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Directly and Indirectly Acting Cholinomimetics

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DRUG LIST

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Cholinomimetic drugs can elicit some or all of the effects that acetylcholine (ACh) produces. This class of drugs includes agents that act directly as agonists at cholinergic receptors and agents that act indirectly by inhibiting the enzymatic destruction of endogenous ACh (i.e., cholinesterase inhibitors). The directly acting cholinomimetics can be subdivided into agents that exert their effects primarily through stimulation of muscarinic receptors at parasympathetic neuroeffector junctions (parasympathomimetic drugs) and agents that stimulate nicotinic receptors in autonomic ganglia and at the neuromuscular junction (see Chapter 9). This chapter focuses on the parasympathomimetic drugs and cholinesterase inhibitors. Drugs acting at nicotinic receptors are presented in Chapters 14 and 28.

Muscarinic Receptors and Signal Transduction

Classical studies by Sir Henry Dale demonstrated that the receptors activated by *muscarine*, an alkaloid isolated from the mushroom *Amanita muscaria*, are the same receptors activated by ACh released from parasympathetic nerve endings, from which the general notion that muscarinic agonists have parasympathomimetic properties was born. This conclusion is true but incomplete, and we now know that muscarinic receptors have a broader distribution and many functional roles. To understand the actions of cholinomimetic drugs it is essential to recognize that muscarinic receptors: (1) mediate the activation of effectors by ACh released from parasympathetic nerve

endings; (2) mediate the activation of sweat glands by ACh released from sympathetic fibers; (3) are found on vascular endothelial cells that receive no cholinergic innervation; (4) are widely distributed in the central nervous system (CNS), from basal ganglia to neocortex; and (5) are present on presynaptic nerve terminals, including terminals that release ACh and terminals associated with other neurotransmitter systems, such as the catecholamines. Therefore, the activation of muscarinic receptors may influence most of the organ systems along with CNS pathways involved in regulating voluntary motor activity, memory, and cognition. Activation of presynaptic muscarinic receptors can inhibit the release of endogenous neurotransmitters, and may account for some paradoxical effects of cholinomimetic stimulation.

Binding studies with high-affinity receptor antagonists revealed four subtypes of muscarinic receptors that can be distinguished on the basis of (1) the rank order of potency of specific antagonists in functional experiments and (2) the affinity of these antagonists for muscarinic receptors in the same tissues. More recently, molecular studies have revealed five genetically distinct receptor subtypes, named M1 through M5, the first four of which correspond to functionally defined receptors. The different subtypes of muscarinic receptors are heterogeneously distributed: (1) M1 receptors are present in brain, exocrine glands, and autonomic ganglia. (2) M2 receptors are found in the heart, brain, autonomic ganglia, and smooth muscle. (3) M3 receptors are present in smooth muscle, exocrine glands, brain, and endothelial cells. (4) M4 receptors are present in brain and autonomic ganglia. (5) M5 receptors are found in the CNS.

All muscarinic receptors are members of the seven transmembrane domain, G protein-coupled receptors, and they are structurally and functionally unrelated to nicotinic ACh receptors. Activation of muscarinic receptors by an agonist triggers the release of an intracellular G-protein complex that can specifically activate one or more signal transduction pathways. Fortunately, the cellular responses elicited by odd- versus even-numbered receptor subtypes can be conveniently distinguished. Activation of M1, M3, and M5 receptors produces an inosine triphosphate (IP3) mediated release of intracellular calcium, the release of diacylglycerol (which can activate protein kinase C), and stimulation of adenylyl cyclase. These receptors are primarily responsible for activating calcium-dependent responses, such as secretion by glands and the contraction of smooth muscle.

Activation of M2 and M4 receptors inhibits adenylyl cyclase, and activation of M2 receptors opens potassium channels. The opening of potassium channels hyperpolarizes the membrane potential and decreases the excitability of cells in the sinoatrial (SA) and atrioventricular (A-V) nodes in the heart. The inhibition of adenylyl cyclase decreases cellular cyclic adenosine

monophosphate (cAMP) levels, which can override the opposing stimulation of adenylyl cyclase by β -adrenoceptor agonists.

Although muscarinic receptors as a class can be selectively activated and they demonstrate strong stereoselectivity among both agonists and antagonists (see Chapter 13), the therapeutic use of cholinomimetics is limited by the paucity of drugs selective for specific *subtypes* of muscarinic receptors. This lack of specificity combined with the broad-ranging effects of muscarinic stimulation on different organ systems makes the therapeutic use of cholinomimetic drugs a challenge, and the careful consideration of the pharmacokinetic properties of the drugs plays an especially important role in making therapeutic decisions.

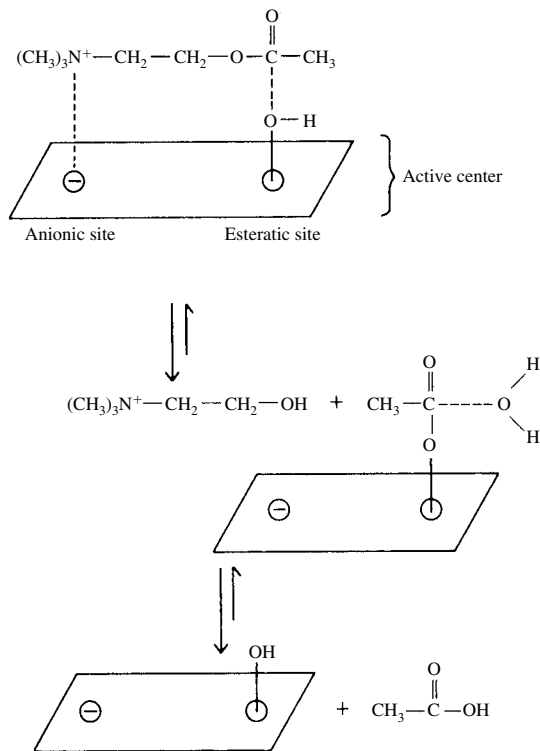
DIRECT-ACTING PARASYMPATHOMIMETIC DRUGS

Acetylcholine

Acetylcholine is an ester of choline and acetic acid, the prototype for a small family of choline ester compounds. The choline moiety of ACh contains a quaternary ammonium group that gives ACh a permanent positive charge, making it very hydrophilic and membrane impermeant.

