Chapter 7. Cholinoceptor-Activating & Cholinesterase-Inhibiting Drugs

Spectrum of Action of Cholinomimetic Drugs

Early studies of the parasympathetic nervous system showed that the alkaloid **muscarine** mimicked the effects of parasympathetic nerve discharge, ie, the effects were **parasympathomimetic.** Application of muscarine to ganglia and to autonomic effector tissues (smooth muscle, heart,

exocrine glands) showed that the parasympathomimetic action of the alkaloid occurred through an action on receptors at effector cells, not those in ganglia. The effects of acetylcholine itself and of other cholinomimetic drugs at autonomic neuroeffector junctions are called parasympathomimetic effects, and are mediated by muscarinic receptors. In contrast, low concentrations of the alkaloid **nicotine** stimulated autonomic ganglia and skeletal muscle neuromuscular junctions but not autonomic effector cells. The ganglion and skeletal muscle receptors were therefore labeled

nicotinic. When acetylcholine was later identified as the physiologic transmitter at both muscarinic and nicotinic receptors, both receptors were recognized as cholinoceptor subtypes.

Cholinoceptors are members of either G protein-linked (muscarinic) or ion channel (nicotinic) families on the basis of their transmembrane signaling mechanisms. Muscarinic receptors contain seven transmembrane domains whose third cytoplasmic loop is coupled to G proteins that function

as intramembrane transducers (see Figure 2–11). In general, these receptors regulate the production

of intracellular second messengers. Agonist selectivity is determined by the subtypes of muscarinic receptors and G proteins that are present in a given cell (Table 7–1). Muscarinic receptors are

located on plasma membranes of cells in the central nervous system, in organs innervated by parasympathetic nerves as well as on some tissues that are not innervated by these nerves, eg, endothelial cells (Table 7–1), and on those tissues innervated by postganglionic sympathetic cholinergic nerves.

Table 7–1. Subtypes and Characteristics of Cholinoceptors.

Receptor	Other Names	Location	Structural Features	Postreceptor
M_1	M_{1a}	Nerves	Seven transmembrane segments, G protein-linked	IP_3, DAG cascade
M_2	M_{2a}, cardiac M_2	Heart, nerves, smooth muscle	Seven transmembrane segments, G protein-linked	Inhibition of cAMP production, activation of K^+ channels
M_3	M_{2b}, glandular	Glands, smooth	Seven transmembrane	IP_3, DAG cascade

	M_2	muscle, endothelium	segments, G protein-linked	
m_4[1]		?CNS	Seven transmembrane segments, G protein-linked	Inhibition of cAMP production
m_5[1]		?CNS	Seven transmembrane segments, G protein-linked	IP_3, DAG cascade
N_M	Muscle type, end plate receptor	Skeletal muscle neuromuscular junction	Pentamer ($_2$ B.F.v)[2]	Na^+, K^+ depolarizing ion channel
N_N	Neuronal type, ganglion receptor	Postganglionic cell body, dendrites	and subunits only as $_2$ $_2$ or $_3$ $_3$	Na^+, K^+ depolarizing ion channel

[1]Genes have been cloned, but functional receptors have not been incontrovertibly identified.

[2]Structure in *Torpedo* electric organ and fetal mammalian muscle. In adult muscle, the subunit is replaced by an subunit. Several different and subunits have been identified in different mammalian tissues (Lukas et al, 1999).

Nicotinic receptors are part of a transmembrane polypeptide whose subunits form cation-selective ion channels (see Figure 2–9). These receptors are located on plasma membranes of postganglionic cells in all autonomic ganglia, of muscles innervated by somatic motor fibers, and of some central nervous system neurons (see Figure 6–1).

Unselective cholinoceptor stimulants in sufficient dosage can produce very diffuse and marked alterations in organ system function because acetylcholine has multiple sites of action where it initiates both excitatory and inhibitory effects. Fortunately, drugs are available that have a degree of

selectivity, so that desired effects can often be achieved while avoiding or minimizing adverse

effects. Selectivity of action is based on several factors. Some drugs stimulate either muscarinic receptors or nicotinic receptors selectively. Some agents stimulate nicotinic receptors at neuromuscular junctions preferentially and have less effect on nicotinic receptors in ganglia. Organ selectivity can also be achieved by using appropriate routes of administration ("pharmacokinetic

selectivity"). For example, muscarinic stimulants can be administered topically to the surface of the eye to modify ocular function while minimizing systemic effects.

Mode of Action of Cholinomimetic Drugs

Direct-acting cholinomimetic agents directly bind to and activate muscarinic or nicotinic receptors

(Figure 7–1). Indirect-acting agents produce their primary effects by inhibiting acetylcholinesterase, which hydrolyzes acetylcholine to choline and acetic acid (see Figure 6–3). By inhibiting acetylcholinesterase, the indirect-acting drugs increase the endogenous acetylcholine concentration

in synaptic clefts and neuroeffector junctions, and the excess acetylcholine in turn stimulates cholinoceptors to evoke increased responses. These drugs act primarily where acetylcholine is physiologically released and are *amplifiers* of endogenous acetylcholine.

Some cholinesterase inhibitors also inhibit butyrylcholinesterase (pseudocholinesterase). However, inhibition of butyrylcholinesterase plays little role in the action of indirect-acting cholinomimetic drugs because this enzyme is not important in the physiologic termination of synaptic acetylcholine action. Some quaternary cholinesterase inhibitors also have a modest direct action as well, eg, neostigmine, which activates neuromuscular nicotinic cholinoceptors directly in addition to

blocking cholinesterase.

Basic Pharmacology of the Direct-Acting Cholinoceptor Stimulants

The direct-acting cholinomimetic drugs can be divided on the basis of chemical structure into esters

of choline (including acetylcholine) and alkaloids (such as muscarine and nicotine). A few of these drugs are highly selective for the muscarinic or for the nicotinic receptor. Many have effects on

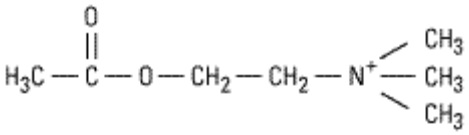
both receptors; acetylcholine is typical.

Chemistry & Pharmacokinetics Structure

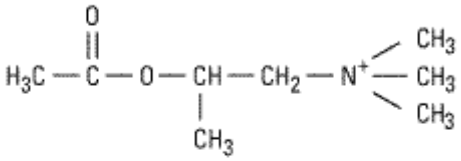
Four important choline esters that have been studied extensively are shown in Figure 7–2. Their permanently charged quaternary ammonium group renders them relatively insoluble in lipids. Many naturally occurring and synthetic cholinomimetic drugs that are not choline esters have been

identified; a few of these are shown in Figure 7–3. The muscarinic receptor is strongly stereoselective: *(S)*-bethanechol is almost 1000 times more potent than *(R)*-bethanechol.

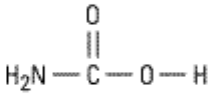
Figure 7–2.



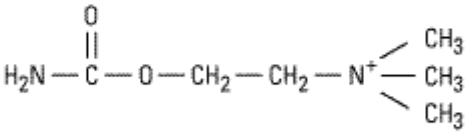
Acetylcholine



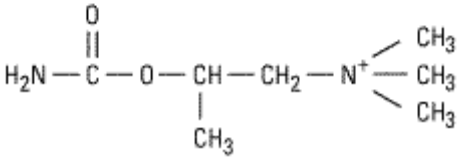
Methacholine
(acetyl-β-methylcholine)



Carbamic acid



Carbachol
(carbamoylcholine)



Bethanechol
(carbamoyl-β-methylcholine)

Molecular structures of four choline esters and carbamic acid. Acetylcholine and methacholine are acetic acid esters of choline and β-methylcholine, respectively. Carbachol and bethanechol are carbamic acid esters of the same alcohols.

