biology led to the cloning of cDNAs that encoded for five muscarinic receptors, designated as m1 through m5; m1, m2, and m3 correspond to the respective M1 through M3 receptors identified by their pharmacological specificity. The International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification has recommended that the uppercase nomenclature M1 through M5 be used to designate both pharmacological as well as molecular subtypes (8).
All the muscarinic receptor subtypes (M₁–M₅) are found in the CNS, and other tissues may contain more than one subtype. These receptors are summarized in Table 12.1 (11). As more muscarinic receptor subtypes have been discovered, it has become apparent that there is a lack of known antagonists exhibiting “very high subtype selectivity” and that there “are no muscarinic agonists with high selectivity” (8). Thus, proof for involvement of any one receptor subtype in a given system currently requires use of more than one antagonist. Additionally, if the selectivity of a novel muscarinic antagonist or putative agonist is to be assessed, it should be through the use of recombinant muscarinic receptors expressed in cell lines rather than with native receptors.

Cloning and sequencing of genes encoding muscarinic receptors have led to major advances in understanding of their chemical nature and function (8,12,13,14). These experiments demonstrated that muscarinic receptors belong to a group of receptors that are coupled to guanine nucleotide–binding proteins and are referred to as G protein–coupled receptors (GPCR) (8,15,16). The guanine nucleotide regulatory protein to which the receptors are coupled has three subunits (α, β, and γ), which link the receptor to effectors that produce second messenger molecules within the cell. Binding of muscarinic agonists to GPCRs leads to a variety of effector responses (see below). The ultimate observable response is a function of the tissue where the receptor is located.

The amino acid sequences (primary structures) of muscarinic receptor proteins expressed by cloned genes for the GPCRs have been deduced from the base sequence of the respective genes. These GPCRs are components of the cell membrane and consist of seven hydrophobic transmembrane helical domains as well as hydrophilic extracellular and intracellular domains (17). The N-terminus of the GPCR protein is extracellular, and the C-terminus is intracellular. This proposed arrangement for the human type 1 muscarinic receptor, including its deduced amino acid sequence, is illustrated in Fig. 12.3 (12). Computer-assisted molecular modeling also has made it possible to obtain three-dimensional models of the muscarinic receptor (17); a proposed top-view model of the M₁ muscarinic receptor is shown in Fig. 12.4 (18). It is interesting to observe that this model suggests that the quaternary nitrogen of acetylcholine participates in an ionic bond with the free carboxylate group of an aspartate residue (D105)—one of the receptor functional groups that was hypothesized to be involved in receptor binding of acetylcholine almost 60 years ago using only SAR data and the powers of deduction.

The current model for muscarinic receptors is much more descriptive than earlier models, and it better
describes ligand binding (molecular recognition) and its effect (signal transduction) (Fig. 12.5) (14). In this model, acetylcholine binds to the muscarinic receptor located in the cell membrane, and this ligand–receptor interaction is translated, presumably by a conformational perturbation, through the receptor protein to the receptor-coupled guanine nucleotide regulatory protein (G protein). A relationship between the guanine nucleotide regulatory protein and the effector is illustrated in Fig. 12.6. In this scheme, the G protein is in the inactive state, with guanosine diphosphate (GDP) bound to its α subunit. On interaction of an agonist with the muscarinic receptor, the α subunit releases GDP and binds GTP. The α subunit–GTP complex then dissociates from the βγ subunits. Both the α subunit–GTP complex and the βγ subunits interact with membrane-bound effectors (phospholipase C or adenylate cyclase) or ion channels (K+ and Ca2+), either independently or in a parallel manner. The α subunit possesses GTPase activity and quickly hydrolyzes the GTP to GDP to terminate signal transmission, at which time the α, β, and γ subunits reassociate and migrate back to the receptor protein. Characteristics of the α subunit determine the classification of the particular G protein:

Table 12.1. Muscarinic Acetylcholine Receptor Subtypes

<table>
<thead>
<tr>
<th>Receptor</th>
<th>G Protein</th>
<th>Tissue Location</th>
<th>Cellular Response</th>
<th>Function</th>
</tr>
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<tbody>
<tr>
<td>M1</td>
<td>Gq11</td>
<td>CNS, gastric and salivary glands, autonomic ganglia, enteric nerves</td>
<td>PLC activation (↑IP3 &amp; ↑DAG → ↑Ca2+ &amp; PKC); depolarization and excitation (↑sEPSP); PLA2 and PLD2 activation; ↑AA</td>
<td>↑Cognitive function; ↑Seizure activity; ↑Secretions</td>
</tr>
<tr>
<td>M3</td>
<td>Gq11</td>
<td>CNS (&lt; other mAChRs), smooth muscle, glands, heart</td>
<td>Same as M1</td>
<td>↑Smooth muscle contraction (e.g., bladder); ↑Salivary gland secretion; ↑Food intake, body fat deposits; Inhibits dopamine release; Synthesis of nitric oxide</td>
</tr>
<tr>
<td>M5</td>
<td>Gq11</td>
<td>Low levels in CNS &amp; periphery; predominate mAChRs in dopaminergic neurons of substantia nigra &amp; ventral tegmentum area</td>
<td>Same as M1</td>
<td>Mediates dilation of cerebral arteries; Facilitates dopamine release; Augments drug seeking behavior and reward</td>
</tr>
<tr>
<td>M4</td>
<td>G1/G0</td>
<td>Autonomic nerve terminals; CNS; heart; smooth muscle</td>
<td>Inhibition of adenyl cyclase (↓cAMP) &amp; voltage gated Ca2+ channels; activation of inwardly rectifying K+ channels</td>
<td>↓Heart rate; ↑Smooth muscle contraction; Neural inhibition in periphery via autoreceptors and heteroreceptor; ↓Ganglionic transmission; Neural inhibition in CNS; ↑Tremors hypothermia &amp; analgesia; Inhibition of autoreceptor- and heteroreceptor-mediated transmitter release in CNS; Analgesia; Cataleptic activity; Facilitates dopamine release</td>
</tr>
<tr>
<td>M2</td>
<td>G1/G0</td>
<td>CNS</td>
<td>Same as M2</td>
<td></td>
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</table>
**CNS**, central nervous system; PLC, phospholipase C; IP$_3$, inositol-1, 4, 5-triphosphate; DAG, diacylglycerol; PLD$_2$, phospholipase D; AA, arachidonic acid; PKC, protein kinase C; sEPSP, slow excitatory postsynaptic potential; mAChRs, muscarinic acetylcholine receptor subtypes; PLA, phospholipase A; cAMP, cyclic adenosine monophosphate; VTA, ventral tegmentum area.


G$_s$ increases adenylyl cyclase activity and increases Ca$^{2+}$ currents

G$_i$ decreases adenylyl cyclase activity and increases K$^+$ currents

G$_o$ decreases Ca$^{2+}$ currents

G$_q$ increases phospholipase C activity

The $\beta_\gamma$ subunits are involved with receptor-operated K$^+$ currents and with activity of adenylyl cyclase and phospholipase C.

Signal transduction at the stimulatory "odd-numbered" muscarinic receptors (i.e., M$_1$, M$_3$, and M$_5$) is via coupling with a G$_{q/11}$ protein that is involved with mobilization of intracellular calcium. Agonist binding to these receptors results in activation of phospholipase C, with subsequent production of the second messengers, diacylglycerol and inositol-1,4,5-triphosphate (IP$_3$). Stimulation of IP$_3$ ion channel receptors leads to the release of intracellular calcium from the endoplasmic reticulum. The diacylglycerol produced, along with calcium, activates protein kinase C, which phosphorylates proteins to afford various physiological responses. The M$_1$, M$_3$, and M$_5$ receptors also stimulate phospholipase A$_2$ and phospholipase D. Activation of phospholipase A$_2$ results in release of arachidonic acid, with subsequent synthesis of eicosanoids (C$_{20}$ fatty acids).

The "even-numbered" muscarinic receptor subtypes (i.e., M$_2$ and M$_4$) are coupled to G$_i$/G$_o$ proteins, the activation of which results in inhibition of adenylyl cyclase.

This results in a decrease in cyclic adenosine monophosphate, inhibition of voltage-gated calcium channels, and activation of inwardly rectifying potassium channels (19). The result is hyperpolarization and inhibition of these excitable membranes.
Fig. 12.3. Deduced amino acid sequence of human muscarinic acetylcholine receptor M₁ and putative arrangement of the seven transmembrane domains, three intracellular domains (i₁–i₃), and three extracellular domains (e₁–e₃). (From Lameh J, Cone RI, Maeda S, et al. Structure and function of G protein coupled receptors. Pharm Res 1990;7:1213–1221; with permission.)
Fig. 12.4. Model of acetylcholine interaction with muscarinic M₁ receptor. Circles represent seven transmembrane domains; D105, T189, and Y381 are aspartate, threonine, and tyrosine residues, respectively.

The M₁ receptors sometimes are termed “neural” because of their abundance in the cerebral cortex, hippocampus, and striatum. The M₁ receptors have been implicated in Alzheimer’s disease and are thought to be involved with such functions as memory and learning. Early studies suggested that the agonist McN-A-343 was selective for the M₁ receptor, but more recent evidence indicates otherwise. It may show moderate selectivity for M₄ receptors. Additionally, M₁ receptors are found at autonomic ganglia, enteric nerves, and salivary and gastric glands. Agonists at M₁ receptors show the greatest promise for treatment of the cholinergic deficit associated with Alzheimer’s disease.

Fig. 12.5. Model of signal transduction by a G protein–coupled receptor. This illustrates a proposed relationship between receptor, G protein, effector, and various second messengers.
Fig. 12.6. Diagram of a GTPase cycle and subunit association/dissociation proposed to control signal transduction between muscarinic G protein–coupled receptors and the effector. The ACh–receptor interaction facilitates GTP binding and activates the α subunit. The α subunit–GTP complex then dissociates from the βγ subunit, and each is free to activate effector proteins. The duration of separation is determined by the rate of α subunit–mediated GTP hydrolysis.

The M2 receptors are found in abundance in the heart, where their activation exerts both negative chronotropic and inotropic actions. In smooth muscle, they stimulate contraction. Activation of M2 autoreceptors located on nerve terminals affords neural inhibition by decreasing acetylcholine release.

The M3 receptors are found in abundance in smooth muscle and glands, where their stimulation leads to contraction and secretion, respectively. Knowledge of this effect on smooth muscle of the bladder has led to the development and subsequent approval of several M3 receptor antagonists for the treatment of overactive bladder (see below). Although widely distributed in the CNS, their concentration there is lower than those of other muscarinic receptors. They function to decrease neurotransmitter release.
The \( M_4 \) receptors are found in the striatum and basal forebrain, where they decrease transmitter release in both the CNS and periphery. Their activation in smooth muscle and secretory glands leads to inhibition of potassium and calcium channels.

The \( M_5 \) receptors are the least characterized of the muscarinic receptors. There is evidence for their existence in the CNS and the periphery, and they may regulate dopamine release in the CNS.

**Nicotinic Receptors**

Nicotinic acetylcholine receptors are found at the skeletal neuromuscular junction, adrenal medulla, and autonomic ganglia. They have been the focus of intensive research interest, even though the majority of clinically effective cholinergic medicinal agents are either muscarinic agonists or antagonists (20). This interest in nicotinic receptors is the result of both the availability of the receptor protein from the electric organs of the electric eel (\textit{Electrophorus electricus}) and the marine ray (\textit{Torpedo californica}) and the important role they play in myasthenia gravis, an autoimmune disease.

The concept of multiple nicotinic receptors is based on the different structural requirements for agonists and antagonists acting at the autonomic ganglia and the skeletal neuromuscular junction. This multiplicity of nicotinic receptors also is supported by molecular biology research (20).