ACh is degraded by a group of enzymes called cholinesterases. These enzymes catalyze the hydrolysis of ACh to choline and acetic acid (Fig. 12.1). The active center of cholinesterase has two areas that interact with ACh: the anionic site and the esteratic site. The anionic site contains a negatively charged amino acid that binds the positively charged quaternary ammonium group of ACh through coulombic forces. This probably serves to bring the ester linkage of ACh close to the esteratic site of the enzyme. The esteratic site contains a serine residue, which is made more reactive by hydrogen bonding to a nearby histidine residue. The nucleophilic oxygen of the serine reacts with the carbonyl carbon of ACh, thereby breaking the ester linkage. During this reaction, choline is liberated and an acetylated enzyme is formed. The latter intermediate is rapidly hydrolyzed to release acetic acid and regenerate the active enzyme. The entire process takes about 150 microseconds, one of the fastest enzymatic reactions known.

There are two major types of cholinesterases: acetylcholinesterase (AChE) and pseudocholinesterase (pseudo-ChE). AChE (also known as true, specific, or erythrocyte cholinesterase) is found at a number of sites in the body, the most important being the cholinergic neuroeffector junction. Here it is localized to the prejunctional and postjunctional membranes, where it rapidly terminates the action of synaptically released ACh. It is essential to recognize that *the action of ACh is ter-*

**FIGURE 12.1**

Simplified scheme of ACh hydrolysis at the active center of ACh. Rectangular area represents the active center of the enzyme with its anionic and esteratic sites. *Top*, the initial bonding of ACh at the active center. The broken line at left represents electrostatic forces. The broken line at right represents the initial interaction between the serine oxygen of the enzyme and the carbonyl carbon of ACh. The ester linkage is broken, choline is liberated, and an acetylated enzyme intermediate is formed (*middle*). Finally, the acetylated intermediate undergoes hydrolysis to free the enzyme and generate acetic acid (*bottom*).

minated only by its hydrolysis. There is no reuptake system in cholinergic nerve terminals to reduce the concentration of ACh in a synaptic cleft, unlike the reuptake systems for other neurotransmitters such as dopamine, serotonin, and norepinephrine. Therefore, inhibition of AChE can greatly prolong the activation of cholinergic receptors by ACh released at a synapse.

Pseudo-ChE (also known as butyryl-, plasma, and nonspecific cholinesterase) has a widespread distribution, with enzyme especially abundant in the liver, where it is synthesized, and in the plasma. In spite of the abundance of pseudo-ChE, its physiological function has not been definitively identified. It does, however, play an important role in the metabolism of such clinically important compounds as succinylcholine, procaine, and numerous other esters.

Derivatives of ACh: Methacholine, Carbachol, and Bethanechol

The therapeutic usefulness of ACh is limited by (1) its lack of selectivity as an agonist for different types of cholinergic receptors and (2) its rapid degradation by cholinesterases. These limitations have been circumvented in part by the development of three *choline ester* derivatives of ACh: methacholine (*Provocholine*), carbachol (*Isopto Carbachol*, *Miostat*) and bethanechol (*Urecholine*). Methacholine differs from ACh only in the addition of a methyl group at the β -carbon of ACh. This modification greatly increases its selectivity for muscarinic receptors relative to nicotinic receptors, and it renders methacholine resistant to the pseudo-ChE in the plasma and decreases its susceptibility to AChE, thereby increasing its potency and duration of action compared to those of ACh. Carbachol differs from ACh only in the substitution of a carbamoyl group for the terminal methyl group of ACh. This substitution makes carbachol completely resistant to degradation by cholinesterases but does not improve its selectivity for muscarinic versus nicotinic receptors. Bethanechol combines the addition of the methyl group and the substitution of the terminal carbamoyl group, producing a drug that is a selective agonist of muscarinic receptors and is resistant to degradation by cholinesterases.

All of these drugs are very hydrophilic and membrane impermeant because they retain the quaternary ammonium group of the choline moiety of ACh.

Pilocarpine is a naturally occurring cholinomimetic alkaloid that is structurally distinct from the choline esters. It is a tertiary amine that crosses membranes relatively easily. Therefore, it is rapidly absorbed by the cornea of the eye, and it can cross the blood-brain barrier. Pilocarpine is a pure muscarinic receptor agonist, and it is unaffected by cholinesterases. *Muscarine* is an alkaloid with no therapeutic use, but it can produce dangerous cholinomimetic stimulation following ingestion of some types of mushrooms (e.g., *Inocybes*).

Basic Pharmacology of the Directly Acting Parasympathomimetic Drugs

Methacholine, bethanechol, and pilocarpine are selective agonists of muscarinic receptors, whereas carbachol and ACh can activate both muscarinic and nicotinic receptors. However, at usual therapeutic doses, the effects of carbachol and ACh are entirely due to the activation of muscarinic receptors. This apparent preference for muscarinic receptors can be attributed to the greater accessibility and abundance of these cholinergic receptors compared with the nicotinic receptors.

Cardiovascular Effects

Low doses of muscarinic agonists given intravenously relax arterial smooth muscle and produce a fall in blood

pressure. These responses result from the stimulation of muscarinic receptors on vascular endothelial cells (Fig. 12.2). Activation of these receptors causes the endothelial cells to synthesize and release nitric oxide. Nitric oxide can diffuse into neighboring vascular smooth muscle cells, where it activates soluble guanylyl cyclase, thereby increasing the synthesis of cyclic guanosine monophosphate (cGMP) and relaxing the muscle fibers. Most of the resistance vasculature is not innervated by cholinergic neurons, and the physiological function of the endothelial muscarinic receptors is not known. However, activation of these receptors by directly acting cholinomimetic drugs has major pharmacological significance, as the potentially dangerous hypotension produced by their activation is an important limitation to the systemic administration of muscarinic agonists.