Choline esters are poorly absorbed and poorly distributed into the central nervous system because

they are hydrophilic. Although all are hydrolyzed in the gastrointestinal tract (and less active by the oral route), they differ markedly in their susceptibility to hydrolysis by cholinesterase in the body. Acetylcholine is very rapidly hydrolyzed (see Chapter 6: Introduction to Autonomic

Pharmacology); large amounts must be infused intravenously to achieve concentrations high

enough to produce detectable effects. A large intravenous bolus injection has a brief effect, typically

5–20 seconds, whereas intramuscular and subcutaneous injections produce only local effects. Methacholine is more resistant to hydrolysis, and the carbamic acid esters carbachol and

bethanechol are still more resistant to hydrolysis by cholinesterase and have correspondingly longer durations of action. The β-methyl group (methacholine, bethanechol) reduces the potency of these drugs at nicotinic receptors (Table 7–2).

Table 7–2. Properties of Choline Esters.

Choline Ester	Susceptibility to Cholinesterase	Muscarinic Action	Nicotinic Action
Acetylcholine chloride	++++	+++	+++
Methacholine chloride	+	++++	None
Carbachol chloride	Negligible	++	+++
Bethanechol chloride	Negligible	++	None

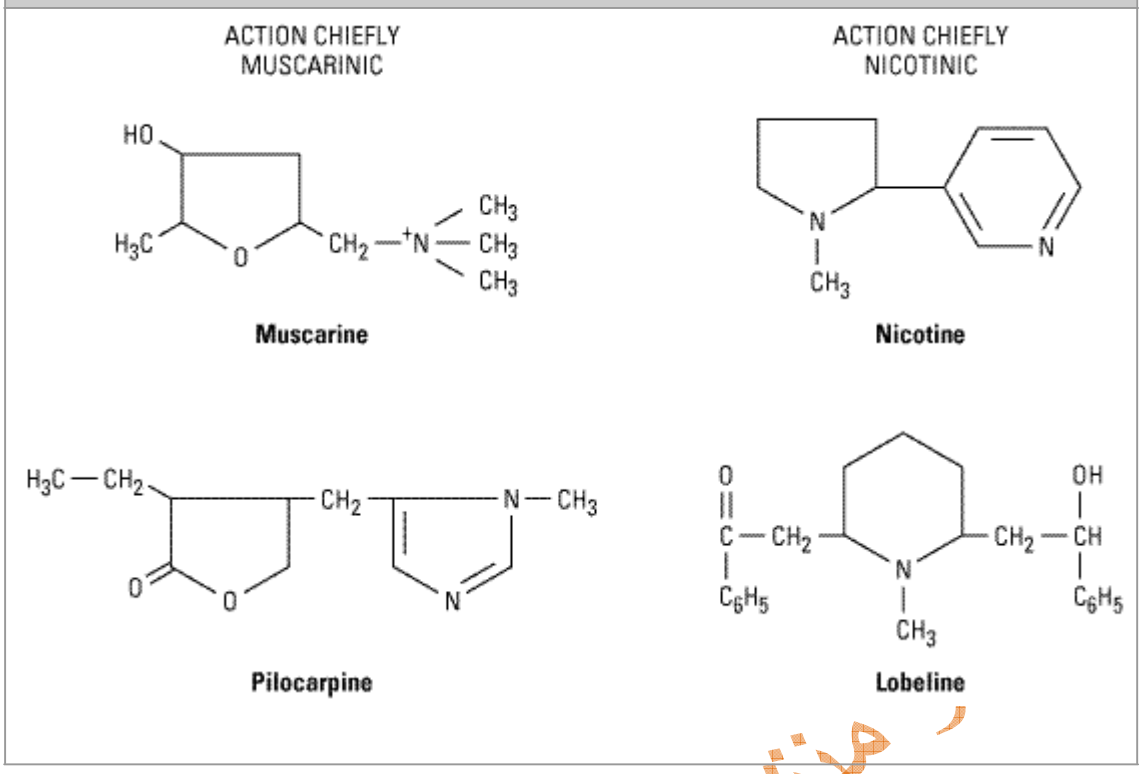
The tertiary natural cholinomimetic alkaloids (pilocarpine, nicotine, lobeline; Figure 7–3) are well

absorbed from most sites of administration. Nicotine, a liquid, is sufficiently lipid-soluble to be absorbed across the skin. Muscarine, a quaternary amine, is less completely absorbed from the gastrointestinal tract than the tertiary amines but is nevertheless toxic when ingested, eg, in certain mushrooms, and even enters the brain. Lobeline is a plant derivative similar to nicotine. These

amines are excreted chiefly by the kidneys. Acidification of the urine accelerates clearance of the

tertiary amines.

Figure 7–3.



Structures of some cholinomimetic alkaloids.

Pharmacodynamics

Mechanism of Action

Activation of the parasympathetic nervous system modifies organ function by two major mechanisms. First, acetylcholine released from parasympathetic nerves activates muscarinic receptors on effector cells to alter organ function directly. Second, acetylcholine released from

parasympathetic nerves interacts with muscarinic receptors on nerve terminals to inhibit the release

of their neurotransmitter. By this mechanism, acetylcholine release and circulating muscarinic agonists indirectly alter organ function by modulating the effects of the parasympathetic and sympathetic nervous systems and perhaps nonadrenergic, noncholinergic systems.

The mechanisms by which muscarinic stimulants alter cellular function continue to be investigated.

As indicated in Chapter 6: Introduction to Autonomic Pharmacology, muscarinic receptor subtypes have been characterized by binding studies and cloned. Several cellular events occur when

muscarinic receptors are activated, one or more of which might serve as second messengers for muscarinic activation. All muscarinic receptors appear to be of the G-protein coupled type (see Chapter 2: Drug Receptors & Pharmacodynamics and Table 7–1). Muscarinic agonist binding activates the IP_3, DAG cascade. Some evidence implicates DAG in the opening of smooth muscle calcium channels; IP_3 releases calcium from endoplasmic and sarcoplasmic reticulum. Muscarinic agonists also increase cellular cGMP concentrations. Activation of muscarinic receptors also increases potassium flux across cardiac cell membranes and decreases it in ganglion and smooth muscle cells. This effect is mediated by the binding of an activated G protein directly to the

channel. Finally, muscarinic receptor activation in some tissues (eg, heart, intestine) inhibits adenylyl cyclase activity. Moreover, muscarinic agonists can attenuate the activation of adenylyl cyclase and modulate the increase in cAMP levels induced by hormones such as catecholamines.

These muscarinic effects on cAMP generation cause a reduction of the physiologic response of the organ to stimulatory hormones.

The mechanism of nicotinic receptor activation has been studied in great detail, taking advantage of three factors: (1) the receptor is present in extremely high concentration in the membranes of the electric organs of electric fish; (2) -bungarotoxin, a component of certain snake venoms, is tightly bound to the receptors and readily labeled as a marker for isolation procedures; and (3) receptor activation results in easily measured electrical and ionic changes in the cells involved. The nicotinic receptor in muscle tissues is a pentamer of four types of glycoprotein subunits (one monomer

occurs twice) with a total molecular weight of about 250,000 (see Figure 2–9). The neuronal nicotinic receptor consists of and subunits only (Table 7–1). Each subunit has four transmembrane segments. Each

subunit has a receptor site that, when occupied by a nicotinic agonist, causes a conformational change in the protein (channel opening) that allows sodium and potassium ions to diffuse rapidly down their concentration gradients. While binding of an agonist molecule by one of the two subunit receptor sites only modestly increases the probability of channel opening, simultaneous binding of agonist by both of the receptor sites greatly enhances opening probability. The primary effect of nicotinic receptor activation is depolarization of the

nerve cell or neuromuscular end plate membrane.