Both neuronal and muscular nicotinic receptors are classified as ligand-gated ion channel receptors, and they are structurally and functionally related to other ligand-gated ion channel receptors, such as \( \gamma \)-aminobutyric acid receptors, 5-hydroxytryptamine receptors, and glycine receptors (21). The receptor creates a transmembrane ion channel (the gate), and acetylcholine (the ligand) serves as a gatekeeper by binding with the receptor to modulate passage of ions, principally K\(^+\) and Na\(^+\), through the channel.

A nicotinic receptor was the first neurotransmitter receptor to be isolated and purified in the active form using the same molecular biological techniques described above for isolation and purification of muscarinic receptors. The primary sequence of nicotinic receptors has been deduced from cloning and sequencing of genes that encode their receptor proteins (22,23). Nicotinic receptors are pentameric transmembrane proteins made up of \( \alpha, \beta, \delta, \) and/or \( \gamma \) subunits (24).

The nicotinic receptor of muscle tissue is a transmembrane glycoprotein consisting of four types of subunits —\( \alpha, \beta, \gamma \) (or \( \epsilon \)), and \( \delta \). Only the \( \alpha_1 \) subtype of the \( \alpha \) subunit is present in muscle. In a mature muscle end plate, the \( \gamma \) subunit is replaced by an \( \epsilon \) subunit. This change in gene expression encoding the \( \gamma \) and \( \epsilon \) subunits affects ligand selectivity along with receptor turnover and/or tissue location.

One class of neuronal nicotinic receptors exists as a heteromeric pentamer composed of \( \alpha \) (\( \alpha_2-\alpha_6 \)) and \( \beta \) (\( \beta_2-\beta_4 \)) subunits (25,26,27)—for example, \( \alpha_4\beta_3 \) with a stoichiometry of two \( \alpha_4 \) and three \( \beta_3 \) subunits. Another class of functional homomeric nicotinic receptors is composed of \( \alpha_7 \) through \( \alpha_{10} \) subunits. The diversity of the subunits and the pentameric structure suggest that a large number of nicotinic receptor subtypes may exist.

The five subunits of each receptor protein in muscle tissue are arranged around a central pore that serves as
the ion channel. Based on molecular modeling of the deduced primary structure of the individual subunits, it has been proposed that each subunit (α, β, γ, δ, or ε) possesses a hydrophilic extracellular N-terminus, a hydrophilic intracellular C-terminus, and four α helical hydrophobic transmembrane domains (M₁–M₄) (Fig. 12.7) (26,28). A pentameric arrangement of these five amphipathic subunits makes up the walls of the ion channel. Two acetylcholine binding sites exist on the extracellular domain of each receptor molecule. In Figure 12.7, one binding site is located on each α subunit at the αγ and αδ interfaces (28). The binding sites show a positive cooperativity, even though the two binding sites are not adjacent to each other in the pentameric receptor. Some central and peripheral nicotinic receptor subtypes are summarized in Table 12.2 (11).

![Fig. 12.7. Nicotinic cholinergic receptor. (A) Longitudinal view (γ subunit removed) showing the internal ion channel. Acetylcholine binding sites on the α subunits are indicated by the arrows. These are located at the αγ and αδ interfaces. (B) Each of the five transmembrane subunits (α, α, β, δ, and γ) are composed of four hydrophobic membrane spanning segments (M₁–M₄). (C) Top view of the nicotinic receptor showing the subunits surrounding the ion channel.](image)

Our knowledge and understanding of the muscarinic and nicotinic receptors have advanced tremendously from the time when these receptors were only ethereal concepts. This understanding provides the basis for the rational design of new selective therapeutic agents to treat diseases associated with cholinergic neurons.

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<tr>
<th>Table 12.2 Nicotinic Acetylcholine Receptor Subtypes</th>
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# Drugs Affecting Cholinergic Neurotransmission

## Acetylcholine Neurochemistry

The neurochemistry of acetylcholine includes its biosynthesis, storage, release, and metabolism. These are illustrated in Figure 12.8.

### Biosynthesis

Acetylcholine is biosynthesized in cholinergic neurons by the enzyme-catalyzed transfer of the acetyl group from acetyl coenzyme A (acetyl-S-CoA) to choline, a quaternary ammonium alcohol (29). The enzyme catalyzing this reaction, choline acetyltransferase, is also biosynthesized in the cholinergic neuron. Some choline is biosynthesized from the amino acid serine (Fig. 12.9), but most of the choline used to form acetylcholine is recycled following AChE-catalyzed hydrolysis of acetylcholine in the synaptic space. Extracellular choline is actively transported into the presynaptic nerve terminal by both high-affinity and low-affinity uptake sites. The high-affinity sites are responsible for the uptake of most of the choline recycled from the synapse. Uptake is considered to be the rate-determining step in biosynthesis of acetylcholine. This uptake is inhibited by hemicholinium.
Fig. 12.8. General cholinergic nerve junction showing location of receptor sites and biosynthesis, storage, release, and hydrolysis of acetylcholine. (Katzung BG. Introduction to autonomic pharmacology. In: Katzung BG, ed. Basic and Clinical Pharmacology, 9th Ed. New York: McGraw-Hill, 2004, pp. 75–93; with permission.)
Efforts to Modulate Acetylcholine Biosynthesis

Efforts to develop therapeutic agents based on regulation of acetylcholine biosynthesis have not been successful. Dexpanthenol, the dextrorotatory enantiomer of the alcohol derived from pantothenic acid (a vitamin), was once used as a cholinomimetic agent to help reestablish normal smooth muscle tone in the gastrointestinal tract following surgery. Pantothenic acid is essential for the biosynthesis of coenzyme A (CoA). The apparent rationale for the therapeutic use of dexpanthenol was that it would be biotransformed to pantothenic acid, which would be incorporated into CoA. This would lead to increased intracellular levels of acetyl CoA, which would facilitate increased biosynthesis of acetylcholine. The limited therapeutic success of dexpanthenol, difficulty with administration, and effectiveness of synthetic cholinergic agonists...
led to its discontinuation. The quaternary pyridinium salt, \textit{trans-N-methyl-4-(1-naphthylvinyl)} pyridinium iodide, is an effective inhibitor of choline acetyltransferase in vitro, but it has proven to be a poor inhibitor in whole animal experiments.

Although efforts have been made to design cholinergic agents based on the mechanism of biosynthesis of acetylcholine, such agents would be expected to have nonselective effects, because it currently is thought that acetylcholine is biosynthesized by the same mechanism in all cholinergic neurons.

\section*{Storage}

Most newly biosynthesized acetylcholine is actively transported into cytosolic storage vesicles located in presynaptic nerve endings, where it is maintained with ATP (10:1 ratio) along with calcium and magnesium ions until it is released. Some acetylcholine remains in the cytosol and eventually is hydrolyzed. Only the stored form of acetylcholine serves as the functional neurotransmitter.

\section*{Release}

Release of acetylcholine from the storage vesicles is initiated by an action potential that has traveled down the axon to the presynaptic nerve membrane. This action potential leads to opening of voltage-dependent calcium channels, affording an influx of Ca$^{2+}$ and exocytotic release of acetylcholine into the synapse. The increase in intracellular Ca$^{2+}$ may induce fusion of acetylcholine storage vesicles with the presynaptic membrane before release of the neurotransmitter. Each synaptic vesicle contains a quantum of acetylcholine; one quantum represents between 12,000 and 60,000 molecules of acetylcholine. A single action potential causes the release of several hundred quanta of acetylcholine into the synapse.

\section*{Metabolism}

Acetylcholine in the synapse can bind with cholinergic receptors on the postsynaptic or presynaptic membranes to produce a response. Free acetylcholine that is not bound to a receptor is hydrolyzed by AChE. This hydrolysis is the physiologic mechanism for terminating the action of acetylcholine. Enough AChE is present in the synapse to hydrolyze approximately $3 \times 10^8$ molecules of acetylcholine in 1 millisecond; thus, adequate enzyme activity exists to hydrolyze all the acetylcholine ($\sim 3 \times 10^6$ molecules) released by one action potential. A number of useful therapeutic cholinomimetic agents have been developed based on the ability of the compounds to inhibit AChE; these agents are addressed later in this chapter.

\section*{Stereochemistry}

One shortcoming of early models for cholinergic receptors was that they did not account for the observed stereoselectivity of the receptors for agonist and antagonist ligands. Even though acetylcholine is achiral,
many synthetic and naturally occurring agonists and antagonists possess chirality; usually, one enantiomer is many times more active than the other. It was apparent to early receptor investigators that the stereochemistry of cholinergic ligands was important for receptor binding. In this regard, the stereochemical–activity relationships of cholinergic ligands have been studied extensively to provide a rational basis for design of cholinergic drugs as well as to describe the properties and functions of cholinergic receptors.

The stereochemistry of acetylcholine resides in the different arrangements in space of its atoms by virtue of rotation about σ bonds (i.e., conformational isomerism). Because of relatively unrestricted rotation about these single covalent bonds, acetylcholine can exist in an infinite number of conformations. Most studies of the conformational isomerism of acetylcholine have focused on torsion angles between the ester oxygen atom and the quaternary nitrogen resulting from rotation about the Cα-Cβ bond. Four of these conformations are illustrated by Newman projections in Fig. 12.10.

![Fig. 12.10. Conformational isomers of acetylcholine.](http://thepointeedition.lww.com/pt/re/9780781768795/bookContentPan.../TXTBKBD[1]/DIVISIONA[2]/CHAPTER[2]&highlightTo=&printPreview=yes)

Nuclear magnetic resonance (NMR) studies in aqueous solution revealed that the preferred conformation between the ester oxygen and the quaternary nitrogen of acetylcholine was synclinal (gauche or skew). Using x-ray data, the synclinal conformation was observed for acetylcholine in the solid crystalline state as well. The same conclusion was also obtained for the preferred conformation of acetylcholine using molecular orbital calculations. These experimental and theoretical determinations of the acetylcholine conformation differ from the antiperiplanar conformation that might be expected when using molecular models to visualize minimum steric interactions. The synclinal conformation would be stabilized by intramolecular electrostatic interactions between the quaternary nitrogen and the carbonyl oxygen.

It must be emphasized that the experimentally determined synclinal conformation of acetylcholine is only that measured in aqueous solution (NMR) or the crystalline state (x-ray). This may not be the conformation preferred by the receptors. Indeed, the conformation of receptor-bound acetylcholine could be much different and might not be a thermodynamically preferred conformation.

In recognition of this possibility, conformationally restricted acetylcholine analogues have been synthesized and pharmacologically evaluated in an effort to determine the conformation of acetylcholine when it binds to cholinergic receptors. The most significant study in this regard is that of Armstrong et al. (30).
synthesized and evaluated the muscarinic and nicotinic activity of *cis*- and *trans*-isomers of a conformationally rigid model of acetylcholine, *cis*- and *trans*-2-acetoxycyclopropyl-1-trimethylammonium iodide. Because this model is based on the cyclopropane ring, the ester and quaternary ammonium functional groups cannot change their relative positions by bond rotation. The *cis*- and *trans*-isomers are rigidly constrained to the conformations shown. The *cis*-isomer is similar to the synperiplanar conformation of acetylcholine, and the *trans*-isomer approximates the anticlinal conformation. The (+)-*trans*-enantiomer was observed to be equally as or more potent, depending on the pharmacological test used, than acetylcholine at muscarinic receptors; it was much more potent than the (–)-*trans*-enantiomer. The racemic *cis*-compound had almost no activity in the same muscarinic receptor test system, and all compounds were very weak nicotinic agonists.