Although the release of ACh onto the heart by the vagus nerve slows the heart rate, a low dose of a muscarinic agonist can sometimes increase the heart rate. This paradoxical effect is produced when the decrease in blood pressure produced by stimulation of endothelial muscarinic receptors, as described earlier, triggers the activation of a compensatory sympathetic reflex stimulation of the heart. Sympathetic stimulation increases heart rate and vasomotor tone, partially counteracting the direct vasodilator response. Therefore, the tachycardia produced by muscarinic agonists is indirect. At higher concentrations of a muscarinic agonist, the direct effects on cardiac muscarinic (M₂) receptors in the

SA node and A-V fibers become dominant. Activation of M₂ receptors increases the potassium permeability and reduces cAMP levels, slowing the rate of depolarization and decreasing the excitability of SA node and A-V fiber cells. This results in marked bradycardia and a slowing of A-V conduction that can override the stimulation of the heart by catecholamines released during sympathetic stimulation. In fact, very high doses of a muscarinic agonist can produce lethal bradycardia and A-V block. Choline esters have relatively minor direct effects on ventricular function, but they can produce negative inotropy of the atria.

The Eye

When solutions of directly acting cholinomimetics are applied to the eye (i.e., conjunctival sac), they cause contraction of the smooth muscle in two important structures, the iris sphincter and the ciliary muscles (Fig. 12.3). Contraction of the iris sphincter decreases the diameter of the pupil (miosis). Contraction of the circular fibers of the ciliary muscle, which encircles the lens, reduces the tension on the suspensory ligaments that normally stretch and flatten the lens, allowing the highly elastic lens to spontaneously round up and focus for near vision (accommodation to near vision).

Other Organ Systems

Prominent effects within the digestive tract include stimulation of salivation and acid secretion, increased intestinal tone and peristaltic activity, and relaxation of most sphincters. Bronchoconstriction and stimulation of secretions are prominent effects in the respiratory system. Muscarinic agonists can also evoke secretion from nasopharyngeal glands. Urination is promoted by stimulation of the detrusor muscle of the bladder and is facilitated by relaxation of the trigone and external sphincter muscles.

Clinical Uses Glaucoma

Cholinomimetic drugs are useful for treating glaucoma because they can decrease the resistance to the movement of fluid (aqueous humor) out of the eye (Fig. 12.3), thereby reducing the intraocular pressure. It is useful to distinguish between open-angle glaucoma, a chronic condition in which the porosity of the trabecular meshwork is insufficient to permit the movement of fluid into the canal of Schlemm, and angle-closure glaucoma, an emergency condition in which an abnormal position of the peripheral iris blocks the access of fluid to the trabecular meshwork. Open-angle glaucoma can be effectively treated with cholinomimetics such as pilocarpine and carbachol, because contraction of the ciliary muscle stretches the trabecular network, increasing its porosity

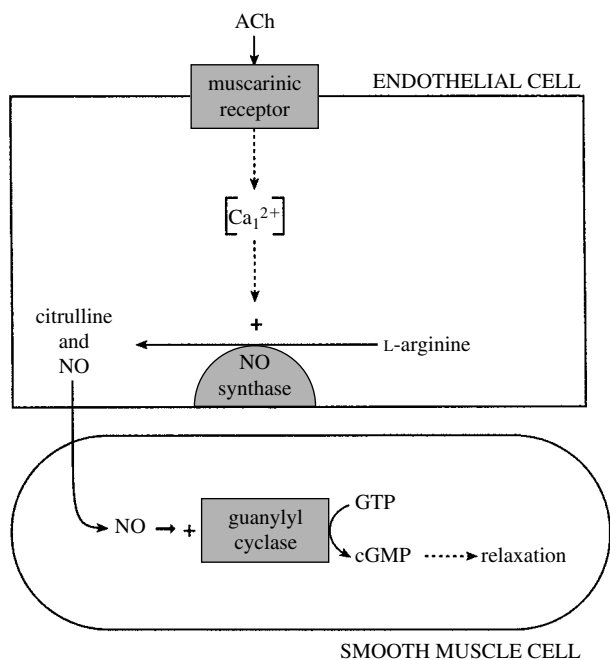


FIGURE 12.2

Mechanism for vasodilation produced by ACh. Arrows with broken tail indicate multiple steps or that other factors are involved. NO, nitric oxide.

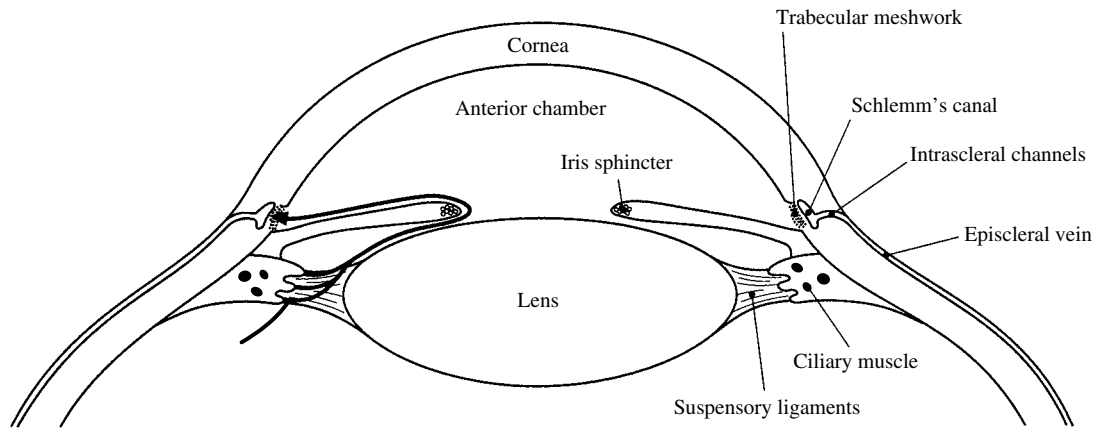
**FIGURE 12.3**

Diagram of eye depicting major pathway for outflow of aqueous humor (*arrow*) and ocular smooth muscles, which contract in response to parasympathomimetics or cholinesterase inhibitors (i.e., iris sphincter and ciliary muscle).

and permeability to the outflow of fluid. This beneficial effect, however, comes at the price of a spasm of accommodation and miosis, which seriously disturb vision. Cholinomimetics, therefore, have been replaced by β -blockers and carbonic anhydrase inhibitors, both of which decrease the formation of aqueous humor without affecting vision. However, some patients simply do not respond to these treatments or do not tolerate the cardiovascular side effects of the β -blockers, and cholinomimetics (most notably pilocarpine) remain as important treatment alternatives.