Prolonged agonist occupancy of the nicotinic receptor abolishes the effector response; ie, the postganglionic neuron stops firing (ganglionic effect), and the skeletal muscle cell relaxes

(neuromuscular end plate effect). Furthermore, the continued presence of the nicotinic agonist prevents electrical recovery of the postjunctional membrane. Thus, a state of "depolarizing

blockade" is induced that is refractory to reversal by other agonists. As noted below, this effect can

be exploited for producing muscle paralysis.

Organ System Effects

Most of the direct organ system effects of muscarinic cholinoceptor stimulants are readily predicted from a knowledge of the effects of parasympathetic nerve stimulation (see Table 6–3) and the distribution of muscarinic receptors. Effects of a typical agent such as acetylcholine are listed in

Table 7–3. The effects of nicotinic agonists are similarly predictable from a knowledge of the physiology of the autonomic ganglia and skeletal muscle motor end plate.

Table 7–3. Effects of Direct-Acting Cholinoceptor Stimulants. Only the Direct Effects Are Indicated; Homeostatic Responses to These Direct Actions May Be Important (See Text).

Organ	Response
Eye	
Sphincter muscle of iris	Contraction (miosis)
Ciliary muscle	Contraction for near vision
Heart	
Sinoatrial node	Decrease in rate (negative chronotropy)
Atria	Decrease in contractile strength (negative inotropy). Decrease in refractory period
Atrioventricular node	Decrease in conduction velocity (negative dromotropy). Increase in refractory period
Ventricles	Small decrease in contractile strength
Blood vessels	
Arteries	Dilation (via EDRF). Constriction (high-dose direct effect)
Veins	Dilation (via EDRF). Constriction (high-dose direct effect)
Lung	
Bronchial muscle	Contraction (bronchoconstriction)
Bronchial glands	Stimulation
Gastrointestinal tract	
Motility	Increase
Sphincters	Relaxation
Secretion	Stimulation
Urinary bladder	
Detrusor	Contraction
Trigone and sphincter	Relaxation

Glands	
Sweat, salivary, lacrimal, nasopharyngeal	Secretion

Eye

Muscarinic agonists instilled into the conjunctival sac cause contraction of the smooth muscle of the iris sphincter (resulting in miosis) and of the ciliary muscle (resulting in accommodation). As a

result, the iris is pulled away from the angle of the anterior chamber, and the trabecular meshwork

at the base of the ciliary muscle is opened. Both effects facilitate aqueous humor outflow into the canal of Schlemm, which drains the anterior chamber.

Cardiovascular System

The primary cardiovascular effects of muscarinic agonists are reduction in peripheral vascular resistance and changes in heart rate. The direct effects listed in Table 7–3 are modified by important homeostatic reflexes, as described in Chapter 6: Introduction to Autonomic Pharmacology and

depicted in Figure 6–7. Intravenous infusions of minimal effective doses of acetylcholine in humans

(eg, 20–50 g/min) cause vasodilation, resulting in a reduction in blood pressure, often

accompanied by a reflex increase in heart rate. Larger doses of acetylcholine produce bradycardia and decrease atrioventricular node conduction velocity in addition to the hypotensive effect.

The direct cardiac actions of muscarinic stimulants include the following: (1) an increase in a potassium current ($I_{K(ACh)}$) in atrial muscle cells and in the cells of the sinoatrial and atrioventricular nodes as well; (2) a decrease in the slow inward calcium current (I_{Ca}) in heart cells; and (3) a

reduction in the hyperpolarization-activated current (I_f) that underlies diastolic depolarization. All

of these actions are mediated by M_2 receptors and contribute to slowing the pacemaker rate. Effects

(1) and (2) cause hyperpolarization and decrease the contractility of atrial cells.

The direct slowing of sinoatrial rate and atrioventricular conduction that is produced by muscarinic agonists is often opposed by reflex sympathetic discharge, elicited by the decrease in blood

pressure. The resultant sympathetic-parasympathetic interaction is complex because of the

muscarinic modulation of sympathetic influences that occurs by inhibition of norepinephrine release and by postjunctional cellular effects. Muscarinic receptors that are present on postganglionic parasympathetic nerve terminals allow neurally released acetylcholine to inhibit its own secretion.

The neuronal muscarinic receptors need not be the same subtype as found on effector cells.

Therefore, the net effect on heart rate depends on local concentrations of the agonist in the heart and

in the vessels and on the level of reflex responsiveness.

Parasympathetic innervation of the ventricles is much less extensive than that of the atria and activation of ventricular muscarinic receptors results in much less physiologic effect than that seen

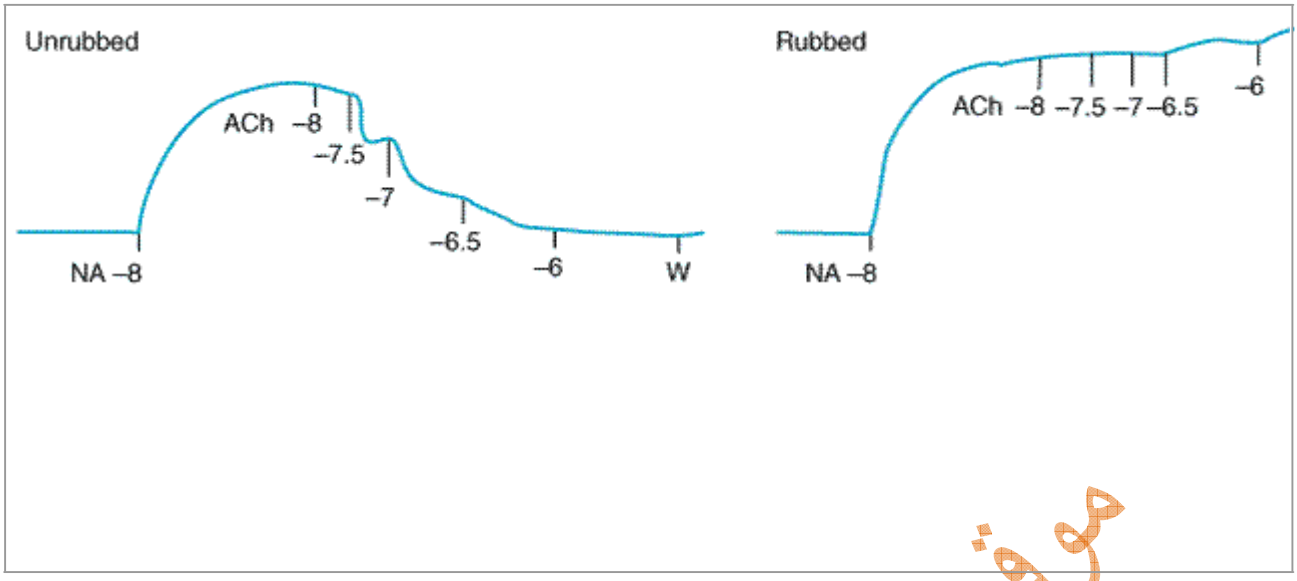
in atria. However, during sympathetic stimulation, the effects of muscarinic agonists on ventricular function are clearly evident because of muscarinic modulation of sympathetic effects ("accentuated antagonism"; Levy et al, 1994).

In the intact organism, muscarinic agonists produce marked vasodilation. However, in earlier

studies, isolated blood vessels often showed a contractile response to these agents. It is now known that acetylcholine-induced vasodilation requires the presence of intact endothelium (Figure 7–4). Muscarinic agonists release a substance (endothelium-derived relaxing factor, or EDRF) from the endothelial cells that relaxes smooth muscle. Isolated vessels prepared with the endothelium

preserved consistently reproduce the vasodilation seen in the intact organism. EDRF appears to be largely nitric oxide (NO). This substance activates guanylyl cyclase and increases cGMP in smooth muscle, resulting in relaxation (see Figure 12–2).

Figure 7–4.