The important conclusion drawn from this study (30) was that acetylcholine would most probably interact with muscarinic receptors in its less favored anticlinal conformation. The most active isomer, the (+)-*trans*-enantiomer, of these cyclopropane analogues was found to have a torsion angle of 137° (anticlinal) between the ester oxygen and the quaternary nitrogen. This is significantly different from the 60° torsion angle in the synclinal conformation found by NMR and x-ray determinations to be the preferred conformation.

Stereochemistry of cholinergic ligands and stereoselectivity of receptors has played an important role in design of cholinergic ligands as therapeutic agents. This role becomes apparent in subsequent sections.

**Acetylcholine Mimetics—Muscarinic Agonists**

Interaction of cholinergic agonists with muscarinic receptors leads to well-defined pharmacological responses depending on the tissue or organ in which the receptor is located. These responses include contractions of smooth muscle, vasodilation, increased secretion from exocrine glands, miosis, and decreased heart rate and force of contraction.

**Acetylcholine**
Acetylcholine is the prototypical muscarinic (and nicotinic) agonist, because it is the physiologic chemical neurotransmitter. It is a poor therapeutic agent, however, both because of its lack of nicotinic or muscarinic receptor specificity and because of the chemical and physicochemical properties associated with its ester and quaternary ammonium salt functional groups. It is quite stable in the solid crystalline form but undergoes rapid hydrolysis in aqueous solution. This hydrolysis is accelerated in the presence of catalytic amounts of either acid or base. For this reason, acetylcholine cannot be administered orally because of rapid hydrolysis in the gastrointestinal tract. Even when administered parenterally, its pharmacological action is fleeting as a result of hydrolysis by butyrylcholinesterase (pseudocholinesterase) in serum. The quaternary ammonium functional group of acetylcholine imparts excellent water solubility, but quaternary ammonium salts are poorly absorbed across lipid membranes because of their high hydrophilic and ionic character. Thus, even if acetylcholine were stable enough to be administered orally, it would be poorly absorbed. When used during ocular surgery to produce complete miosis within seconds, acetylcholine must be directly instilled into the anterior chamber. It cannot be administered topically, because it is not lipophilic enough to penetrate the cornea. It requires aqueous reconstitution immediately before instillation because of its chemical hydrolytic lability.

**Structure–Activity Relationship**

The necessity to design compounds that would serve as therapeutic alternatives to acetylcholine and as probes to study the role of acetylcholine in neurotransmission led to an exhaustive study of the structural features required for the action of acetylcholine. Structure–activity relationships that developed from these studies have provided the basis for the design of all muscarinic agonists currently used as therapeutic agents.

To review the SAR, it is logical to divide the structure of acetylcholine into the three components shown below to examine the effects of chemical modification of each group:

![Structure of Acetylcholine](http://thepointiedition.lww.com/pt/re/9780781768795/bookContentPan.../DIVISIONA[2]/CHAPTER[2]&highlightTo=&printPreview=yes)

**Modification of the quaternary ammonium group**

Analogues of acetylcholine in which the nitrogen atom was replaced by arsenic, phosphorus, or sulfur have
been synthesized (10,31). Although they exhibited some of the activity of acetylcholine, these compounds are less active and are not used clinically. It was concluded that only compounds possessing a positive charge on the atom in the position of the nitrogen had appreciable muscarinic activity.

Compounds in which all three methyl groups on the nitrogen are replaced by larger alkyl groups are inactive as agonists. When the methyl groups are replaced by three ethyl groups, the resulting compound is a cholinergic antagonist. Replacement of only one methyl group by an ethyl or propyl group affords a compound that is active, but much less so than acetylcholine (32). Furthermore, successive replacement of one, two, or three of the methyl groups with hydrogen atoms to afford a tertiary, secondary, or primary amine, respectively, leads to successively diminishing muscarinic activity (33,34).

**Modification of the ethylene bridge**

Synthesis of acetic acid esters of quaternary ammonium alcohols of greater length than choline led to a series of compounds with activity that was rapidly reduced as the chain length increased. This observation led Ing (10) to postulate his Rule of Five. This rule suggests that there should be no more than five atoms between the nitrogen and the terminal hydrogen atom for maximal muscarinic potency. Present concepts suggest that the muscarinic receptor cannot successfully accommodate molecules larger than acetylcholine and still produce its physiologic effect. Although larger molecules may bind to the receptor, they lack efficacy and demonstrate antagonist properties.

Replacement of the hydrogen atoms of the ethylene bridge by alkyl groups larger than methyl affords compounds that are much less active than acetylcholine. Introduction of a methyl group on the carbon β to the quaternary nitrogen affords acetyl-β-methylcholine (methacholine), which has muscarinic potency almost equivalent to that of acetylcholine and much greater muscarinic than nicotinic selectivity.

A methyl group on the carbon α to the quaternary nitrogen affords acetyl-α-methylcholine. Although activity relative to acetylcholine is reduced at both muscarinic and nicotinic receptors, it exhibits greater nicotinic than muscarinic potency. This compound is not currently used as a therapeutic agent.

Addition of methyl groups to either one or both of the ethylene carbons results in chiral molecules. Muscarinic receptors (see below) display stereoselectivity for the enantiomers of methacholine. The S-(+)

enantiomer is equipotent with acetylcholine, and the R-(−)-enantiomer is approximately 20-fold less potent. Acetylcholinesterase hydrolyzes the S-(+)-isomer much slower (approximately half the rate) than acetylcholine. The R-(−)-isomer is not hydrolyzed by AChE and even acts as a weak competitive inhibitor of the enzyme. This stability toward AChE hydrolysis as well as the AChE inhibitory effect of the R-(−)-enantiomer may explain why racemic methacholine produces a longer duration of action than acetylcholine. The nicotinic receptor and AChE exhibit little stereoselectivity for the optical isomers of acetyl-α-methylcholine.
Modification of the acyloxy group

As would be predicted by the Rule of Five (10), when the acetyl group is replaced by higher homologues (i.e., the propionyl or butyryl groups), the resulting esters are less potent than acetylcholine. Choline esters of aromatic or higher-molecular-weight acids possess cholinergic antagonist activity.

Because the fleeting pharmacological action and chemical instability of acetylcholine result from its rapid hydrolysis, a logical approach to the development of better muscarinic therapeutic agents was to replace the acetyloxy functional group with a functional group more resistant to hydrolysis. This led to synthesis of the carbamic acid ester of choline (carbachol), a potent cholinergic agonist possessing both muscarinic and nicotinic activity. Esters derived from carbamic acid are referred to as carbamates, and because their carbonyl carbon is less electrophilic, they are more stable than carboxylate esters to hydrolysis. Carbachol is less readily hydrolyzed by gastric acid, AChE, or butyrylcholinesterase than acetylcholine is, and it can be administered orally.

This same chemical logic was extended to methacholine and led to synthesis of its carbamate ester,
bethanechol, an orally effective potent muscarinic agonist with almost no nicotinic activity at therapeutic doses. Muscarinic receptors exhibit stereoselectivity for the two optical isomers of bethanechol, and similar to methacholine, the S-(+)-enantiomer exhibits greater binding affinity at muscarinic receptors than the R-(−)-enantiomer in isolated receptor preparations.

The profound muscarinic activity of the alkaloid muscarine provided substantial rationale for synthesizing ethers of choline. Muscarine, which is obtained from the red variety of mushroom (*Amanita muscaria*) and other mushrooms is one of the oldest known cholinergic agonists and is the compound for which muscarinic receptors were named. It was used in many pharmacological experiments during the latter 19th century and the early part of the 20th century, and its use preceded the discovery and chemical characterization of acetylcholine (2). The chemical structure of muscarine (see above), however, was not completely characterized until 1957. Muscarine possesses three chiral centers (C2, C3, and C5). Thus, eight optical isomers (four enantiomeric pairs) are possible. Of these, only the naturally occurring alkaloid, (2S,3R,5S)-(+)-muscarine (also called L-(+)-muscarine), is correctly referred to as muscarine. The C5 carbon of (+)-muscarine has the same absolute configuration as the analogous chiral β carbon in S-(+)-methacholine.

Other choline ethers as well as alkylaminoketones have been synthesized and evaluated for muscarinic activity. Choline ethyl ether exhibits significant muscarinic activity and is chemically stable, but it has not been used clinically. The most potent ketone derivatives possess the carbonyl on the carbon δ to the quaternary nitrogen; this is the same relative position as the carbonyl in acetylcholine. This suggests that these carbonyl groups bind by either a hydrogen bond or other dipole–dipole interaction with an appropriate group on the muscarinic receptor. Furthermore, the activity of these ethers and ketones demonstrates that neither the ester functional group nor a carbonyl is required for muscarinic agonist activity.

The classic SAR for muscarinic agonist activity can be summarized as follows:

- The molecule must possess a nitrogen atom capable of bearing a positive charge, preferably a quaternary ammonium salt.
For maximum potency, the size of the alkyl groups substituted on the nitrogen should not exceed the size of a methyl group.

The molecule should have an oxygen atom, preferably an ester-like oxygen, capable of participating in a hydrogen bond.

There should be a two-carbon unit between the oxygen atom and the nitrogen atom.

It is important to note that this SAR was based on in vitro and in vivo pharmacological evaluations performed over a 60-year period. Scientists conducting this research did not have the luxury of modern, highly refined biological testing systems (i.e., protein-binding assays, cell membrane–binding assays, and single-cell models) that are considered to be state-of-the-art today for pharmacological evaluation of new medicinal agents. This is why some classic muscarinic agonists and many of the more modern agents do not adhere to this SAR. Indeed, SAR rules are not static; they should change as new experimental data refine the structural and stereochemical requirements for muscarinic agonist activity.

**Specific Muscarinic Agonists**

**Methacholine chloride (Provocholine)**

Methacholine, acetyl β-methylcholine, (see previous structure and SAR discussion) is marketed as the racemic mixture. It is a selective muscarinic agonist with very little activity at nicotinic receptors. Although methacholine chloride is marketed as the racemic mixture, the S-(+)-enantiomer is 240-fold more potent than the R-(–)-isomer at muscarinic receptors. In addition, AChE hydrolyzes S-(+)–methacholine at approximately 54% the rate of acetylcholine, whereas the R-(–)–enantiomer is a weak inhibitor. Methacholine chloride is used via inhalation for the diagnosis of asthma. The resulting bronchospasm may be relieved with bronchodilators. Methacholine chloride is available as a powder that is reconstituted for inhalation.

**Carbachol chloride (Isopto Carbachol)**

Carbachol (see structure above), the carbamate analogue of acetylcholine, shows no selectivity for muscarinic or nicotinic receptors. Because it is a carbamate ester, carbachol is more resistant toward acid-, base-, or enzyme (AChE)-catalyzed hydrolysis than acetylcholine. It also is reported to exhibit weak anticholinesterase activity. Both of these actions work to prolong the duration of action of carbachol. Because of erratic absorption and its actions at nicotinic receptors, use of carbachol has been limited to the treatment of glaucoma and for the induction of miosis in ocular surgery. Carbachol is available as an intraocular solution and an ophthalmic solution.

**Bethanechol chloride (Urecholine)**

Bethanechol (see structure above), the carbamate analogue of methacholine, is selective for muscarinic
receptors and exhibits almost no activity at nicotinic receptors. It is used to treat postsurgical and postpartum urinary retention and abdominal distention. Bethanechol is administered orally, because there is danger of a cholinergic crisis if it is given by intravenous or intramuscular injection.

**Pilocarpine hydrochloride (Isopto Carpine)**

Pilocarpine, the salt of an alkaloid obtained from *Pilocarpus jaborandi*, is an example of a muscarinic agonist that does not adhere to the traditional SAR. In 1876, Langley reported that extracts containing the alkaloid stimulated the end organs of parasympathetic neurons. The structure of pilocarpine was reported in 1901.