Contraction of the iris sphincter (miosis) by cholinomimetic stimulation is less important than contraction of the ciliary muscle for treating angle-closure glaucoma, but it may be essential as emergency therapy for acute-angle glaucoma to reduce intraocular pressure prior to surgery (iridectomy). Contraction of the iris sphincter by pilocarpine pulls the peripheral iris away from the trabecular meshwork, thereby opening the path for aqueous outflow.

Pilocarpine is the first choice among cholinomimetics for the treatment of glaucoma. Pilocarpine can be applied to the eye as a gel (Pilopine HS Gel) or time-release system (Ocusert) for the chronic treatment of open-angle glaucoma, or as drops (Pilocar) for an acute reduction of intraocular pressure, as in the emergency management of angle-closure glaucoma. Carbachol is sometimes effective in treating cases of open-angle glaucoma that are resistant to pilocarpine.

Surgery of the Eye

Because ACh is rapidly inactivated by cholinesterases, its use is best suited for clinical applications requiring only a brief duration of action, such as when it is employed to cause miosis during cataract surgery. ACh

(Miochol) can produce a brief (10 minutes) miosis, and carbachol is used during eye surgery necessitating miosis of a longer duration.

Urinary Retention

Bethanechol is used to treat postsurgical bladder dysfunction associated with the retention of urine. It is most commonly given orally for this purpose, although the subcutaneous route is also used. Effects are more rapid and intense after subcutaneous administration, but the duration of action is shorter.

Diagnosis of Bronchial Hyperreactivity

Methacholine is used to identify bronchial hyperreactivity in patients without clinically apparent asthma. For this indication, the drug is administered by inhalation, and patients who may be developing asthma usually produce an exaggerated airway contraction. Upon completion of the test, a rapid-acting bronchodilator (e.g., inhaled β -adrenoceptor agonist) can be given to counter the bronchoconstrictor effect of methacholine and relieve the patient's discomfort.

Adverse Effects

Potentially severe adverse effects can result from systemic administration of cholinomimetic drugs, and none should be administered by intramuscular or intravenous injection. If significant amounts of these drugs enter the circulation, nausea, abdominal cramps, diarrhea, salivation, hypotension with reflex tachycardia, cutaneous vasodilation, sweating, and bronchoconstriction can result. Pilocarpine can cross the blood-brain barrier and affect cognitive function. Even the topical application of cholinomimetics to the eyes can present

some risk, and the escape of cholinomimetics into the circulatory system following topical application to the eye can be minimized by pressure applied to the lacrimal duct. Within the eye, cholinomimetics elicit miosis and spasm of accommodation, both of which disturb vision.

Bethanechol is relatively selective in activating cholinergic receptors in the gastrointestinal and urinary tracts when taken orally, but it is less selective when given subcutaneously, and it is very dangerous when given intramuscularly or intravenously, having the potential to produce circulatory collapse and cardiac arrest. Systemic poisoning with cholinomimetics can be treated with the muscarinic receptor antagonist atropine.

Bethanechol should not be used in patients with possible mechanical obstruction of the bladder or gastrointestinal tract or when contraction of smooth muscles in these tissues may be harmful (e.g., recent intestinal resection). It is also contraindicated in patients with bronchial asthma, peptic ulcer disease, coronary artery disease, gastrointestinal hypermotility or inflammatory disease, hypotension or marked bradycardia, hyperthyroidism, parkinsonism, or epilepsy. Care should be exercised in administering pilocarpine to elderly patients because it can enter the CNS and affect memory and cognition, even when applied topically to the eye.

CHOLINESTERASE INHIBITORS

Inhibition of AChE slows or prevents the degradation of ACh released at synapses, and this can greatly prolong the activation of cholinergic receptors produced by synaptically released ACh. In a functional sense, the indirect cholinergic effect of AChE inhibitors is more selective than the effect of directly acting cholinomimetics, because the inhibitors of AChE increase the activation of cholinergic receptors only at active cholinergic synapses. This permits strengthening of the phasic stimulation of synaptically activated cholinergic receptors rather than the persistent activation by directly acting cholinomimetics. At therapeutic concentrations, inhibitors of AChE do not activate cholinergic receptors at sites that do not receive cholinergic synaptic input, such as endothelial muscarinic receptors, and therefore do not present the same risk of eliciting large vasodilator responses.

Acetylcholinesterase can be inhibited by two general mechanisms. In the first mechanism, positively charged quaternary ammonium compounds bind to the anionic site and prevent ACh from binding—a simple competitive inhibition. In the second mechanism, the agents act either as a false substrate for the cholinesterase or directly attack the esteratic site; in both cases they covalently modify the esteratic site and non-competitively prevent further hydrolytic activity. Either mechanism can be effective in preventing the hydroly-

sis of ACh, but they differ markedly in their pharmacokinetic properties.

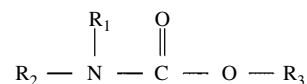
Inhibition of AChE can increase the stimulation of both muscarinic and nicotinic receptors produced by synaptically released ACh. Nicotinic receptors can also be stimulated directly by AChE inhibitors with a quaternary ammonium group, and this can potentiate their cholinergic effect. Finally, although inhibition of true AChE is most important for potentiating the synaptic activity of ACh, several AChE inhibitors also inhibit the pseudo-ChE in plasma. This can permit plasma concentrations of ACh to rise markedly and activate endothelial muscarinic receptors.

Quaternary Ammonium Agents

Edrophonium (*Enlon*, *Tensilon*) and ambenonium (*Mytelase*) are monoquaternary and bisquaternary ammonium alcohols, respectively. Their positive charge allows them to bind to the anionic site at the reactive center, competitively displacing ACh from the active site without covalent modification of the site. Edrophonium has a very short duration of action, lasting only 5 to 10 minutes, whereas inhibition by ambenonium can last 4 to 8 hours. These drugs have direct agonist activity at nicotinic receptors.