Activation of endothelial cell muscarinic receptors by acetylcholine releases endothelium-derived relaxing factor (nitric oxide) (EDRF [NO]), which causes relaxation of vascular smooth muscle precontracted with norepinephrine. Removal of the endothelium by rubbing eliminates the relaxant effect and reveals contraction caused by direct action of acetylcholine on vascular smooth muscle.

(Modified and reproduced, with permission, from Furchgott RF, Zawadzki JV: The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980;288:373.)

The cardiovascular effects of all of the choline esters are similar to those of acetylcholine, the main difference being in their potency and duration of action. Because of the resistance of methacholine, carbachol, and bethanechol to acetylcholinesterase, lower doses given intravenously are sufficient to produce effects similar to those of acetylcholine, and the duration of action of these synthetic choline esters is longer. The cardiovascular effects of most of the cholinomimetic natural alkaloids and the synthetic analogs are also generally similar to those of acetylcholine.

Pilocarpine is an interesting exception to the above statement. If given intravenously (an experimental exercise), it may produce hypertension after a brief initial hypotensive response. The longer-lasting hypertensive effect can be traced to sympathetic ganglionic discharge caused by activation of postganglionic cell membrane M_1 receptors, which close K^+ channels and elicit slow excitatory (depolarizing) postsynaptic potentials. This effect, like the hypotensive effect, can be blocked by atropine, an antimuscarinic drug.

Respiratory System

Muscarinic stimulants contract the smooth muscle of the bronchial tree. In addition, the glands of

the tracheobronchial mucosa are stimulated to secrete. This combination of effects can occasionally cause symptoms, especially in individuals with asthma.

Gastrointestinal Tract

Administration of muscarinic agonists, like parasympathetic nervous system stimulation, increases

the secretory and motor activity of the gut. The salivary and gastric glands are strongly stimulated;

the pancreas and small intestinal glands less so. Peristaltic activity is increased throughout the gut, and most sphincters are relaxed. Stimulation of contraction in this organ system involves depolarization of the smooth muscle cell membrane and increased calcium influx.

Genitourinary Tract

Muscarinic agonists stimulate the detrusor muscle and relax the trigone and sphincter muscles of the bladder, thus promoting voiding. The human uterus is not notably sensitive to muscarinic agonists.

Miscellaneous Secretory Glands

Muscarinic agonists stimulate secretion by thermoregulatory sweat, lacrimal, and nasopharyngeal glands.

Central Nervous System

The central nervous system contains both muscarinic and nicotinic receptors, the brain being relatively richer in muscarinic sites and the spinal cord containing a preponderance of nicotinic sites. The physiologic roles of these receptors are discussed in Chapter 21: Introduction to the Pharmacology of CNS Drugs.

The role of muscarinic receptors in the central nervous system has been confirmed by experiments

in knockout mice (see Chapter 1: Introduction). Predictably, carbachol did not inhibit atrial rate in animals with mutated M_2 receptors. The central nervous system effects of the synthetic muscarinic agonist oxotremorine (tremor, hypothermia, and antinociception) were also lacking in mice with homozygously mutated M_2 receptors. Knockout of M_1 receptors is associated with different changes

in the peripheral and central nervous systems. Oxotremorine did not suppress M current in

sympathetic ganglia, and pilocarpine did not induce epileptic seizures in M_1 mutant mice.

In spite of the smaller ratio of nicotinic to muscarinic receptors in the brain, nicotine and lobeline

(Figure 7–3) have important effects on the brainstem and cortex. The mild alerting action of nicotine absorbed from inhaled tobacco smoke is the best-known of these effects. In larger concentrations, nicotine induces tremor, emesis, and stimulation of the respiratory center. At still

higher levels, nicotine causes convulsions, which may terminate in fatal coma. The lethal effects on

the central nervous system and the fact that nicotine is readily absorbed form the basis for the use of nicotine as an insecticide. Dimethylphenylpiperazinium (DMPP), a synthetic nicotinic stimulant

used in research is relatively free of these central effects because it does not cross the blood-brain barrier.

Peripheral Nervous System

The autonomic ganglia are important sites of nicotinic synaptic action. The nicotinic agents shown

in Figure 7–3 cause marked activation of these nicotinic receptors and initiate action potentials in postganglionic neurons. Nicotine itself has a somewhat greater affinity for neuronal than for skeletal muscle nicotinic receptors. The action is the same on both parasympathetic and sympathetic

ganglia. The initial response therefore often resembles simultaneous discharge of both the parasympathetic

and the sympathetic nervous systems. In the case of the cardiovascular system, the effects of nicotine are chiefly sympathomimetic. Dramatic hypertension is produced by parenteral injection of nicotine; sympathetic tachycardia may alternate with a vagally mediated bradycardia. In

the gastrointestinal and urinary tracts, the effects are largely parasympathomimetic: nausea,

vomiting, diarrhea, and voiding of urine are commonly observed. Prolonged exposure may result in depolarizing blockade of the ganglia.

Neuronal nicotinic receptors are present on sensory nerve endings—especially afferent nerves in coronary arteries and the carotid and aortic bodies as well as on the glomus cells of the latter. Activation of these receptors by nicotinic stimulants and of muscarinic receptors on glomus cells by muscarinic stimulants elicits complex medullary responses, including respiratory alterations and

vagal discharge.

Neuromuscular Junction

The nicotinic receptors on the neuromuscular end plate apparatus are similar but not identical to the receptors in the autonomic ganglia (see Table 7–1). Both types respond to acetylcholine and

nicotine. (However, as discussed in Chapter 8: Cholinoceptor-Blocking Drugs, the receptors differ

in their structural requirements for nicotinic blocking drugs.) When a nicotinic agonist is applied directly (by iontophoresis or by intra-arterial injection), an immediate depolarization of the end

plate results, caused by an increase in permeability to sodium and potassium ions. Depending on the synchronization of depolarization of end plates throughout the muscle, the contractile response will vary from disorganized fasciculations of independent motor units to a strong contraction of the

entire muscle. Depolarizing nicotinic agents that are not rapidly hydrolyzed (like nicotine itself)

cause rapid development of depolarization blockade; transmission blockade persists even when the membrane has repolarized (discussed further in Chapters 8 and 27). In the case of skeletal muscle,

this block is manifested as flaccid paralysis.

Basic Pharmacology of the Indirect-Acting Cholinomimetics

The actions of acetylcholine released from autonomic and somatic motor nerves are terminated by enzymatic destruction of the molecule. Hydrolysis is accomplished by the action of acetylcholinesterase, which is present in high concentrations in cholinergic synapses. The indirect- acting cholinomimetics have their primary effect at the active site of this enzyme, although some

also have direct actions at nicotinic receptors. The chief differences between members of the group are chemical and pharmacokinetic—their pharmacodynamic properties are almost identical.