Pilocarpine is marketed as tablets (Salogen), an ophthalmic solution, and gel. It penetrates the eye well and is the miotic of choice for open-angle glaucoma and to terminate acute angle closure attacks. It also is used for the treatment of xerostomia (dryness of the mouth) caused by radiation therapy of the head and neck, Sjogren's syndrome, or as a side effect of some psychotropic drugs.

Because pilocarpine is a lactone, its solutions are subject to hydrolysis to afford the pharmacologically inactive pilocarpic acid and to base-catalyzed epimerization at C3 in the lactone to give isopilocarpine, an inactive stereoisomer of pilocarpine. Epimerization is not believed to be a serious problem if the drug is properly stored. Its solutions can be stored at room temperature, but the gel should be refrigerated and labeled with a 2-week expiration date when dispensed.

**Cevimeline hydrochloride (Evoxac)**

Cevimeline is a nonclassical muscarinic agonist. It is a quinuclidine derivative that exhibits partial direct M1 receptor agonist activity in the CNS and affinity for M3 receptors in epithelial tissue of lacrimal and salivary glands. Its elimination half-life is 3 to 5 hours. It is metabolized by CYP2D6, CYP3A3, and CYP3A4 to inactive metabolites, the cis- and trans-sulfoxide, N-oxide, and glucuronide. Cevimeline hydrochloride is available as an oral capsule for the treatment of xerostomia (dry mouth) associated with Sjogren's syndrome.
syndrome. Before its approval, pilocarpine was the only drug for this condition.

Future Muscarinic Agonists

Current research interest in the design and synthesis of new muscarinic agonists is focused on discovering agents that might be effective in the treatment of Alzheimer's disease and other cognitive disorders. Investigators are searching actively for muscarinic agonists that exhibit selectivity for muscarinic receptors in the brain. Among these compounds are analogues of arecoline, oxotremorine, and McN-A-343 as well as many other novel chemical structures possessing muscarinic agonist activity.

Arecoline is of historical interest, because its structure, like those of many other early medicinal agents, was determined and confirmed by a 19th-century German pharmacist, E. Jahns (2). Xanomeline may be viewed as a nonclassical bio-isostere of the ester moiety of arecoline. It is a muscarinic M₁/M₄ agonist that is showing promise in clinical trials for the treatment of Alzheimer’s disease (35). Although it is not tolerated at orally effective doses, transdermal delivery systems are showing promise.
Acetylcholinesterase Inhibitors

Another means of producing a cholinergic response is to interfere with the mechanism by which the action of acetylcholine is terminated. Thus, inhibition of its rapid hydrolysis by AChE increases the concentration of acetylcholine in the synapse and results in production of both muscarinic and nicotinic effects.

Therapeutic Application

Acetylcholinesterase inhibitors (AChEIs), sometimes referred to as anticholinesterases, are classified as indirect cholinomimetics, because their principle mechanism of action does not involve binding to cholinergic receptors. These agents are used therapeutically to improve muscle strength in myasthenia gravis. They also are used in open-angle glaucoma to decrease intraocular pressure by stimulating contraction of the ciliary muscle and sphincter of the iris. This facilitates outflow of aqueous humor via the canal of Schlemm. Recently, AChEIs have found use in the treatment of symptoms of Alzheimer's disease.
and similar cognitive disorders (36,37), which are conditions characterized by a cholinergic deficiency in the
cortex and basal forebrain. They are used extensively as insecticides and are in military arsenals for use as
chemical warfare agents.

**Mechanism of Acetylcholinesterase Hydrolysis**

Extensive studies of AChE have resulted in the purification and amino acid sequencing of the enzyme from
several sources as well as the description of its quaternary structure from x-ray crystallographic and
molecular modeling studies (38). To understand the mode of action of AChEIs, it is necessary to examine
the mechanism by which AChE catalyzes hydrolysis of acetylcholine. This enzymatically controlled
hydrolysis parallels the two chemical mechanisms for hydrolysis of esters. The first mechanism is acid-
catalyzed hydrolysis, in which the initial step involves protonation of the carbonyl oxygen. The transition
state is formed by the attack of a molecule of water at the electrophilic carbonyl carbon atom. Collapse of
the transition state affords the carboxylic acid and the alcohol (Fig. 12.11). The second mechanism, base-
catalyzed hydrolysis, involves the nucleophilic attack

![Fig. 12.11. Mechanism of acid-catalyzed hydrolysis of an ester.](image)

Both mechanisms for ester hydrolysis are proposed to be involved in the mechanism for AChE-catalyzed
hydrolysis of acetylcholine. Fig. 12.13 is a schematic illustration of the binding of acetylcholine to the
catalytic (active) site of AChE, which consists of an ester-binding site and an “anionic-binding site.” This
figure reflects binding of the quaternary nitrogen of acetylcholine to an area that has been described as an
“anionic site” on the enzyme. Originally, this “anionic site” was proposed to be contributed by the free
carboxylate group of a glutamate residue. Current evidence using selective mutagenesis, however,
suggests that rather than ionic bonding between the quaternary nitrogen and an anionic site, there is a
cation–π interaction between the quaternary nitrogen and the aromatic rings of tryptophan and
phenylalanine of the enzyme (39). In Fig. 12.13, there is a concerted protonation of the carbonyl oxygen by
an imidazole proton from a histidine residue and nucleophilic attack on the partial positive carbon of the
carbonyl group by the hydroxyl group of a serine residue. The remainder of the hydrolysis mechanism is described in Fig. 12.14: Transition state B is unstable and collapses to form choline and acetylated AChE (C); this form of the enzyme is referred to as the acetylated enzyme. As long as the enzyme is acetylated, it cannot bind another molecule of acetylcholine; the enzyme is in an inactive state. The acetylated enzyme undergoes rapid hydrolysis to regenerate the original, active form of AChE and a molecule of acetic acid.

The latter step in the mechanism—the regeneration of the active enzyme—is important in the development of AChEIs. If the enzyme becomes acylated by a functional group (i.e., carbamyl or phosphate) that is more stable to hydrolysis than a carboxylate ester, the enzyme remains inactive for a longer period of time. Application of this chemical principle regarding rates of hydrolysis led to discovery and design of two classes of AChEIs, the reversible inhibitors and the irreversible inhibitors.

**Fig. 12.12.** Mechanism of base-catalyzed hydrolysis of an ester.
Reversible Inhibitors of Acetylcholinesterase

Mechanism of action

Reversible AChEIs are those compounds that are substrates for and react with AChE to form an acylated enzyme, which is more stable than the acetylated enzyme but still capable of undergoing hydrolytic regeneration, or that bind to AChE with greater affinity than acetylcholine but do not react with the enzyme as a substrate. Inhibitors of both types have found clinical application. Those that acylate AChE include the aryl carbamates, such as esters of carbamic acid and phenols (e.g., physostigmine). Alkyl carbamates (esters of carbamic acid and alcohols), such as carbachol and bethanechol, both of which are structurally related to acetylcholine, also are substrates for and competitively inhibit AChE, because they are hydrolyzed very slowly by AChE. For reasons previously discussed, carbachol and bethanechol are more resistant than acetylcholine to AChE-catalyzed hydrolysis.

When aryl carbamate AChEIs, such as physostigmine and its analogues, bind to the catalytic site of AChE, hydrolysis of the carbamate occurs. This transesterifies the serine residue with carbamic acid, forming what is termed a “carbamylated enzyme.” The rate of carbamylation follows the order of carbamic acid esters > methylcarbamic acid esters > dimethylcarbamic acid esters (40).
Regeneration of active AChE by hydrolysis of the carbamylated enzyme is much slower than hydrolysis of the acetylated enzyme. The rate for hydrolytic regeneration of the carbamylated AChE is measured in minutes (e.g., the half-life for methyl carbamylated enzyme is ~15 minutes); the rate of hydrolytic regeneration of acetylated AChE is measured in milliseconds (e.g., the half-life for acetylated enzyme is ~0.2 milliseconds). Despite the longer time required to regenerate the carbamylated enzyme, the active form of AChE eventually is regenerated. Therefore, these inhibitors are considered to be reversible.

Aryl carbamates are superior to alkyl carbamates as AChEIs, because they have better affinity for AChE and, therefore, carbamylate AChE more efficiently. Physostigmine and other aryl carbamates exhibit inhibition constants ($K_i$) on the order of $10^{-9}$ to $10^{-8}$ M and are three to four orders of magnitude more effective than alkyl carbamates, such as carbachol ($K_i \sim 10^{-5}$ M). This is to be expected, because phenoxide anions are more stable than and, hence, are better leaving groups than alkoxide anions. Phenoxide anions are stabilized through resonance with the aromatic ring. Thus, the therapeutically effective carbamate inhibitors of AChE are derived from phenols.
Specific Agents

Reversible acetylcholinesterase inhibitors

Physostigmine

The classic AChEI, physostigmine, is an alkaloid obtained from seeds of the Calabar bean (*Physostigma venenosum*) (37). Its parasympathomimetic effects were recognized long before its structure was elucidated in 1923. In 1929, Stedman found that the mechanism of the parasympathomimetic effects of physostigmine was inhibition of AChE; it inhibits AChE by acting as a substrate and carbamylating the enzyme. Acetylcholinesterase is carbamylated at a slow rate, but physostigmine has exceptionally high affinity ($K_i \sim 10^{-9}$ M) for the catalytic site of the enzyme. By comparison, the $K_s$ for acetylcholine is on the order of $10^{-4}$ M. Thus, physostigmine is classified as a reversible AChEI that carbamylates the enzyme at a slow rate; the carbamylated AChE also is regenerated quite slowly. Because physostigmine is a tertiary amine with a $pK_a$ of 8.2 (+BH) rather than a quaternary ammonium salt, it is more lipophilic than many other AChEIs and can diffuse across the blood-brain barrier. The tertiary amine also imparts pH dependence to its ability to inhibit AChE, because its affinity for AChE is greater when the amine is protonated. Physostigmine is metabolized in vivo by esterases to the phenol and has an elimination half-life of 1 to 2 hours. Its aqueous solutions are subject to hydrolytic decomposition to form eseroline, which undergoes light-catalyzed oxidation to form rubreserine, a red-colored compound (Fig. 12.15). Both degradation products are inactive as AChEIs.

Physostigmine has been used for many years in ophthalmology for the treatment of glaucoma. More recently, the salicylate salt has been used in hospital emergency rooms to treat overdoses of compounds possessing significant anticholinergic CNS effects (for example, antidepressants), such as atropine and tricyclic antidepressants. Physostigmine’s ability to cross the blood-brain barrier has led to renewed interest in this molecule, and it also is one of a number of centrally active AChEIs being investigated as indirect cholinomimetics for use in the treatment of Alzheimer’s disease and other cognitive disorders.
Neostigmine (Prostigmin)

The discovery that physostigmine and other aryl carbamates inhibit AChE reversibly led to research to find other AChEIs possessing this activity. Most of this research involved incorporation of the required structural features of both physostigmine and acetylcholine into the new molecules. This led to synthesis of neostigmine, a compound resembling physostigmine but having a much simpler structure. Neostigmine retains the substituted carbamate group, the benzene ring, and the nitrogen atom of the first heterocyclic ring of physostigmine. The distance between the ester and the quaternary ammonium group is approximately the same as that found in acetylcholine and physostigmine. Because of its quaternary ammonium group, it lacks central activity. Neostigmine is metabolized to 3-hydroxyphenyltrimethylammonium, 3-hydroxyphenyldimethylamine, and its glucuronide conjugate, and it has an elimination half-life of 15 to 90 minutes. Neostigmine is indicated for prophylaxis of postoperative
abdominal distension and urinary retention, myasthenia gravis, reversal of neuromuscular blockade.