Carbamates

Carbamate anticholinesterase agents are carbamic acid esters that are hydrolyzed by AChE in a manner similar to that of ACh. Carbamates have this general structure:



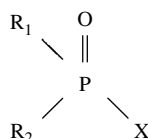
The clinically useful carbamates generally contain a tertiary or quaternary amine group that can bind non-covalently to the anionic site of the enzyme. The inhibition of AChE by *neostigmine* (Prostigmin) illustrates the general mechanism. The quaternary ammonium group of neostigmine binds electrostatically to the anionic site of the enzyme, thereby orienting the drug. The serine oxygen at the esteratic site of the enzyme then reacts with the carbonyl carbon of neostigmine, just as it did with ACh (Fig. 12.1). However, a carbamylated intermediate is formed instead of an acetylated one, and this carbamylated enzyme undergoes hydrolysis much more slowly. Whereas the acetylated enzyme is hydrolyzed nearly instantly, the half-life for hydrolysis of this particular carbamylated intermediate is about an hour. The carbamates generally inhibit pseudo-ChE as well as true AChE, and their suicidal degradation by cholinesterases contributes importantly to terminating their duration of effect. Physostigmine (also called eserine) (*Antilirium*) is a tertiary amine that can inhibit

AChE in the CNS, and it can be used in life-threatening cases to treat antimuscarinic poisoning.

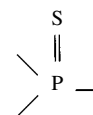
Pyridostigmine (*Mestinon*) is a quaternary ammonium carbamate. Neostigmine and pyridostigmine also have direct agonist activity at nicotinic receptors on skeletal muscle. Rivastigmine (*Exelon*) is a carbamate cholinesterase inhibitor with good penetration into the brain.

Organophosphates

The organophosphate compounds also react at the esteratic site of AChE (Fig. 12.4). In general, however, they are much less selective than are the carbamates, inhibiting many enzymes that contain a serine molecule at an active center. The organophosphate compounds have this general structure:



Examples of X groups are fluorine in isofluorophate (*Floropryl*, no longer available in the United States or Canada) and echothiophate (*Phospholine*). Parathion and malathion (insecticides) are thiophosphates



that must be converted to oxyanalogues to become active. The organophosphates, except for echothiophate, are very lipid soluble.

In the interaction of isofluorophate with AChE, a phosphorylated intermediate is formed and fluoride is released. An important characteristic of the organophosphate-induced inhibition is that the bond between the phosphate and the enzyme is very stable. While the regeneration of most carbamylated enzymes occurs with a half-life of minutes or hours, the recovery of a phosphorylated enzyme is generally measured in days. These agents are referred to, therefore, as irreversible inhibitors.

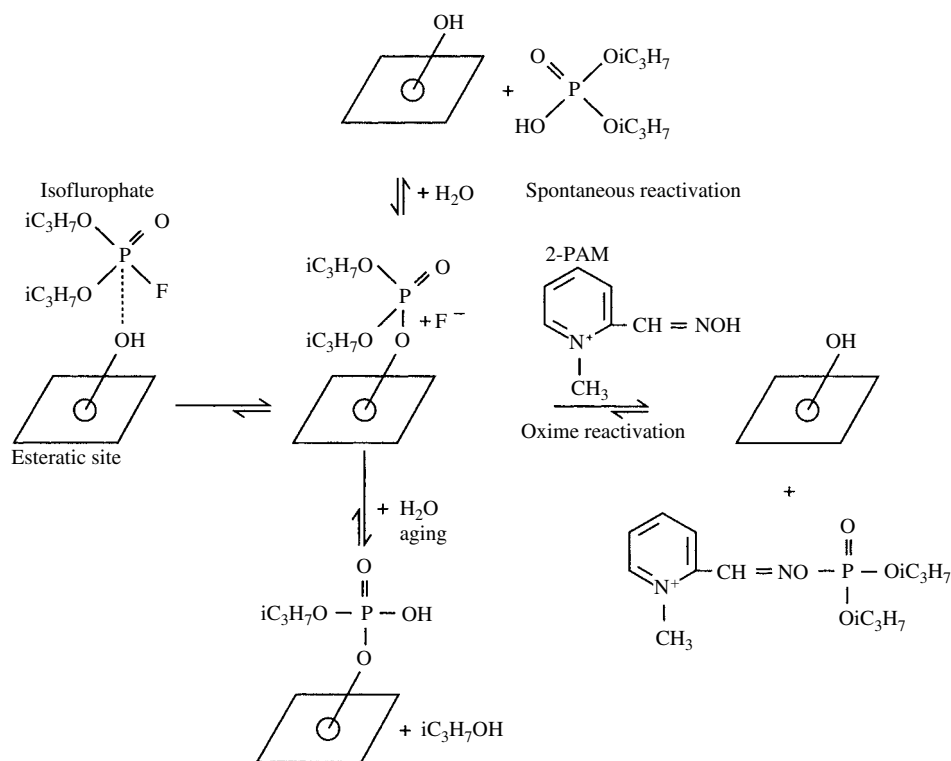


FIGURE 12.4

Isofluorophate reaction at AChE esteratic site, aging, spontaneous reactivation, and oxime reactivation. *Left*, the nucleophilic attack on the phosphorus of isofluorophate by the serine oxygen. This results in a stable phosphorylated enzyme intermediate, which undergoes dephosphorylation at a negligible rate (*top*). A more favorable reaction is the loss of an isopropoxy group, a process termed aging (*bottom*). This renders the phosphorylated enzyme resistant to dephosphorylation by an oxime. The original phosphorylated intermediate (*center*) will react with the nucleophilic oxygen of pralidoxime (2-PAM), resulting in dephosphorylation of the enzyme and formation of an oxime phosphonate (*lower right*).