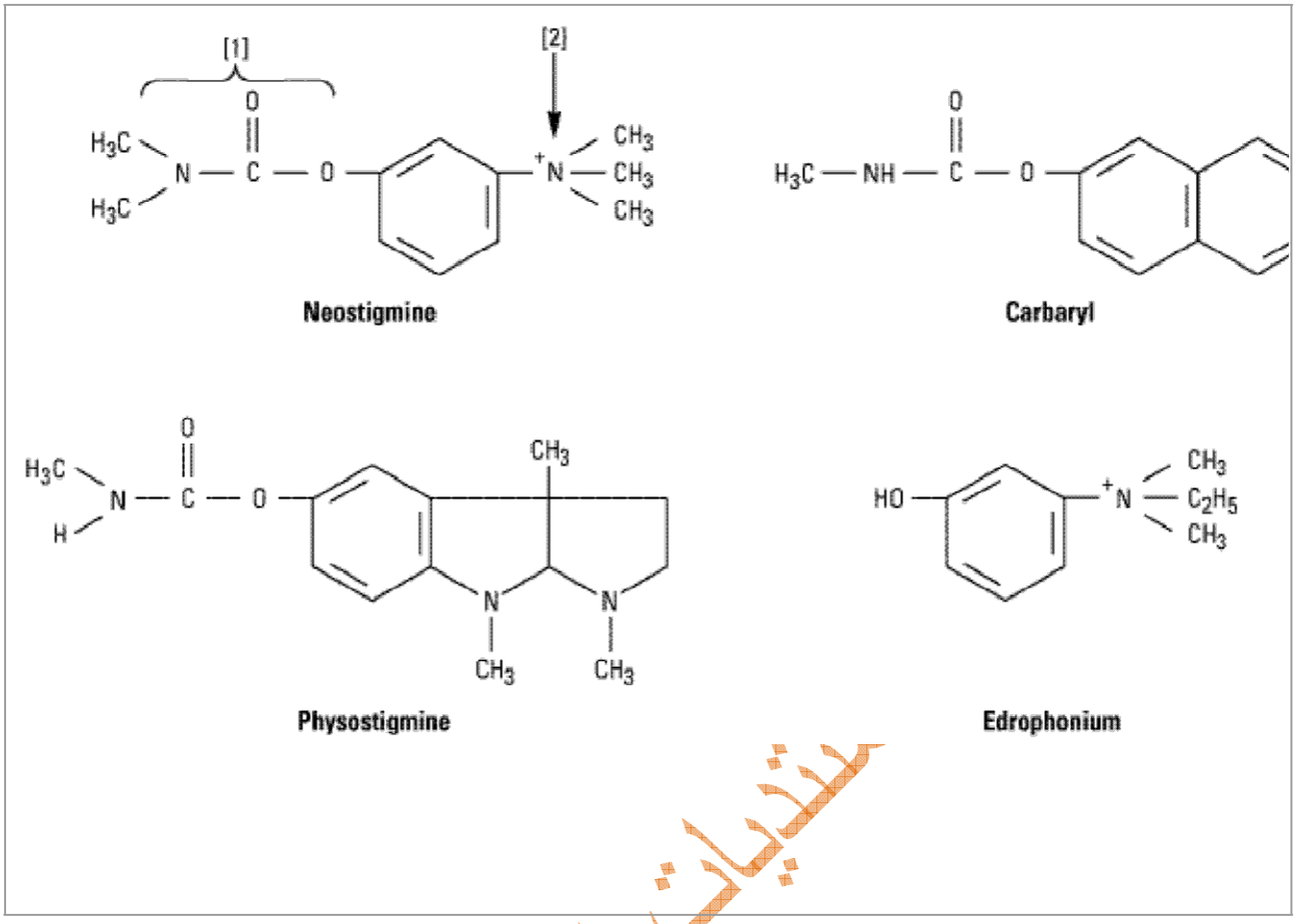
Chemistry & Pharmacokinetics

Structure

The commonly used cholinesterase inhibitors fall into three chemical groups: (1) simple alcohols bearing a quaternary ammonium group, eg, edrophonium; (2) carbamic acid esters of alcohols bearing quaternary or

tertiary ammonium groups (carbamates, eg, neostigmine); and (3) organic derivatives of phosphoric acid (organophosphates, eg, echothiophate). Examples of the first two groups are shown in Figure 7–5. Edrophonium, neostigmine, and ambenonium are synthetic quaternary ammonium agents used in medicine. Physostigmine (eserine) is a naturally occurring tertiary amine of greater lipid solubility that is also used in therapeutics. Carbaryl (carbaril) is typical of a large group of carbamate insecticides designed for very high lipid solubility, so that absorption into the insect and distribution to its central nervous system are very rapid.

Figure 7–5.



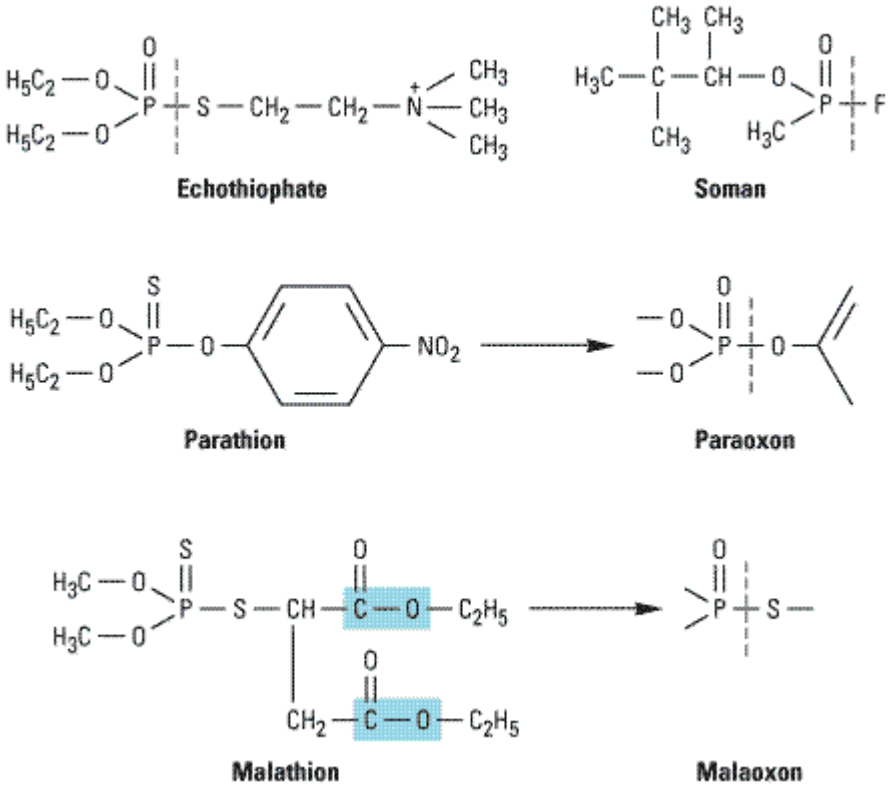
Cholinesterase inhibitors. Neostigmine exemplifies the typical compound that is an ester of carbamic acid ([1]) and a phenol bearing a quaternary ammonium group ([2]). Physostigmine, a naturally occurring carbamate, is a tertiary amine. Edrophonium is not an ester but binds to the active site of the enzyme.

A few of the estimated 50,000 organophosphates are shown in Figure 7–6. Many of the

organophosphates (echothiophate is an exception) are highly lipid-soluble liquids. Echothiophate, a thiocholine derivative, is of clinical value because it retains the very long duration of action of other organophosphates but is more stable in aqueous solution. Soman is an extremely potent "nerve gas." Parathion and malathion are thiophosphate insecticides that are inactive as such; they are converted

to the phosphate derivatives in animals and plants and are used as insecticides.

Figure 7–6.



Structures of some organophosphate cholinesterase inhibitors. The dashed lines indicate the bond that is hydrolyzed in binding to the enzyme. The shaded ester bonds in malathion represent the points of detoxification of the molecule in mammals and birds.

Absorption, Distribution, and Metabolism

Absorption of the quaternary carbamates from the conjunctiva, skin, and lungs is predictably poor, since their permanent charge renders them relatively insoluble in lipids. Similarly, much larger

doses are required for oral administration than for parenteral injection. Distribution into the central nervous system is negligible. Physostigmine, in contrast, is well absorbed from all sites and can be used topically in the eye (Table 7–4). It is distributed into the central nervous system and is more toxic than the more polar quaternary carbamates. The carbamates are relatively stable in aqueous solution but can be metabolized by nonspecific esterases in the body as well as by cholinesterase. However, the duration of their effect is determined chiefly by the stability of the inhibitor-enzyme complex (see Mechanism of Action, below), not by metabolism or excretion.