**Pyridostigmine (Mestinon)**

Pyridostigmine, a closely related structure to neostigmine that incorporates the charged nitrogen into a pyridine ring, acts by the same mechanism as physostigmine, but it lacks CNS activity. It is orally effective and, compared to neostigmine, has a longer duration of action and a lower incidence of side effects. Thus, it is a better choice for oral therapy of myasthenia gravis. It is approved for U.S. military use as an adjunct for prophylaxis of soman nerve gas exposure. It is also administered parenterally to reverse the effects of nondepolarizing neuromuscular blocking agents. Its elimination half-life is 1 to 2 hours.

![Pyridostigmine](image)

**Carbaryl**

Carbaryl is a reversible, carbamate-derived AChEI that has tremendous economic impact as an insecticide for use on houseplants and vegetables as well as for control of fleas and ticks on pets. Its structural relationship to physostigmine and neostigmine is readily apparent. A number of other carbamate AChEIs also are commercially available for this use.

![Carbaryl](image)
Edrophonium chloride (Enlon, Reversol)

Edrophonium is a quaternary ammonium-substituted phenol. Because it is a phenol derivative rather than a carbamate ester of a phenol, it does not carbamylate AChE. It does, however, inhibit AChE in a reversible manner, and it also exhibits a direct cholinomimetic effect at skeletal muscle. Edrophonium is used intravenously for the diagnosis of myasthenia gravis, where it acts rapidly to increase muscle strength. It also is administered intramuscularly to rapidly reverse the effects of nondepolarizing neuromuscular blocking agents like \( d \)-tubocurarine and gallamine. It is not effective, however, at reversing the effects of the depolarizing blockers such as succinylcholine and decamethonium. Its elimination half-life is 1.3 to 2.4 hours.

Reversible acetylcholinesterase inhibitors for treatment of Alzheimer's disease

Of all the age-related disorders in which dementia is a component, Alzheimer's disease (AD) is probably the best known. Much effort has been expended to discover the cause of AD. Autopsy examination of the brains of patients who had AD has revealed microscopic structural changes characteristic of the disease. In addition, neurotransmitter dysfunction involving reduction in acetylcholine, serotonin, norepinephrine, dopamine, and glutamate levels have been reported. For a review of AD and the search for therapies, see Rzeszotarski (41). It is known that in AD patients, there is widespread atrophy in the primary motor and sensory cortices and cerebellum. There is a disruption in cholinergic innervation in these areas of the brain, along with decreases in choline acetyltransferase, high-affinity nicotinic acetylcholine receptor binding, and choline transporter sites (42,43,44,45). Impairment of short-term memory is the first observable symptom of the disease, and progressive memory impairment, severe mood changes, and depression coupled with loss of judgment and reasoning ability follow. The U.S. Food and Drug Administration has approved four AChEIs for the treatment of AD: tacrine, donepezil, rivastigmine, and galantamine. Although these four AChEIs are not without problems, they do provide some benefit in early to mild AD. Their clinical effectiveness in advanced AD is yet to be shown.
**Tacrine hydrochloride (Cognex)**

Tacrine, an aminoacridine synthesized in the 1930s, is a nonclassic cholinesterase inhibitor that binds to both AChE or butyrylcholinesterase (46). It was approved in 1993 for the treatment of AD. Approximately 20% of tacrine-treated patients may show improvement, but its use has been limited because of hepatotoxicity. Use of tacrine has greatly decreased because of the recent development of safer AChEIs. Tacrine is extensively metabolized by CYP450 to at least three metabolites. The major metabolite, 1-hydroxy-tacrine, is active. Its elimination half-life is between 1.5 and 4 hours, with metabolites being excreted via the urine.

![Donepezil hydrochloride](image)

**Donepezil (Aricept)**

Donepezil is another “nonclassic,” centrally acting, reversible, noncompetitive AChEI that was approved in 1997 for treatment of mild-to-moderate AD and dementia. Its selectivity for AChE is 570- to 1,250-fold that for butyrylcholinesterase, and it also exhibits greater affinity for brain AChE than for AChE in the periphery (47). When compared to tacrine, donepezil exhibits greater CNS AChE selectivity, longer elimination half-life (70–104 hours in subjects older than 55 years) and little or no potential for hepatotoxicity. Donepezil is metabolized by CYP2D6 and CYP3A4 via demethylation, debenzylation, hydroxylation, oxidation to the cis-N-oxide, and glucuronidation. The 6-O-desmethyl metabolite accounts for 11% of a dose, and it exhibits AChE inhibitory activity comparable to that of the parent compound.
Rivastigmine (Exelon)

Rivastigmine is a centrally selective, arylcarbamate AChEI that was approved in 2000 for oral administration in the treatment of AD. It has an elimination half-life of 1.4 to 1.7 hours but is able to inhibit AChE for up to 10 hours. Because of the slow dissociation of the carbamylated enzyme, it has been referred to as a pseudo-irreversible AChEI (47). Like donepezil, rivastigmine exhibits a low level of hepatotoxicity. It is rapidly and extensively hydrolyzed in the CNS by cholinesterase with minimal involvement of CYP450. The phenolic metabolite is excreted primarily via the kidneys.

Galantamine hydrobromide (Razadyne)

Galantamine, which was introduced in 2001, is an alkaloid found in plants of the family Amaryllidaceae, which includes the daffodil (Narcissus pseudonarcissus) and snowflake (Leucojum aestivum). It is a reversible inhibitor of AChE, but it does not appear to inhibit butyrylcholinesterase. Because it is a tertiary amine and can cross the blood-brain barrier, it is indicated for treatment of mild-to-moderate AD and
dementia. It has been used outside the U.S. for more than 30 years as an anticholinesterase agent in anesthesia. Galantamine differs from other cholinesterase inhibitors, because it allosterically binds to nicotinic receptors, giving it a dual cholinergic action. It is metabolized (75%) by CYP2D6 and CYP3A4 to afford the normethyl, O-desmethyl, and O-desmethylnormethyl metabolites, along with some other minor metabolites. Unlike tacrine, galantamine is not associated with hepatotoxicity. Its elimination half-life is 5.7 hours.

**Irreversible Inhibitors of Acetylcholinesterase**

**Mechanism of action**

The chemical logic involved in the development of effective AChEIs was to synthesize compounds that would be substrates for AChE and result in an acylated enzyme more stable to hydrolysis than a carboxylate ester. Phosphate esters are very stable to hydrolysis, being even more stable than many amides. Application of this chemical property to the design of AChEI compounds led to derivatives of phosphoric, pyrophosphoric, and phosphonic acids that are effective inhibitors of AChE. These act as inhibitors by the same mechanism as the carbamate inhibitors, except that they leave the enzyme esterified as phosphate esters. The rate of hydrolytic regeneration of the phosphorylated enzyme is much slower than that of the carbamylated enzyme, and its rate is measured in hours (e.g., the half-lives for diethyl phosphates are ~8 hours). Because the duration of action of these compounds is much longer than that of carbamate esters, they are referred to as irreversible inhibitors of AChE.

An important difference between irreversible phosphoester-derived AChEIs and reversible AChEIs is that the phosphorylated AChE can undergo a process known as aging (Fig. 12.16). The aging process plays an important role in the toxicity of these irreversible AChEIs. Aging is the result of cleavage of one or more of the phosphoester bonds while the AChE is phosphorylated. This reaction affords an anionic phosphate that possesses a phosphorus atom that is much less electrophilic and, therefore, much less likely to undergo hydrolytic regeneration than the original phosphoester. Thus, the aged phosphorylated enzyme does not undergo nucleophilic attack and regeneration by antidotes (see next section) for phosphate ester AChEIs. This aging process occurs over a period of time, which depends on the rate of the P-O bond cleavage reaction; during this time, the antidotes to phosphate ester poisoning may be effective.
Only those phosphorus-derived AChEIs that have at least one phosphoester group undergo the aging process. Knowledge of the chemical mechanisms associated with irreversible inhibition of AChE and the aging process led to the development of deadly phosphorus-derived chemical warfare agents, one of which is sarin (GB is the two letter NATO designation for this nerve agent). When this compound phosphorylates AChE, only one aging reaction takes place, and then the enzyme becomes refractory to regeneration by the currently available antidotal agents.

**Specific agents**

**Echothiophate iodide (Phospholine Iodide)**
Echothiophate iodide has found therapeutic application for the treatment of glaucoma and strabismus. Echothiophate is applied topically as a solution and is the only irreversible AChEI for the treatment of glaucoma. The decrease in intraocular pressure observed can last up to 4 weeks. Phosphoester AChEIs exhibit cataractogenic properties; thus, their use should be reserved for patients who are refractory to other forms of treatment (i.e., short-acting miotics, β-blockers, epinephrine, and possibly, carbonic anhydrase inhibitors). Because of its toxicity, echothiophate is not used for its systemic action. Selectivity of echothiophate for the AChE catalytic site was enhanced by incorporation in the molecule of a quaternary ammonium salt functional group two carbons removed from the phosphoryl group.

![Fig. 12.17. Irreversible acetylcholinesterase inhibitors used as insecticides.](http://thepointeedition.lww.com/pt/re/97807817668795/bookContentPan.../DIVISIONA[2]/CHAPTER[2]&highlightTo=&printPreview=yes)

**Insecticidal AChEIs**

A number of lipophilic derivatives of phosphoester AChEIs have been designed as insecticides; the structures of some of these are shown in Fig. 12.17. This group of irreversible AChEI insecticides is beneficial to agricultural production throughout the world. In addition to being extremely lipophilic, another physicochemical property common to these compounds is a high vapor pressure. This combination of physicochemical properties makes it imperative that these compounds be used with extreme caution in the presence of humans and other mammals to prevent inhalation of the vapors and their absorption through the skin. Both routes of exposure cause a number of poisoning accidents every year, some of which are fatal.

Some of these irreversible AChEI insecticides have a sulfur atom bonded to the phosphorus atom with a coordinate-covalent bond. These compounds exhibit little AChEI activity, but they are rapidly bioactivated via desulfurization by microsomal oxidation in insects to afford the corresponding oxo derivatives (phosphate esters), which are quite potent. A good example of this bioactivation phenomenon is illustrated by the commercially available insecticide parathion and its bioactivation to a toxic metabolite paraoxon.
Malathion (Ovide)

Malathion (Fig. 12.17) is a dithiophosphate ester that has found use both as an aerial insecticide and clinically as a miticide for topical treatment of lice infestations of the hair and scalp. It will kill both hatched lice and their eggs (nits) within 3 seconds after application. Compared to other organophosphate AChEIs, malathion exhibits lower transdermal absorption. On intact skin, less than 10% of a topical dose is systemically absorbed. Similar to parathion, malathion is bioactivated in insects to its toxic phosphate ester metabolite. It is much less toxic in humans, mammals, and birds than in insects. Selective toxicity with malathion is achieved because plasma esterases hydrolyze the carboxylate esters to less toxic carboxylic acid metabolites that are rapidly eliminated in urine as carboxylate anions in humans but not in insects. Acute toxicity with malathion is rare and usually occurs only after oral ingestion. The lethal dose in mammals is approximately 1 g/kg.

Antidotes for Irreversible AChEIs

Background

The marked toxicity of the phosphate ester irreversible AChEIs, their widespread use as insecticides, and their proliferation as chemical warfare agents posed serious problems that stimulated research to develop antidotes for these agents. This required rational use of reaction kinetics, organic reaction mechanisms, and synthetic organic chemistry. Water is a nucleophile capable of rapidly hydrolyzing acetylated AChE and regenerating the active enzyme. Phosphorylated AChE (irreversibly inhibited), however, was known to involve a phosphate ester of serine. It is well established from reaction kinetic studies that the rate of hydrolysis is much slower for organic phosphate esters than for carboxylate esters and that a significantly stronger nucleophile than water would be required for efficient hydrolysis of phosphate esters. The problem required the design of reagents capable of efficiently catalyzing phosphate ester cleavage to regenerate active AChE while being safe enough for use as therapeutic agents. The resolution of this problem is an elegant example of the application of chemical principles to the solution of a therapeutic problem (48,49,50).