Although the spontaneous hydrolysis of a phosphorylated enzyme is generally very slow, compounds called oximes can cause dephosphorylation (Fig. 12.4). Pralidoxime chloride (2-PAM) (Protopam chloride) is an oxime used therapeutically to reactivate phosphorylated AChE. It has the additional feature that its quaternary ammonium group binds to the anionic site of the enzyme and thereby promotes dephosphorylation. If the oxime is not administered soon enough (minutes to hours) after AChE has been inhibited, an alkoxy group may be lost from the phosphorylated enzyme. This reaction is called aging. Once aging has occurred, oximes can no longer regenerate free enzymes. The rate of aging appears to depend both on the nature of the enzyme (AChE or pseudo-ChE) and on the particular inhibitor employed. Since pralidoxime is a quaternary amine, it does not cross the blood-brain barrier, and it is not useful for reactivating cholinesterases in the CNS.

Inhibitors targeted at AChE in the CNS

Several inhibitors of AChE have been developed for use in treating Alzheimer's disease, which requires that the drugs readily enter the CNS. These inhibitors are structurally unrelated and vary in their mechanism of inhibition, although all are reversible inhibitors. Tacrine (*Cognex*) is a monoamine acridine. Donepezil (*Aricept*) is a piperidine derivative that is a relatively specific inhibitor of AChE in the brain, with little effect on pseudo-ChE in the periphery. Galanthamine (*Reminyl*) is a tertiary alkaloid and phenanthrene derivative extracted from daffodil bulbs that is a reversible competitive inhibitor of AChE; it also acts on nicotinic receptors.

Absorption, Metabolism, and Excretion

Physostigmine and rivastigmine are tertiary amines that are rapidly absorbed from the gastrointestinal tract, as are tacrine, donepezil, and galanthamine, whereas quaternary ammonium compounds are poorly absorbed after oral administration. Nevertheless, quaternary ammonium compounds like neostigmine and pyridostigmine are orally active if larger doses are employed. Only the quaternary ammonium inhibitors do not readily enter the CNS. Because of their high lipid solubility and low molecular weight, most of the organophosphates are absorbed by all routes of administration; even percutaneous exposure can result in the absorption of sufficient drug to permit the accumulation of toxic levels of these compounds.

Edrophonium is partially metabolized to a glucuronide conjugate in the liver. Some of this metabolite is excreted in bile. Carbamates undergo both nonenzymatic and enzymatic hydrolysis, with enzymatic hydrolysis generally resulting from an interaction of the drug with the pseudo-ChE in plasma and liver. Organo-

phosphates are metabolized to inactive products by hydrolytic enzymes in the plasma, kidney, liver, and lungs. In contrast, the organophosphate insecticide parathion requires metabolism (oxidative desulfuration) to become an effective insecticide.

Metabolites of the cholinesterase inhibitors and in some instances significant amounts of the parent compound are eliminated in the urine. Renal excretion is very important in the clearance of agents such as neostigmine, pyridostigmine, and edrophonium. This is demonstrated by a twofold to threefold increase in elimination half-lives for these drugs in anephric patients. Renal elimination is largely the result of glomerular filtration but probably also involves, at least in the case of quaternary amines, secretion via the renal cationic transport system.

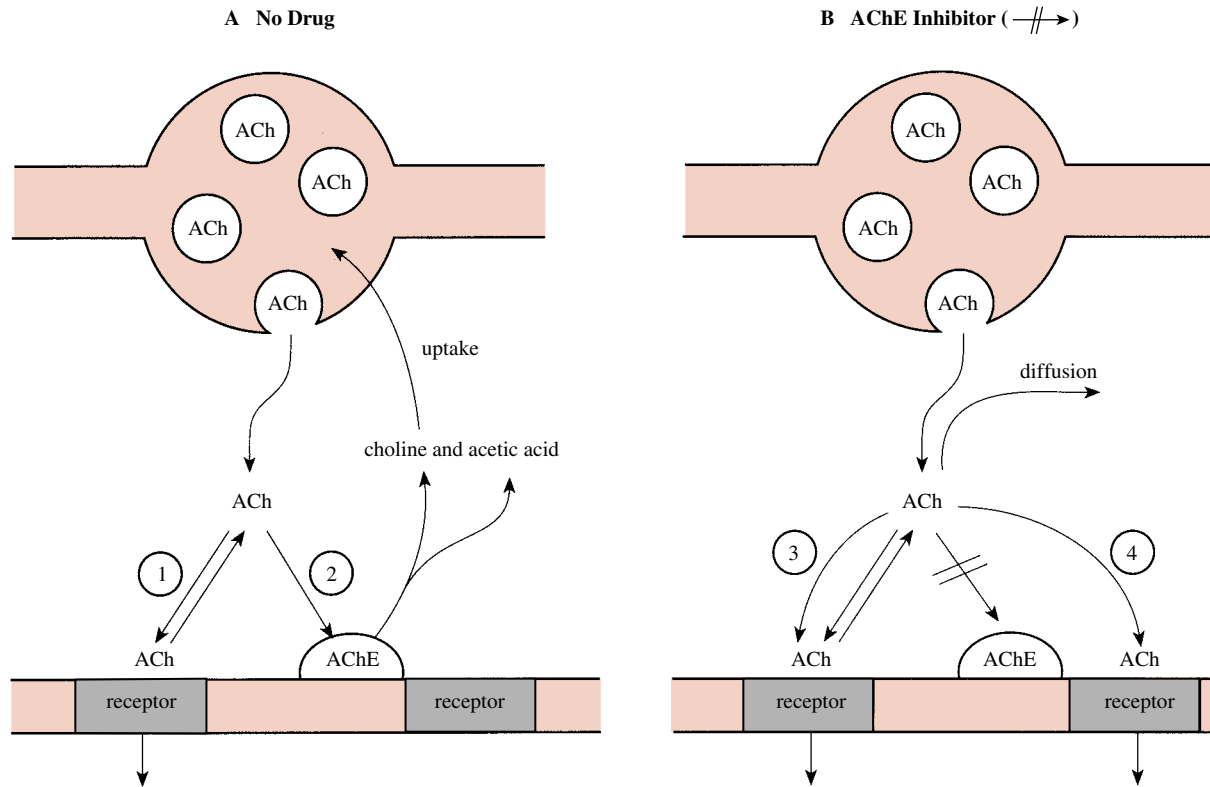
Basic Pharmacology

Inhibition of AChE potentiates and prolongs the stimulation of cholinergic receptors resulting from ACh released at cholinergic synapses (Fig. 12.5). These synapses include those found at the skeletal neuromuscular junction, adrenal medulla, autonomic ganglia, cholinergic neuroeffector junctions of the autonomic nervous system, and cholinergic synapses in the CNS. The degree and range of effects observed depend on the inhibitor chosen, the dose employed, and the route of exposure or administration.