Table 7–4. Therapeutic Uses and Durations of Action of Cholinesterase Inhibitors.		
	Uses	**Approximate Duration of**
Alcohols		
Edrophonium	Myasthenia gravis, ileus, arrhythmias	5–15 minutes
Carbamates and related agents		
Neostigmine	Myasthenia gravis, ileus	0.5–2 hours

Pyridostigmine	Myasthenia gravis	3–6 hours
Physostigmine	Glaucoma	0.5–2 hours
Ambenonium	Myasthenia gravis	4–8 hours
Demecarium	Glaucoma	4–6 hours
Organophosphates		
Echothiophate	Glaucoma	100 hours

The organophosphate cholinesterase inhibitors (except for echothiophate) are well absorbed from the skin, lung, gut, and conjunctiva—thereby making them dangerous to humans and highly effective as insecticides. They are relatively less stable than the carbamates when dissolved in water and thus have a limited half-life in the environment (compared with the other major class of insecticides, the halogenated hydrocarbons, eg, DDT). Echothiophate is highly polar and more stable than most other organophosphates. It can be made up in aqueous solution for ophthalmic use and retains its activity for weeks.

The thiophosphate insecticides (parathion, malathion, and related compounds) are quite lipid-soluble and are rapidly absorbed by all routes. They must be activated in the body by conversion to the oxygen analogs (Figure 7–6), a process that occurs rapidly in both insects and vertebrates. Malathion and certain other organophosphate insecticides are also rapidly metabolized by other pathways to inactive products in birds and mammals but not in insects; these agents are therefore considered safe enough for sale to the general public. Unfortunately, fish cannot detoxify malathion, and significant numbers of fish have died from the heavy use of this agent on and near waterways. Parathion is not detoxified effectively in vertebrates; thus, it is considerably more dangerous than malathion to humans and livestock and is not available for general public use.

All of the organophosphates except echothiophate are distributed to all parts of the body, including the central nervous system. Poisoning with these agents therefore includes an important component of central nervous system toxicity.

Pharmacodynamics

Mechanism of Action

Acetylcholinesterase is the primary target of these drugs, but butyrylcholinesterase is also inhibited. Acetylcholinesterase is an extremely active enzyme. In the initial step, acetylcholine binds to the enzyme's active site and is hydrolyzed, yielding free choline and the acetylated enzyme. In the

second step, the covalent acetylenzyme bond is split, with the addition of water (hydration). The entire process takes place in approximately 150 microseconds.

All of the cholinesterase inhibitors increase the concentration of endogenous acetylcholine at cholinoceptors by inhibiting acetylcholinesterase. However, the molecular details of their interaction with the enzyme vary according to the three chemical subgroups mentioned above.

The first group, of which edrophonium is the major example, consists of quaternary alcohols. These agents reversibly bind electrostatically and by hydrogen bonds to the active site, thus preventing

access of acetylcholine. The enzyme-inhibitor complex does not involve a covalent bond and is correspondingly short-lived (on the order of 2–10 minutes). The second group consists of carbamate esters, eg, neostigmine and physostigmine. These agents undergo a two-step hydrolysis sequence

analogous to that described for acetylcholine. However, the covalent bond of the *carbamoylated* enzyme is considerably more resistant to the second (hydration) process, and this step is correspondingly prolonged (on the order of 30 minutes to 6 hours). The third group consists of the organophosphates. These agents also undergo initial binding and hydrolysis by the enzyme,

resulting in a *phosphorylated* active site. The covalent phosphorus-enzyme bond is extremely stable and hydrolyzes in water at a very slow rate (hundreds of hours). After the initial binding-hydrolysis step, the phosphorylated enzyme complex may undergo a process called **aging.** This process

apparently involves the breaking of one of the oxygen-phosphorus bonds of the inhibitor and further strengthens the phosphorus-enzyme bond. The rate of aging varies with the particular

organophosphate compound. If given before aging has occurred, strong nucleophiles like pralidoxime are able to split the phosphorus-enzyme bond and can be used as "cholinesterase regenerator" drugs for organophosphate insecticide poisoning (see Chapter 8: Cholinoceptor-

Blocking Drugs). Once aging has occurred, the enzyme-inhibitor complex is even more stable and

is more difficult to split, even with oxime regenerator compounds.

Because of the marked differences in duration of action, the organophosphate inhibitors are sometimes referred to as "irreversible" cholinesterase inhibitors, and edrophonium and the

carbamates are considered "reversible" inhibitors. However, the molecular mechanisms of action of the three groups do not support this simplistic description.

Organ System Effects

The most prominent pharmacologic effects of cholinesterase inhibitors are on the cardiovascular

and gastrointestinal systems, the eye, and the skeletal muscle neuromuscular junction. Because the primary action is to amplify the actions of endogenous acetylcholine, the effects are similar (but not always identical) to the effects of the direct-acting cholinomimetic agonists.

Central Nervous System

In low concentrations, the lipid-soluble cholinesterase inhibitors cause diffuse activation on the electroencephalogram and a subjective alerting response. In higher concentrations, they cause generalized convulsions, which may be followed by coma and respiratory arrest.

Eye, Respiratory Tract, Gastrointestinal Tract, Urinary Tract

The effects of the cholinesterase inhibitors on these organ systems, all of which are well innervated

by the parasympathetic nervous system, are qualitatively quite similar to the effects of the direct- acting cholinomimetics.

Cardiovascular System

The cholinesterase inhibitors can increase activation in both sympathetic and parasympathetic ganglia supplying the heart and at the acetylcholine receptors on neuroeffector cells (cardiac and vascular smooth muscles) that receive cholinergic innervation.

In the heart, the effects on the parasympathetic limb predominate. Thus, cholinesterase inhibitors

such as edrophonium, physostigmine, or neostigmine mimic the effects of vagal nerve activation on the heart. Negative chronotropic, dromotropic, and inotropic effects are produced, and cardiac

output falls. The fall in cardiac output is attributable to bradycardia, decreased atrial contractility, and some reduction in ventricular contractility. The latter effect occurs as a result of prejunctional

inhibition of norepinephrine release as well as inhibition of postjunctional cellular sympathetic effects.

Cholinesterase inhibitors have less marked effects on vascular smooth muscle and on blood pressure than direct-acting muscarinic agonists. This is because indirect-acting drugs can modify

the tone of only those vessels that are innervated by cholinergic nerves and because the net effects

on vascular tone may reflect activation of both the parasympathetic and sympathetic nervous systems. The cholinomimetic effect at the smooth muscle effector tissue is minimal since few vascular beds receive cholinergic innervation. Activation of sympathetic ganglia may increase vascular resistance.

The *net* cardiovascular effects of moderate doses of cholinesterase inhibitors therefore consist of modest bradycardia, a fall in cardiac output, and no change or a modest fall in blood pressure. Large

(toxic) doses of these drugs cause more marked bradycardia (occasionally tachycardia) and hypotension.

Neuromuscular Junction

The cholinesterase inhibitors have important therapeutic and toxic effects at the skeletal muscle neuromuscular junction. Low (therapeutic) concentrations moderately prolong and intensify the actions of physiologically released acetylcholine. This increases strength of contraction, especially

in muscles weakened by curare-like neuromuscular blocking agents or by myasthenia gravis. At higher concentrations, the accumulation of acetylcholine may result in fibrillation of muscle fibers. Antidromic firing of the motor neuron may also occur, resulting in fasciculations that involve an

entire motor unit. With marked inhibition of acetylcholinesterase, depolarizing neuromuscular blockade occurs and that may be followed by a phase of nondepolarizing blockade as seen with succinylcholine (see Table 27–2 and Figure 27–6).

Some quaternary carbamate cholinesterase inhibitors, eg, neostigmine, have an additional *direct* nicotinic agonist effect at the neuromuscular junction. This may contribute to the effectiveness of these agents as therapy for myasthenia.