Hydroxylamine (NH$_2$OH) is a strong nucleophilic compound that efficiently cleaves phosphate esters. It significantly increases the rate of hydrolysis of phosphorylated AChE, but only at toxic concentrations (51). This prompted the development of a number of structurally related compounds in the hope of eliminating toxicity. The toxicity inherent in hydroxylamine would most probably be present in any structurally related compound, but this toxicity might be minimized if sufficiently small doses could be used. It would be logical to design a compound that would have a high degree of selectivity and strong binding affinity for AChE and also carry a hydroxylamine-like nucleophile into close proximity to the phosphorylated serine residue. This was achieved by synthesis of hydroxylamine derivatives of organic compounds possessing a functional group bearing a positive charge.

Reaction of hydroxylamine with aldehydes or ketones affords oximes, which possess the desired nucleophilic oxygen atom. A pyridine ring was considered an attractive carrier for the oxime function, because such groups are common in a number of biochemical systems (e.g., NAD and NADP), indicating a possible low order of toxicity. Furthermore, three readily available positional isomers of pyridine aldehyde can be converted easily to oximes. Finally, the nitrogen atom of the pyridine ring can be converted to a
quaternary ammonium salt by treatment with methyl iodide. This cationic charge would be expected to increase affinity of the compound for the anionic-binding site of the phosphorylated AChE.

The three isomeric pyridine aldoxime methiodides were synthesized and biologically evaluated. Of these, the most effective is the isomer derived from 2-pyridinylaldehyde. This compound, known as pralidoxime chloride (2-PAM, or 2-pyridine aldoxime methyl chloride) currently is the only available agent proven to be clinically effective as an antidote for poisoning by phosphate ester AChEIs. The proposed mechanism for regeneration of AChE by 2-PAM is illustrated in Fig. 12.18. The initial step involves binding of the quaternary ammonium nitrogen of 2-PAM to the anionic-binding site of phosphorylated AChE. This places the nucleophilic oxygen of 2-PAM in close proximity to the electrophilic phosphorus atom. Nucleophilic attack of the oxime oxygen results in breaking of the ester bond between the serine oxygen atom and the phosphorus atom. The final products of the reaction are the regenerated active form of AChE and phosphorylated 2-PAM.

Pralidoxime is administered subcutaneously, intramuscularly, or intravenously, and it must be given within a short
period of time after enzyme phosphorylation, generally a few hours after exposure, for it to be effective because of the aging process of the phosphorylated enzyme. Little reactivation is likely if given 36 hours after exposure. If the phosphorylated AChE has aged, 2-PAM will not regenerate the enzyme. For this reason, as well as because new phosphate ester AChEIs capable of aging rapidly are being developed as insecticides and chemical warfare agents, there is a continuing effort to discover new and better substitutes for 2-PAM. This research is focused on finding substitutes for 2-PAM that are better nucleophiles and, therefore, more effective generators of active AChE as well as compounds that cross the blood-brain barrier to regenerate phosphorylated AChE in the brain.

**Acetylcholine Antagonists—Muscarinic Antagonists**

Muscarinic antagonists are compounds that have high binding affinity for muscarinic receptors but have no intrinsic activity. When the antagonist binds to the receptor, it is proposed that the receptor protein undergoes a conformational perturbation that is different from that produced by an agonist. Therefore, antagonist binding to the receptor produces no response. Muscarinic antagonists commonly are referred to as anticholinergics, antimuscarinics, cholinergic blockers, antispasmodics, or parasympatholytics. The term “anticholinergic” refers, in a pure sense, to medicinal agents that are antagonists at both muscarinic and nicotinic receptors. Common usage of the term, however, has become synonymous with muscarinic antagonist, and it is used as such in this section.

**Therapeutic Application**

Muscarinic antagonists frequently are employed as both prescription drugs and over-the-counter medications. Because they act as competitive (reversible) antagonists of acetylcholine, these compounds have pharmacological effects that are opposite those of muscarinic agonists. Responses of muscarinic antagonists include decreased contractions of smooth muscle of the gastrointestinal and urinary tracts,
dilation of the pupils, and reduced gastric, mucociliary, and salivary secretions. It follows that these compounds have therapeutic value in treating smooth muscle spasms associated with increased tone of the gastrointestinal tract or with overactive bladder, in ophthalmologic examinations, and in treatment of gastric ulcers. Compounds possessing muscarinic antagonist activity are common components of cold and flu remedies that act to reduce nasal and upper respiratory tract secretions.

In addition to reducing gastric motility, anticholinergic agents decrease gastric acid secretion and were once widely used to manage peptic ulcers. Histamine H₂ antagonists and, more recently, the proton pump inhibitors have largely replaced them for this use. When used systemically, they tend to produce undesirable side effects, such as blurred vision, photophobia, dry mouth, and difficulty in urination. These side effects tend to reduce patient compliance.

Anticholinergic agents exhibit a mydriatic action and, thus, must be used with caution because of their effect on intraocular pressure. Drainage of the canal of Schlemm is restricted by the iris when the pupil is dilated, and this can cause an increase in intraocular pressure. Hence, muscarinic antagonists are contraindicated in patients with glaucoma.

The aforementioned side effect of causing difficulty in urination has been used to advantage with the recent approval of several anticholinergic agents—darifenacin trospium, solifenacin, tolterodine, and oxybutynin—for the treatment of overactive bladder.

Centrally acting belladonna alkaloids, such as scopolamine, have been used in transdermal delivery systems for the prevention of motion sickness. They are most effective when used prophylactically; they have less effect when used after nausea and vomiting have begun. Several of the synthetic muscarinic antagonists have been used to treat parkinsonism and to block the extrapyramidal effects of antipsychotic agents. The anticholinergic alkaloid atropine is used for treatment of central and peripheral symptoms associated with poisoning by organophosphorus anticholinesterase agents.

**Specific Agents—Solanaceous Alkaloids**

The earliest known anticholinergic agents were alkaloids found in the family Solanaceae, a large family of plants that includes potatoes. *Atropa belladonna* (deadly nightshade), *Hyoscymus niger* (black henbane), and *Datura stramonium* (jimsonweed, thorn apple) are plants that have significant historical importance to our understanding of the parasympathetic nervous system. Pharmacological effects of extracts from these plants have been recognized since the Middle Ages, although these effects were not associated with the autonomic nervous system until the latter part of the 19th century. (−)-Hyoscyamine, isolated as atropine, and scopolamine are the two alkaloids that have found the widespread clinical applications.
Atropine

Atropine is the tropic acid ester of tropine and is marketed as the sulfate salt. The naturally occurring alkaloid, (−)-hyoscyamine, undergoes base-catalyzed racemization during isolation from plants of the Solanaceae to give (±)-hyoscyamine or atropine. It was the first compound shown to block the effects of electrical stimulation and muscarine on the parasympathetic nervous system. Atropine sulfate has a number of clinical uses; two of the most common are treatment of bradycardia and as a preoperative agent to reduce secretions before surgery. Its use for management of parkinsonism has been supplanted by newer agents with fewer peripheral side effects. It has been used in ophthalmology as a cycloplegic agent to paralyze the iris and ciliary muscle in the treatment of iritis and uveitis and as a cycloplegic/mydriatic agent. Atropine is contraindicated in glaucoma because of its ability to increase intraocular pressure during mydriasis. Its prolonged duration of mydriasis makes other drugs more attractive for this purpose. In poisoning by organophosphate nerve agents and insecticides, atropine is used to decrease the muscarinic cholinergic actions (e.g., lacrimation, salivation, sweating, bradycardia, and breathing problems) associated with this poisoning. It only treats the symptoms and does not reverse the underlying AChE inhibition. Atropine undergoes nonenzymatic ester hydrolysis in vivo and has an elimination half-life of 4 hours in adults and 6.5 hours in children.

Scopolamine

Scopolamine, another Solanaceous alkaloid, is chemically and pharmacologically similar to atropine. Scopolamine is the generic name given to (−)-hyoscine, the naturally occurring alkaloid. The racemic compound, isolated during extraction of the alkaloid from plants, is atroscine. Scopolamine is marketed as the hydrobromide salt, because it is less deliquescent than some of its other salts. Scopolamine is a CNS depressant at usual therapeutic doses, whereas atropine and other antimuscarinic agents are CNS stimulants. It has been used for the treatment of uveitis, iritis, and parkinsonism, but its most widespread use is for the treatment of motion sickness. For this indication, scopolamine is used in a transdermal patch.
applied behind the ear. It is almost completely metabolized in the liver and is excreted via the kidneys. Its elimination half-life is approximately 8 hours.

**Structure–activity relationship**

Atropine, the prototype anticholinergic agent, provided the structural model that guided the design of synthetic muscarinic antagonists for almost 70 years. The circled portion of the atropine molecule depicts the segment resembling acetylcholine.

Although the amine functional group is separated from the ester oxygen by more than two carbons, the conformation assumed by the tropine ring orients these two atoms such that the intervening distance is similar to that in acetylcholine. One important structural difference between atropine and acetylcholine, both of which are esters of amino alcohols, is the size of the acyl portion of the molecules. Based on the assumption that size was a major factor in blocking action, many substituted acetic acid esters of amino alcohols were prepared and evaluated for biological activity.

It became apparent that the most potent compounds were those that possessed two lipophilic ring substituents on the carbon α to the carbonyl of the ester moiety. This is the first of the classic SARs for muscarinic antagonist activity, and this SAR became defined more precisely as research on these antagonists continued. The SAR for muscarinic antagonists can be summarized as follows:
Substituents $R_1$ and $R_2$ should be carbocyclic or heterocyclic rings for maximal antagonist potency. The rings may be identical, but the more potent compounds have different rings. Generally, one ring is aromatic and the other saturated or possessing only one olefinic bond. Substituents $R_1$ and $R_2$, however, may be combined into a fused aromatic tricyclic ring system, such as that found in propantheline (Table 12.1). The size of these substituents is limited. For example, substitution of naphthalene rings for $R_1$ and $R_2$ affords compounds that are inactive, apparently because of steric hindrance of the binding of these compounds to the muscarinic receptor.

The $R_3$ substituent may be a hydrogen atom, a hydroxyl group, a hydroxymethyl group, or a carboxamide, or it may be a component of one of the $R_1$ and $R_2$ ring systems. When this substituent is either a hydroxyl group or a hydroxymethyl group, the antagonist usually is more potent than the same compound without this group. The hydroxyl group presumably increases binding strength by participating in a hydrogen bond interaction at the receptor.

The $X$ substituent in the most potent anticholinergic agents is an ester, but an ester functional group is not an absolute necessity for muscarinic antagonist activity. This substituent may be an ether oxygen, or it may be absent completely.

The $N$ substituent is a quaternary ammonium salt in the most potent anticholinergic agents. This is not a requirement, however, because tertiary amines also possess antagonist activity, presumably by binding to the receptor in the cationic (conjugate acid) form. The alkyl substituents usually are methyl, ethyl, propyl, or isopropyl.

The distance between the ring-substituted carbon and the amine nitrogen apparently is not critical; the length of the alkyl chain connecting these may

P.383
be from two to four carbons. The most potent anticholinergic agents have two methylene units in this chain.