Neuromuscular transmission in skeletal muscle is enhanced by low concentrations of anticholinesterase agents, whereas high concentrations result in cholinergic blockade. This blockade is initially due to a persistent membrane depolarization and inactivation of voltage-gated sodium channels, but if ACh levels remain high, the nicotinic cholinergic receptors can quickly become desensitized. Although anticholinesterase agents will facilitate cholinergic transmission at autonomic ganglia, their action at these sites is less marked than at the neuromuscular junction. See Chapter 28 for further discussion of this topic. Muscarinic receptors do not exhibit comparable desensitization.

Anticholinesterase agents of all classes can initiate antidromic firing of action potentials in motor neurons, possibly due to an activation of prejunctional ACh receptors that are activated by the elevated synaptic ACh. Quaternary ammonium inhibitors can also act as agonists at these receptors. The initiation of antidromic firing may be a mechanism by which cholinesterase inhibitors produce fasciculation of skeletal muscle.

The actions of anticholinesterase agents on the cardiovascular system are complex. The primary effect produced by potentiation of vagal stimulation is bradycardia with a consequent decrease in cardiac output and blood pressure. However, potentiation of both parasympathetic and sympathetic ganglionic transmis-

**FIGURE 12.5**

Action of AChE at a cholinergic neuroeffector function and effects of AChE inhibition. **A.** Key features of cholinergic neurotransmission in the absence of drugs. After release from a cholinergic nerve terminal or varicosity, ACh can (1) bind reversibly to cholinergic receptors in the postsynaptic membrane and elicit a response or (2) bind to AChE and undergo hydrolysis to choline and acetic acid (inactive metabolites). The survival time of released ACh is quite brief because of the abundance and effectiveness of AChE. **B.** The consequences of inhibiting AChE. Since ACh no longer has access to the active site of AChE, the concentration of ACh in the synaptic cleft increases. This can result in enhanced transmission due to (3) repeated activation of receptors and (4) activation of additional cholinergic receptors.

sion, including that in the adrenal medulla, can produce complicated effects on the cardiovascular system, including vasoconstrictor responses. The activation of reflexes can also complicate the total cardiovascular response to cholinesterase inhibitors.

Clinical Uses **Myasthenia Gravis**

Myasthenia gravis is an autoimmune disease in which antibodies recognize nicotinic cholinergic receptors on skeletal muscle. This decreases the number of functional receptors and consequently decreases the sensitivity of the muscle to ACh. Muscle weakness and rapid fatigue of muscles during use are characteristics of the disease. Anticholinesterase agents help to alleviate the weakness by elevating and prolonging the concentration of ACh in the synaptic cleft, producing a greater activation

of the remaining nicotinic receptors. By contrast, thymectomy, plasmapheresis, and corticosteroid administration are treatments directed at decreasing the autoimmune response.

Anticholinesterase agents play a key role in the diagnosis and therapy of myasthenia gravis, because they increase muscle strength. During diagnosis, the patient's muscle strength is examined before and immediately after the intravenous injection of edrophonium chloride. In myasthenics, an increase in muscle strength is obtained for a few minutes.

The pronounced weakness that may result from inadequate therapy of myasthenia gravis (myasthenic crisis) can be distinguished from that due to anticholinesterase overdose (cholinergic crisis) by the use of edrophonium. In cholinergic crisis, edrophonium will briefly cause a further weakening of muscles, whereas improvement in muscle strength is seen in the

myasthenic patient whose anticholinesterase therapy is inadequate. Means for artificial respiration should be available when patients are being tested for cholinergic crisis.

Pyridostigmine and neostigmine are the major anticholinesterase agents used in the therapy of myasthenia gravis, but ambenonium can be used when these drugs are unsuitable. When it is feasible, these agents are given orally. Pyridostigmine has a slightly longer duration of action than neostigmine, with smoother dosing, and it causes fewer muscarinic side effects. Ambenonium may act somewhat longer than pyridostigmine, but it produces more side effects and tends to accumulate.

Smooth Muscle Atony

Anticholinesterase agents can be employed in the treatment of adynamic ileus and atony of the urinary bladder, both of which may result from surgery. Neostigmine is most commonly used, and it can be administered subcutaneously or intramuscularly in these conditions. Cholinesterase inhibitors are, of course, contraindicated if mechanical obstruction of the intestine or urinary tract is known to be present.

Antimuscarinic Toxicity

A number of drugs in addition to atropine and scopolamine have antimuscarinic properties. These include tricyclic antidepressants, phenothiazines, and antihistamines. Physostigmine has been used in the treatment of acute toxicity produced by these compounds. However, physostigmine can produce cardiac arrhythmias and other serious toxic effects of its own, and therefore, it should be considered as an antidote only in life-threatening cases of anticholinergic drug overdose.

Alzheimer's Disease

Alzheimer's disease is a slowly developing neurodegenerative disease that produces a progress loss of memory and cognitive function, that is, dementia. These functional changes appear to result primarily from the loss of cholinergic transmission in the neocortex. The four cholinesterase inhibitors that have been approved for use in the palliative treatment of Alzheimer's disease are tacrine, donepezil, rivastigmine, and galanthamine. These agents can cross the blood-brain barrier to produce a reversible inhibition of AChE in the CNS. These compounds produce modest but significant improvement in the cognitive function of patients with mild to moderate Alzheimer's disease, but they do not delay progression of the disease. Donepezil, rivastigmine, and galanthamine are as effective as tacrine in increasing cognitive performance but do not share tacrine's hepatotoxic effects.