Clinical Pharmacology of the Cholinomimetics

The major therapeutic uses of the cholinomimetics are for diseases of the eye (glaucoma, accommodative esotropia), the gastrointestinal and urinary tracts (postoperative atony, neurogenic bladder), the neuromuscular junction (myasthenia gravis, curare-induced neuromuscular paralysis), and rarely, the heart (certain atrial arrhythmias). Cholinesterase inhibitors are occasionally used in

the treatment of atropine overdosage. Several newer cholinesterase inhibitors are being used to treat patients with Alzheimer's disease.

Clinical Uses

the Eye

Glaucoma is a disease characterized by increased intraocular pressure. Muscarinic stimulants and cholinesterase inhibitors reduce intraocular pressure by causing contraction of the ciliary body so as

to facilitate outflow of aqueous humor and perhaps also by diminishing the rate of its secretion (see

Figure 6–9). In the past, glaucoma was treated with either direct agonists (pilocarpine,

methacholine, carbachol) or cholinesterase inhibitors (physostigmine, demecarium, echothiophate, isoflurophate). For chronic glaucoma, these drugs have been largely replaced by topical β-blockers and prostaglandin derivatives.

Acute angle-closure glaucoma is a medical emergency that is frequently treated initially with drugs but usually requires surgery for permanent correction. Initial therapy often consists of a

combination of a direct muscarinic agonist and a cholinesterase inhibitor (eg, pilocarpine plus physostigmine) as well as other drugs. Once the intraocular pressure is controlled and the danger of vision loss is diminished, the patient can be prepared for corrective surgery (iridectomy). Open-

angle glaucoma and some cases of secondary glaucoma are chronic diseases that are not amenable

to traditional surgical correction although newer laser techniques appear to be useful. Other treatments for glaucoma are described in the section Treatment of Glaucoma in Chapter 10: Adrenoceptor Antagonist Drugs.

Accommodative esotropia (strabismus caused by hypermetropic accommodative error) in young children is sometimes diagnosed and treated with cholinomimetic agonists. Dosage is similar to or higher than that used for glaucoma.

Gastrointestinal and Urinary Tracts

In clinical disorders that involve depression of smooth muscle activity *without obstruction,* cholinomimetic drugs with direct or indirect muscarinic effects may be helpful. These disorders include postoperative ileus (atony or paralysis of the stomach or bowel following surgical manipulation) and congenital megacolon. Urinary retention may occur postoperatively or postpartum or may be secondary to spinal cord injury or disease (neurogenic bladder).

Cholinomimetics are also sometimes used to increase the tone of the lower esophageal sphincter in patients with reflux esophagitis. Of the choline esters, bethanechol is the most widely used for these disorders. For gastrointestinal problems, it is usually administered orally in a dose of 10–25 mg

three or four times daily. In patients with urinary retention, bethanechol can be given subcutaneously in a dose of 5 mg and repeated in 30 minutes if necessary. Of the cholinesterase

inhibitors, neostigmine is the most widely used for these applications. For paralytic ileus or atony of

the urinary bladder, neostigmine can be given subcutaneously in a dose of 0.5–1 mg. If patients are able to take the drug by mouth, neostigmine can be given orally in a dose of 15 mg. In all of these situations, the clinician must be certain that there is no mechanical obstruction to outflow prior to using the cholinomimetic. Otherwise, the drug may exacerbate the problem and may even cause perforation as a result of increased

pressure.

Pilocarpine has long been used to increase salivary secretion. Cevimeline is a new direct-acting muscarinic agonist used for the treatment of dry mouth associated with Sjgren's syndrome.

Neuromuscular Junction

Myasthenia gravis is a disease affecting skeletal muscle neuromuscular junctions. An autoimmune process causes production of antibodies that decrease the number of functional nicotinic receptors

on the postjunctional end plates. Frequent findings are ptosis, diplopia, difficulty in speaking and swallowing, and extremity weakness. Severe disease may affect all the muscles, including those necessary for respiration. The disease resembles the neuromuscular paralysis produced by *d*- tubocurarine and similar nondepolarizing neuromuscular blocking drugs (see Chapter 27: Skeletal Muscle Relaxants). Patients with myasthenia are exquisitely sensitive to the action of curariform drugs and other drugs that interfere with neuromuscular transmission, eg, aminoglycoside

antibiotics.

Cholinesterase inhibitors—but not direct-acting acetylcholine receptor agonists—are extremely valuable as therapy for myasthenia. Almost all patients are also treated with immunosuppressant drugs and some with thymectomy.

Edrophonium is sometimes used as a diagnostic test for myasthenia. A 2 mg dose is injected intravenously after baseline measurements of muscle strength have been obtained. If no reaction occurs after 45 seconds, an additional 8 mg may be injected. Some clinicians divide the 8 mg dose into two doses of 3 and 5 mg given at 45-second intervals. If the patient has myasthenia gravis, an improvement in muscle strength that lasts about 5 minutes will usually be observed.

Edrophonium is also used to assess the adequacy of treatment with the longer-acting cholinesterase inhibitors in patients with myasthenia gravis. If excessive amounts of cholinesterase inhibitor have been used, patients may become paradoxically weak because of nicotinic depolarizing blockade of

the motor end plate. These patients may also exhibit symptoms of excessive stimulation of muscarinic receptors (abdominal cramps, diarrhea, increased salivation, excessive bronchial

secretions, miosis, bradycardia). Small doses of edrophonium (1–2 mg intravenously) will produce

no relief or even worsen weakness if the patient is receiving excessive cholinesterase inhibitor

therapy. On the other hand, if the patient improves with edrophonium, an increase in cholinesterase inhibitor dosage may be indicated. Clinical situations in which severe myasthenia (myasthenic

crisis) must be distinguished from excessive drug therapy (cholinergic crisis) usually occur in very

ill myasthenic patients and must be managed in hospital with adequate emergency support systems

(eg, mechanical ventilators) available.

Long-term therapy for myasthenia gravis is usually accomplished with neostigmine, pyridostigmine, or ambenonium. The doses are titrated to optimum levels based on changes in muscle strength. These agents are relatively short-acting and therefore require frequent dosing

(every 4 hours for neostigmine and every 6 hours for pyridostigmine and ambenonium; Table 7–4). Sustained-release preparations are available but should be used only at night and if needed. Longer- acting cholinesterase inhibitors such as the organophosphate agents are not used, because the dose requirement in this disease changes too rapidly to permit smooth control with long-acting drugs.

If muscarinic effects of such therapy are prominent, they can be controlled by the administration of antimuscarinic drugs such as atropine. Frequently, tolerance to the muscarinic effects of the cholinesterase inhibitors develops, so atropine treatment is not required.

Neuromuscular blockade is frequently produced as an adjunct to surgical anesthesia, using nondepolarizing neuromuscular relaxants such as pancuronium and newer agents (see Chapter 27: Skeletal Muscle Relaxants). Following surgery, it is usually desirable to reverse this pharmacologic paralysis promptly. This can be easily accomplished with cholinesterase inhibitors; neostigmine and edrophonium are the drugs of choice. They are given intravenously or intramuscularly for prompt effect.

Heart

The short-acting cholinesterase inhibitor edrophonium had been used to treat supraventricular tachyarrhythmias, particularly paroxysmal supraventricular tachycardia. In this application, edrophonium has been replaced by newer drugs (adenosine and the calcium channel blockers verapamil and diltiazem).

Antimuscarinic Drug Intoxication

Atropine intoxication is potentially lethal in children (see Chapter 8: Cholinoceptor-Blocking Drugs) and may cause prolonged severe behavioral disturbances and arrhythmias in adults. The tricyclic antidepressants, when taken in overdosage (often with suicidal intent), also cause severe muscarinic blockade (see Chapter 30: Antidepressant Agents). The muscarinic receptor blockade

produced by all these agents is competitive in nature and can be overcome by increasing the amount

of endogenous acetylcholine present at the neuroeffector junctions. Theoretically, a cholinesterase inhibitor could be used to reverse these effects. Physostigmine has been used for this application, because it enters the central nervous system and reverses the central as well as the peripheral signs

of muscarinic blockade. However, as noted previously, physostigmine itself can produce dangerous central nervous system effects, and such therapy is therefore used only in patients with dangerous elevation of body temperature or very rapid supraventricular tachycardia.