![Diagram of anticholinergic aminoalcohol esters]

Muscarinic antagonists must compete with agonists for a common receptor. Their ability to do this effectively is because the large groups R₁ and R₂ enhance binding to the receptor. Because antagonists are larger than agonists, this suggests that groups R₁ and R₂ bind outside the binding site of acetylcholine. It has been suggested that the area surrounding the binding site of acetylcholine is hydrophobic in nature (52). This accounts for the fact that in potent cholinergic antagonists, groups R₁ and R₂ must be hydrophobic (usually phenyl, cyclohexyl, or cyclopentyl). This concept also is supported by the current models for muscarinic receptors.

Figures 12.19 and 12.20 and Table 12.3 include structures and pharmacological properties of some of the anticholinergic agents that have found clinical application. These compounds reflect the SAR features that have been described. All these compounds are effective when administered orally or parenterally. Anticholinergic agents possessing a quaternary ammonium functional group generally are not well
absorbed from the gastrointestinal tract because of their ionic character. These drugs are useful primarily in the treatment of ulcers or other conditions for which a reduction in gastric secretions and reduced motility of the gastrointestinal tract are desired. Those antagonists having a tertiary nitrogen are much better absorbed and distributed following all routes of administration and are especially useful when systemic distribution is desired. The tertiary amino-derived anticholinergic agents readily cross the blood-brain barrier. These have proven to be particularly beneficial in the treatment of Parkinson's disease and other diseases requiring a central anticholinergic effect.

<table>
<thead>
<tr>
<th>Anticholinergic aminoalcohols:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Procyclidine Hydrochloride salt (Kemadrin)</td>
<td>Trihexyphenidyl Hydrochloride salt (Artane)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Anticholinergic aminoethers:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orphenadrine Citrate salt (Norflex)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Miscellaneous anticholinergic agents:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solifenacin Succinate salt (Vericare)</td>
</tr>
<tr>
<td>Propantheline Bromide salt (Pro-Banthine)</td>
</tr>
</tbody>
</table>

**Fig. 12.20.** Aminoalcohol, aminoether, and miscellaneous anticholinergic agents.
All these drugs display pronounced selectivity for muscarinic receptors; however, some of those possessing the quaternary ammonium functional group exhibit nicotinic antagonist activity at high doses. With the exception of the M₃ antagonists, solifenacin and darifenacin, these agents display no marked selectivity for muscarinic receptor subtypes.

**Recent muscarinic antagonists**

More recently discovered muscarinic antagonists display a higher affinity for the receptors compared with the older agents, as exemplified by quinuclidinylbenzilate (QNB), which has structural features common to the classic anticholinergic agents. Radiolabeled QNB was instrumental in the development of muscarinic receptor labeling techniques as well as the discovery of subtypes of muscarinic receptors. This latter research also depended on the M₁-selective antagonist pirenzepine, a compound having a novel structure for muscarinic antagonist activity. A number of compounds structurally related to pirenzepine have demonstrated a similar M₁ selectivity; among these is telenzepine (53). Because of their selectivity for muscarinic M₁ receptors, pirenzepine and telenzepine have been evaluated in clinical trials for the treatment of duodenal ulcers. It is of interest to note that AFDX-116, structurally similar to pirenzepine, is a muscarinic antagonist exhibiting selectivity for cardiac M₂ receptors.

<table>
<thead>
<tr>
<th>Name</th>
<th>Calculateda Log P</th>
<th>Log D (pH 7)</th>
<th>Half-life</th>
<th>Metabolism</th>
<th>Indications</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atropine</td>
<td>1.53–1.21</td>
<td>3.5 ± 1.5 hours</td>
<td>Hydrolysis; N-dealkylation; N-oxide</td>
<td>Bradycardia; parkinsonism; cycloplegic/mydriatic</td>
<td>Nonselective muscarinic antagonist; stimulates the CNS</td>
<td></td>
</tr>
<tr>
<td>Scopolamine</td>
<td>0.76</td>
<td>0.29</td>
<td>8 hours</td>
<td>Almost completely metabolized (liver)</td>
<td>Uveitis; iritis; parkinsonism; motion sickness</td>
<td>Nonselective muscarinic antagonist; CNS depressant</td>
</tr>
<tr>
<td>Homatropine (Isopto Homatropine)</td>
<td>1.57–1.17</td>
<td>—</td>
<td>—</td>
<td>Cycloplegic/mydriatic</td>
<td>Nonselective muscarinic antagonist; less potent an shorter duration than atropine</td>
<td></td>
</tr>
<tr>
<td>Ipratropium bromide (Atrovent)</td>
<td>—</td>
<td>2 hours</td>
<td>Hydrolysis</td>
<td>Bronchodilator (oral inhalation); seasonal rhinitis (nasal spray)</td>
<td>Nonselective muscarinic antagonist; slow onset after inhalation</td>
<td></td>
</tr>
<tr>
<td>Tiotropium bromide (Spiriva)</td>
<td>—</td>
<td>5–6 days</td>
<td>CYP2D6 and CYP3A4 Hydrolysis; N-dealkylation; glucuronide conjugation</td>
<td>Chronic obstructive pulmonary disease (oral inhalation)</td>
<td>Equal affinity for M₁, M₂ and M₃ receptors</td>
<td></td>
</tr>
<tr>
<td>Drug</td>
<td>M1 affinity</td>
<td>M2 affinity</td>
<td>M3 affinity</td>
<td>Elimination</td>
<td>Route of Elimination</td>
<td>Indication</td>
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</tr>
<tr>
<td>Trospium Chloride</td>
<td>High</td>
<td>Lesser</td>
<td>High</td>
<td>Hydrolysis; conjugation</td>
<td>Urinary and gastrointestinal antispasmodic</td>
<td>M1 and M3 receptors, lesser affinity for M2.</td>
</tr>
<tr>
<td>Oxybutynin (Sanctura)</td>
<td>5.19</td>
<td>3.93</td>
<td>3.70</td>
<td>2–5 hours</td>
<td>CYP3A4 Hydrolysis; N-dealkylation</td>
<td>Overactive bladder</td>
</tr>
<tr>
<td>Oxybutynin (transdermal: Oxytrol)</td>
<td>3.93</td>
<td>2–5 hours</td>
<td>CYP3A4 Hydrolysis; N-dealkylation</td>
<td>Overactive bladder</td>
<td>Nonselective muscarinic antagonist</td>
<td></td>
</tr>
<tr>
<td>Solifenacin (Vesicare)</td>
<td>3.70</td>
<td>1.70</td>
<td>55 hours</td>
<td>4 R-Hydroxy (active); N-glucuronide; N-oxide; 4R-hydroxy-N-oxide</td>
<td>Overactive bladder</td>
<td>Selective M3 antagonist</td>
</tr>
<tr>
<td>Tolterodine (Detrol)</td>
<td>5.77</td>
<td>2.79</td>
<td>2–4 hours</td>
<td>Primary pathway: CYP2D6 (primary); 7% of Caucasians &amp; 2% of African Americans lack CYP2D6; CYP3A4 is the primary pathway in the latter. Metabolites: 5-hydroxymethyl (active), 5-carboxylic acid, N-dealkylated-5-carboxylic acid</td>
<td>Overactive bladder</td>
<td>Nonselective muscarinic antagonist</td>
</tr>
<tr>
<td>Darifenacin</td>
<td>4.50</td>
<td>2.25</td>
<td>—</td>
<td>CYP2D6 (primary; see tolterodine above); hydroxylation of the dihydrobenzofuran; ring opening (dihydrobenzofuran); N-dealkylation</td>
<td>Overactive bladder</td>
<td>Selective M3 antagonist</td>
</tr>
</tbody>
</table>

^aValues calculated using ACD Lab Solarius, Chemical Abstracts Service, 2006, Columbus, OH (values for quaternary compounds are not listed).

**Nicotinic Antagonists—Neuromuscular Blocking Agents**

Nicotinic antagonists are chemical compounds that bind to cholinergic nicotinic receptors but have no
efficacy. All therapeutically useful nicotinic antagonists are competitive antagonists; in other words, the effects are reversible with acetylcholine. There are two subclasses of nicotinic antagonists—skeletal neuromuscular blocking agents and ganglionic blocking agents—classified according to the two populations of nicotinic receptors. This section emphasizes nicotinic antagonists used clinically as neuromuscular blocking agents. These medicinal agents should not be confused with those skeletal muscle relaxant compounds that produce their effects through the CNS.

**History**

In terms of the historical perspective, tubocurarine, the first known neuromuscular blocking drug, was as important to the understanding of nicotinic antagonists as atropine was to that of muscarinic antagonists. The neuromuscular blocking effects of extracts of curare were first reported as early as 1510, when explorers of the Amazon River region of South America found natives using these plant extracts as arrow poisons. Early research with these crude plant extracts indicated that the active components caused muscle paralysis by effects on either the nerve or the muscle (remember that the concept of neurochemical transmission was not introduced until the late 19th century). In 1856, however, Bernard (54) described the results of his experiments, which demonstrated unequivocally that curare extracts prevented skeletal muscle contractions by an effect at the neuromuscular junction, rather than the nerve innervating the muscle or the muscle itself.

Much of the early literature concerning the effects of curare is confusing and difficult to interpret. This is not at all surprising considering that this research was performed using crude extracts, many of which came from different plants. It was not until the late 1800s that scientists recognized that curare extracts contained quaternary ammonium salts. This knowledge prompted the use of other quaternary ammonium compounds to explore the neuromuscular junction. In the meantime, curare extracts continued to be used to block the effects of nicotine and acetylcholine at skeletal neuromuscular junctions and to explore the nicotinic receptors.

In 1935, King (55) isolated a pure alkaloid, which he named \( d \)-tubocurarine, from a tube curare of unknown botanical origin. The word “tube” refers to the container in which the South American natives transported their plant extract. It was almost 10 years later that the botanical source for \( d \)-tubocurarine was clearly
identified as *Chondodendron tomentosum*. The structure that King assigned to tubocurarine possessed two nitrogen atoms, both of which were quaternary ammonium salts (e.g., a bis-quaternary ammonium compound). It was not until 1970 that the correct structure was reported by Everett et al. (56). The correct structure, shown here, has only one quaternary ammonium nitrogen; the other nitrogen is a tertiary amine salt. Nevertheless, the incorrect structure of tubocurarine served as the model for the synthesis of all the neuromuscular blocking agents in use today. These compounds have been of immense therapeutic value for surgical and orthopedic procedures and have been essential to research that led to the isolation and purification of nicotinic receptors.

The potential therapeutic benefits of the neuromuscular blocking effects of tubocurarine as well as the difficulty in obtaining pure samples of the alkaloid encouraged medicinal chemists to design structurally related compounds possessing nicotinic antagonist activity. Using the incorrectly assigned bis-quaternary ammonium structure of tubocurarine (as reported by King) as a guide, a large number of compounds were synthesized and evaluated. It became apparent that a bis-quaternary ammonium compound having two quaternary ammonium salts separated by 10 to 12 carbon atoms (similar to the distance between the nitrogen atoms in tubocurarine) was a requirement for neuromuscular blocking activity. The rationale for this structural requirement was that in contrast to muscarinic receptors, nicotinic receptors possessed two anionic-binding sites, both of which had to be occupied for a neuromuscular blocking effect. It is important to observe that the current transmembrane model for the nicotinic receptor protein has two anionic sites in the extracellular domain.

Some of the new bis-quaternary ammonium agents produced depolarization of the postjunctional membrane at the neuromuscular junction before causing blockade; other compounds, such as tubocurarine, did not produce this depolarization. Thus, the structural features of the remainder of the molecule determined whether the nicotinic antagonist was a depolarizing or a nondepolarizing neuromuscular blocker.

**Therapeutic Application**

Neuromuscular blocking agents are used primarily as an adjunct to general anesthesia. They produce skeletal muscle relaxation that facilitates operative procedures such as abdominal surgery. Furthermore, they reduce the depth requirement for general anesthetics; this decreases the overall risk of a surgical procedure and shortens the postanesthetic recovery time. Muscles producing rapid movements are the first to be affected by neuromuscular blocking agents. These include muscles of the face, eyes, and neck. Muscles of the limbs, chest, and abdomen are affected next, with the diaphragm (respiration) being affected last. Recovery generally is in the reverse order.

Neuromuscular blocking agents also have been used in the correction of dislocations and the realignment of fractures. Short-acting neuromuscular blocking agents, such as succinylcholine, are routinely used to assist in tracheal intubation. When choosing a neuromuscular blocking agent, four questions must be considered:

- Will the compound produce the desired neuromuscular blockade?
What is its duration of action?

- What are its adverse effects?

- What is its relative cost?

**Side Effects**

Adverse reactions to most, but not all, of the neuromuscular blocking agents may include hypotension, bronchospasm, and cardiac disturbances. The depolarizing agents also cause an initial muscle fasciculation before relaxation. Many of these agents cause release of histamine and subsequent cutaneous (flushing, erythema, urticaria, and pruritus), pulmonary (bronchospasm and wheezing), and cardiovascular (hypotension) effects.

**Specific Depolarizing Neuromuscular Blocking Agents**

Decamethonium bromide

Decamethonium was one of the first neuromuscular blocking agents to be synthesized. An SAR study on a series of bis-quaternary ammonium compounds with varying numbers of methylene groups separating the nitrogen atoms demonstrated that maximal neuromuscular blockade occurred with 10 to 12 unsubstituted methylene groups. Activity diminished as the number of carbons was either decreased or increased. The compound with six methylene groups, hexamethonium, is a nicotinic antagonist at autonomic ganglia (ganglionic blocking agent). All the compounds in this series that possessed neuromuscular blocking activity also caused depolarization of the postjunctional membrane.
Succinylcholine chloride (Anectine)

Succinylcholine is a depolarizing neuromuscular blocking agent that represents a dimer of acetylcholine bonded through their $\alpha$ carbons. The molecule can exist in an extended conformation (antiperiplanar), as shown in the Newman projection. This would account for the appropriate separation of the quaternary nitrogens. Succinylcholine is rapidly hydrolyzed and rendered inactive both in aqueous solution and by plasma esterases; this chemical instability must be considered when preparing solutions for parenteral administration. This same chemical property, however, gives the compound a brief duration of action. As a result, succinylcholine is frequently used for the rapid induction of neuromuscular blockade and when blockade of short duration is desired (Table 12.4). As such, it is used primarily to produce muscle relaxation during endotracheal intubation or endoscopic procedures. The depolarizing property is undesirable in neuromuscular blockers, so most research efforts have been directed toward the design of nondepolarizing agents.

Specific Nondepolarizing Neuromuscular Blocking Agents

Compounds in this class have one or two quaternary ammonium groups. Those with only one quaternary ammonium group, however, exist as bis-cations in vivo because of the second positive charge being on a protonated tertiary amine. The various structures of these compounds serve primarily as a “scaffold” to position two positive charges in the correct three-dimensional orientation for interaction with the transmembrane nicotinic receptors.

**Table 12.4. Properties of Clinically Useful Neuromuscular Blocking Agents**
### Table: Agents Acting at the Neuromuscular Junction and Autonomic Ganglia

<table>
<thead>
<tr>
<th>Agent</th>
<th>Time of Onset (min)</th>
<th>Duration of Action (min)</th>
<th>Half-life (min)</th>
<th>Mode of Elimination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Succinylcholine</td>
<td>1–1.5</td>
<td>6–8</td>
<td>&lt;1</td>
<td>Hydrolysis by plasma cholinesterases</td>
</tr>
<tr>
<td><em>d</em>-Tubocurarine</td>
<td>4–6</td>
<td>80–120</td>
<td>173</td>
<td>Renal elimination, liver clearance</td>
</tr>
<tr>
<td>Vecuronium</td>
<td>2–4</td>
<td>30–40</td>
<td>65–80</td>
<td>Liver metabolism and clearance</td>
</tr>
<tr>
<td>Pancuronium</td>
<td>4–6</td>
<td>120–180</td>
<td>89–140</td>
<td>Renal elimination, liver metabolism and clearance</td>
</tr>
<tr>
<td>Pipecuronium</td>
<td>2–4</td>
<td>80–100</td>
<td>137–161</td>
<td>Renal elimination, liver metabolism and clearance</td>
</tr>
<tr>
<td>Rocuronium</td>
<td>1–2</td>
<td>30–40</td>
<td>84–131</td>
<td>Liver metabolism and clearance</td>
</tr>
<tr>
<td>Atracurium</td>
<td>2–4</td>
<td>30–40</td>
<td>16–20</td>
<td>Hofmann degradation, hydrolysis by plasma cholinesterases</td>
</tr>
<tr>
<td>Mivacurium</td>
<td>2–4</td>
<td>12–18</td>
<td>1.8–2.0</td>
<td>Hydrolysis by plasma cholinesterases</td>
</tr>
<tr>
<td>Doxacurium</td>
<td>4–6</td>
<td>90–120</td>
<td>72–96</td>
<td>Renal elimination, liver metabolism and clearance</td>
</tr>
</tbody>
</table>


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**d-Tubocurarine and metocurine (Metubine Iodide)**

The prototype of this class is *d*-tubocurarine. It is administered intravenously and has a relatively long duration of action. Only approximately 1% of a dose is demethylated in the liver, and it is excreted primarily as unchanged drug in the urine and bile. *d*-Tubocurarine preparations contain bisulfites and, thus, may potentiate allergic reactions in patients with bisulfate allergy. It is the most potent inducer of histamine release of all the nondepolarizing neuromuscular blockers.

Reaction of *d*-tubocurarine with methyl iodide affords metocurine iodide (see above), in which the two phenolic hydroxyl groups of *d*-tubocurarine are changed to the methyl ethers and the tertiary amine becomes quaternary. This agent is approximately fourfold more potent than *d*-tubocurarine in neuromuscular blocking activity. Like *d*-tubocurarine, it has a long duration of action and is eliminated (predominantly unchanged) via the kidney.
Steroid-based neuromuscular blocking agents

An ideal neuromuscular blocking agent would be a nondepolarizing compound that is metabolically inactivated and rapidly eliminated. Efforts to design such a neuromuscular blocker have resulted in the development of several synthetic neuromuscular agents. Those that have found clinical use are either aminosteroids derived from (+)-malouetine (an aminosteroid found in the rain forest of central Africa) (Fig. 12.21) or tetrahydroisoquinoline derivatives (Fig. 12.22).

Pancuronium bromide (Pavulon)

Pancuronium, a long-acting agent, is more active than tubocurarine. It may cause increases in heart rate and blood pressure and should not be used in patients with coronary artery disease. Pancuronium undergoes hydrolysis in the liver to the active 3-hydroxy metabolite and the inactive 17-hydroxy and 3,17-dihydroxy metabolites; it is excreted primarily in the urine, with small amounts in the bile.

Vecuronium bromide (Norcuron)

Removal of the methyl group from the quaternary piperidinium group at position 3 of pancuronium affords vecuronium, an intermediate-acting agent. Vecuronium has the advantage of not inducing histamine release at normal doses and of not exhibiting significant cardiovascular effects. One-third of an administered dose of vecuronium is hydrolyzed to the 3-hydroxy, 17-hydroxy, and 3,17-dihydroxy metabolites, all of which are active. Accumulation of the 3,17-dihydroxy metabolite is responsible for prolonged neuromuscular blockade in patients receiving long-term therapy with vecuronium.

Pipecuronium bromide (Arduan)

Pipecuronium bromide, a long-acting neuromuscular blocking agent, exhibits minimal cardiovascular effects. Like pancuronium and vecuronium, pipecuronium undergoes some hydrolysis but is excreted primarily unchanged in the urine with very small amounts in the bile. Pipecuronium may be used in patients with coronary artery disease, but neuromuscular blockade is prolonged in patients with renal failure.

Rocuronium bromide (Zemuron)

Rocuronium bromide is an intermediate-acting agent with a duration of action similar to vecuronium and atracurium but with a more rapid onset. It does not appear to cause significant histamine release.
A amino steroid–based neuromuscular blocking agents.

Tetrahydroisoquinoline-based neuromuscular blocking agents

Atracurium besylate (Tracrium)

Atracurium besylate is a nondepolarizing neuromuscular blocker in which the quaternary ammonium groups are located in two substituted tetrahydroisoquinoline rings separated by an aliphatic diester. It has a duration of action slightly longer than that of succinylcholine. Atracurium is not metabolized in the liver; rather, it undergoes hydrolysis of the ester functional groups that connect the two quaternary nitrogens. It also undergoes Hofmann elimination, a nonenzymatic, base-catalyzed decomposition, to yield laudanosine, which is inactive (Fig. 12.23) (57,58). Thus, termination of the effects of atracurium are independent of renal elimination. Because of this unusual metabolic profile, it is useful in patients with hepatic or renal disease.
Mivacurium chloride (Mivacron)

Mivacurium chloride is a mixture of three stereoisomers, with the trans-trans (92–96%) and the cis-trans diesters being equipotent. The cis-cis diester produces only minimal (<5%) neuromuscular blockade. It is hydrophilic, has a small volume of distribution, and is distributed primarily to extracellular fluids. Mivacurium is short acting (Table 12.4), with mean elimination half-lives for the trans-trans and cis-trans stereoisomers of 2.0 and 1.8 minutes, respectively, in adults receiving opioid/nitrous oxide/oxygen anesthesia. It is rapidly hydrolyzed and does not undergo Hofmann elimination like atracurium.

Doxacurium chloride (Nuromax)

Doxacurium is a mixture of three trans, trans-stereoisomers, a dl pair [(1R,1’R,2S,2’S) and (1S,1’S,2R,2’R)] and a meso form (1R,1’S,2S,2’R). Doxacurium is hydrophilic, has a small volume of distribution, and is distributed primarily to extracellular fluids. It is not metabolized by plasma cholinesterase or hepatic enzymes and does not undergo Hofmann elimination.
Fig. 12.23. Inactivation of atracurium by Hofmann elimination and hydrolysis.

Case Study

Victoria F. Roche
S. William Zito
PJ was brought to the emergency department where you work by his fellow housemates. PJ is a 29-year-old graduate student at the local university who has been studying for his Ph.D. in anthropology for the past 7 years. His housemates say he is a devotee of “natural highs” and is known to consume 6 to 12 beers per day and for his stash of natural substances, including marijuana. In the emergency department, he is extremely agitated, confused, and combative. His friends say he became that way soon after he ingested a handful of brown, kidney-shaped seeds. On examination, PJ's vital signs showed a temperature of 102°F, with tachycardia, hypertension, and unresponsive, dilated pupils. Urine was collected for routine drug screening and serum for liver function tests. Test results determined that he had elevated aspartate aminotransferase and lactate dehydrogenase, a prolongation of his prothrombin time, and atropine and scopolamine (structures 1 and 2, respectively) in the urine. A diagnosis of anticholinergic overdose was made, and PJ was administered gastric lavage with 2.5 L of normal saline followed by activated charcoal. The physician wants to administer a cholinergic agonist to counter the effects of the anticholinergic overdose. Evaluate structures 3 to 5 for possible use in this case.

- Identify the therapeutic problem(s) where the pharmacist's intervention may benefit the patient.
- Identify and prioritize the patient-specific factors that must be considered to achieve the desired therapeutic outcomes.
- Conduct a thorough and mechanistically oriented SAR analysis of all therapeutic alternatives provided in the case.
- Evaluate the SAR findings against the patient-specific factors and desired therapeutic outcomes, and make a therapeutic decision.
- Counsel your patient.
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Suggested Readings