Central Nervous System

Tacrine is a drug with anticholinesterase and other cholinomimetic actions that has been used for

the treatment of mild to moderate Alzheimer's disease. Evidence for tacrine's efficacy is modest and hepatic toxicity is significant. Donepezil, galantamine, and rivastigmine are newer, more selective acetylcholinesterase inhibitors that appear to have the same modest clinical benefit as tacrine in treatment of cognitive dysfunction in Alzheimer's patients. Donepezil may be given once daily

because of its long half-life, and it lacks the hepatotoxic effect of tacrine. However, no comparative trials of these newer drugs and tacrine have been reported. These drugs are discussed in Chapter 61: Special Aspects of Geriatric Pharmacology.

Toxicity

The toxic potential of the cholinoceptor stimulants varies markedly depending on their absorption, access to the central nervous system, and metabolism.

Direct-Acting Muscarinic Stimulants

Drugs such as pilocarpine and the choline esters cause predictable signs of muscarinic excess when given in overdosage. These effects include nausea, vomiting, diarrhea, salivation, sweating,

cutaneous vasodilation, and bronchial constriction. The effects are all blocked competitively by atropine and its congeners.

Certain mushrooms, especially those of the genus *Inocybe,* contain muscarinic alkaloids. Ingestion of these mushrooms causes typical signs of muscarinic excess within 15–30 minutes. Treatment is with atropine, 1–2 mg parenterally. (*Amanita muscaria,* the first source of muscarine, contains very low concentrations of the alkaloid.)

Direct-Acting Nicotinic Stimulants

Nicotine itself is the only common cause of this type of poisoning. The acute toxicity of the alkaloid is well-defined but much less important than the chronic effects associated with smoking. In addition to tobacco products, nicotine is also used in insecticides.

Acute Toxicity

The fatal dose of nicotine is approximately 40 mg, or 1 drop of the pure liquid. This is the amount of nicotine in two regular cigarettes. Fortunately, most of the nicotine in cigarettes is destroyed by

burning or escapes via the "sidestream" smoke. Ingestion of nicotine insecticides or of tobacco by infants and children is usually followed by vomiting, limiting the amount of the alkaloid absorbed.

The toxic effects of a large dose of nicotine are simple extensions of the effects described

previously. The most dangerous are (1) central stimulant actions, which cause convulsions and may progress to coma and respiratory arrest; (2) skeletal muscle end plate depolarization, which may

lead to depolarization blockade and respiratory paralysis; and (3) hypertension and cardiac arrhythmias.

Treatment of acute nicotine poisoning is largely symptom-directed. Muscarinic excess resulting

from parasympathetic ganglion stimulation can be controlled with atropine. Central stimulation is usually treated with parenteral anticonvulsants such as diazepam. Neuromuscular blockade is not responsive to pharmacologic treatment and may require mechanical respiration.

Fortunately, nicotine is metabolized and excreted relatively rapidly. Patients who survive the first 4

hours usually recover completely if hypoxia and brain damage have not occurred. Chronic

Nicotine Toxicity

The health costs of tobacco smoking to the smoker and its socioeconomic costs to the general public are still incompletely understood. However, the 1979 *Surgeon General's Report on Health*

Promotion and Disease Prevention stated that "cigarette smoking is clearly the largest single preventable cause of illness and premature death in the United States." This statement has been supported by numerous studies. Unfortunately, the fact that the most important of the tobacco-

associated diseases are delayed in onset reduces the health incentive to stop smoking.

It is clear that the addictive power of cigarettes is directly related to their nicotine content. It is not known to what extent nicotine per se contributes to the other well-documented adverse effects of chronic tobacco use. It appears highly probable that nicotine contributes to the increased risk of vascular disease and sudden coronary death associated with smoking. It is also probable that

nicotine contributes to the high incidence of ulcer recurrences in smokers with peptic ulcer.

Cholinesterase Inhibitors

The acute toxic effects of the cholinesterase inhibitors, like those of the direct-acting agents, are

direct extensions of their pharmacologic actions. The major source of such intoxications is pesticide use in

agriculture and in the home. Approximately 100 organophosphate and 20 carbamate cholinesterase inhibitors are available in pesticides and veterinary vermifuges used in the USA.

Acute intoxication must be recognized and treated promptly in patients with heavy exposure. The dominant initial signs are those of muscarinic excess: miosis, salivation, sweating, bronchial constriction, vomiting, and diarrhea. Central nervous system involvement usually follows rapidly, accompanied by peripheral nicotinic effects, especially depolarizing neuromuscular blockade.

Therapy always includes (1) maintenance of vital signs—respiration in particular may be impaired;

(2) decontamination to prevent further absorption—this may require removal of all clothing and washing of the skin in cases of exposure to dusts and sprays; and (3) atropine parenterally in large doses, given as often as required to control signs of muscarinic excess. Therapy often also includes treatment with pralidoxime as described in Chapter 8: Cholinoceptor-Blocking Drugs.

Chronic exposure to certain organophosphate compounds, including some organophosphate cholinesterase inhibitors, causes neuropathy associated with demyelination of axons.

Triorthocresylphosphate, an additive in lubricating oils, is the prototype agent of this class. The effects are not caused by cholinesterase inhibition.

Preparations Available

Direct-Acting Cholinomimetics

Acetylcholine (Miochol-E)

Ophthalmic: 1:100 (10 mg/mL) intraocular solution

Bethanechol (generic, Urecholine) Oral:

5, 10, 25, 50 mg tablets Parenteral: 5

mg/mL for SC injection **Carbachol**

Ophthalmic (topical, Isopto Carbachol, Carboptic): 0.75, 1.5, 2.25, 3% drops

Ophthalmic (intraocular, Miostat, Carbastat): 0.01% solution

Cevimeline (Evoxac)

Oral: 30 mg capsules

Pilocarpine (generic, Isopto Carpine)

Ophthalmic (topical): 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10% solutions, 4% gel

Ophthalmic sustained-release inserts (Ocusert Pilo-20, Ocusert Pilo-40): release 20 and 40 g pilocarpine per hour for 1 week, respectively

Oral (Salagen): 5 mg tablets

Cholinesterase Inhibitors

Ambenonium (Mytelase) Oral:

10 mg tablets **Demecarium**

(Humorsol)

Ophthalmic: 0.125, 0.25% drops

Donepezil (Aricept)

Oral: 5, 10 mg tablets

Echothiophate (Phospholine)

Ophthalmic: Powder to reconstitute for 0.03, 0.06, 0.125, 0.25% drops

Edrophonium (generic, Tensilon)

Parenteral: 10 mg/mL for IM or IV injection

Galantamine (Reminyl)

Oral: 4, 8, 12 mg capsules; 4 mg/mL solution

Neostigmine

(generic, Prostigmin)

Oral: 15 mg tablets

Parenteral: 1:1000 in 10 mL; 1:2000, 1:4000 in 1 mL

Physostigmine, eserine (generic)

Parenteral: 1 mg/mL for IM or slow IV injection

Pyridostigmine (Mestinon, Regonol)

Oral: 60 mg tablets; 180 mg sustained-release tablets; 15 mg/mL syrup

Parenteral: 5 mg/mL for IM or slow IV injection

Rivastigmine (Exelon)

Oral: 1.5, 3, 4.5, 6 mg tablets; 2 mg/mL solution

Tacrine (Cognex)

Oral: 10, 20, 30, 40 mg tablets

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