Glaucoma

Long-lasting AChE inhibitors, such as demecarium (*Humorsol*), echothiophate, and physostigmine are also effective in treating open-angle glaucoma, although they have now been largely replaced by less toxic drugs. Topical application of long-acting cholinesterase inhibitors to the eye not only presents the risk of systemic effects, but they can cause cataracts; this is a primary reason for reluctance to use these drugs even in resistant cases of glaucoma. Pilocarpine should be used rather than AChE inhibitors for treating angle-closure glaucoma.

Strabismus

Drug treatment of *strabismus* (turning of one or both eyes from the normal position) is largely limited to certain cases of accommodative *esotropia* (inward deviation). Long-acting anticholinesterase agents, such as echothiophate or demecarium, are employed to potentiate accommodation by blocking ACh hydrolysis at the ciliary muscle and decreasing the activity of extraocular muscles of convergence. This results in reduced accommodative convergence. The same side effects and precautions mentioned for the use of these drugs in glaucoma apply to the therapy of strabismus.

Reversal of Neuromuscular Blockade

Anticholinesterase agents are widely used in anesthesiology to reverse the neuromuscular blockade caused by nondepolarizing muscle relaxants (see Chapter 28). The blockade by these drugs is competitive and can be overcome by increasing the concentration of ACh available to stimulate nicotinic cholinergic receptors at the neuromuscular junction. Neostigmine, pyridostigmine, and edrophonium are anticholinesterase agents that are used for this purpose. Atropine or glycopyrrolate is administered in conjunction with the anticholinesterase agents to prevent the bradycardia and other side effects that result from excessive stimulation of muscarinic receptors.

Adverse Effects

Accidental poisoning by cholinesterase inhibitors can arise in many settings, since these agents are not only used clinically but also widely used as agricultural and household insecticides, and accidental poisoning sometimes occurs during their manufacture and use. In addition, a number of cholinesterase inhibitors, including the nerve gases *sarin* and *soman*, have been used in chemical warfare. The acute toxicity of all of these agents results from the accumulation of ACh at cholinergic synapses. With increasing inhibition of AChE and accumulation of ACh, the first signs are muscarinic stimulation, followed by nicotinic receptor stimulation and then desensitiza-

tion of nicotinic receptors. Excessive inhibition can ultimately lead to a *cholinergic crisis* that includes gastrointestinal distress (nausea, vomiting, diarrhea, excessive salivation), respiratory distress (bronchospasm and increased bronchial secretions), cardiovascular distress (bradycardia or tachycardia, A-V block, hypotension), visual disturbance (miosis, blurred vision), sweating, and loss of skeletal motor function (progressing through incoordination, muscle cramps, weakness, fasciculation, and paralysis). CNS symptoms include agitation, dizziness, and mental confusion. Death usually results from paralysis of skeletal muscles required for respiration but may also result from cardiac arrest. The progression through the adverse effects can be rapid.

Although anticholinesterase agents can be used in the treatment of atony of the bladder and adynamic ileus, they are contraindicated in cases of mechanical obstruction of the intestine or urinary tract. Caution should also be used in giving these drugs to a patient with bronchial asthma or other respiratory disorders, since they will further constrict the smooth muscle of the bronchioles and stimulate respiratory secretions.

Because anticholinesterase agents also inhibit plasma pseudo-ChE, they will potentiate the effects of succinylcholine by inhibiting its breakdown. This is important, for example, when succinylcholine is to be employed in patients who have previously received cholinesterase inhibitors for the treatment of myasthenia gravis or glaucoma.

Adverse effects unrelated to inhibition of AChE can also occur. Tacrine presents a high risk of hepatotoxic-

ity, which limits its use. Some organophosphorous compounds produce delayed neurotoxicity unrelated to inhibition of any cholinesterase. Clinically, this syndrome is characterized by muscle weakness that begins a few weeks after acute poisoning and may progress to flaccid paralysis and eventually to spastic paralysis. This syndrome appears to result from changes in axonal transport. There is no specific therapy for organophosphate-induced neuropathy, and clinical recovery occurs only in the mildest cases.

Treatment of Anticholinesterase Poisoning

The first step in treatment of anticholinesterase poisoning should be injection of increasing doses of atropine sulfate to block all adverse effects resulting from stimulation of muscarinic receptors. Since atropine will not alleviate skeletal and respiratory muscle paralysis, mechanical respiratory support may be required.

If the poisoning is due to an organophosphate, prompt administration of pralidoxime chloride will result in dephosphorylation of cholinesterases in the periphery and a decrease in the degree of the blockade at the skeletal neuromuscular junction. Since pralidoxime is a quaternary amine, it will not enter the CNS and therefore cannot reactivate central cholinesterases. In addition, pralidoxime is effective only if there has been no aging of the phosphorylated enzyme. Pralidoxime has a greater effect at the skeletal neuromuscular junction than at autonomic effector sites.

Study QUESTIONS

1. A young patient is being treated for myasthenia gravis, which requires frequent adjustment of the optimal dose of neostigmine. The patient is challenged with edrophonium to evaluate the effectiveness of the cholinesterase inhibition. Optimal dosing will be indicated by
 - (A) An increase in muscle strength
 - (B) A decrease in muscle strength
 - (C) No change in muscle strength
2. A young man broke his leg in a skiing accident, causing severe muscular spasm that necessitated relaxation of the muscle with a competitive nicotinic receptor antagonist before the fracture could be set. At the end of the orthopaedic procedure, the doctor restored neuromuscular transmission by administering:
 - (A) Succinylcholine
 - (B) Carbachol
 - (C) Physostigmine
 - (D) Neostigmine
3. A patient has developed glaucoma that is refractory to noncholinergic therapies. You decide to prescribe eyedrops containing pilocarpine, but you are concerned about the patient's ability to self-administer the drops. The most sensitive indicator of excessive administration of pilocarpine is
 - (A) An increased heart rate
 - (B) A decreased heart rate
 - (C) Mental confusion
 - (D) Constriction of the pupil

4. An 80-year-old man is increasingly forgetful, and his wife is afraid he is developing Alzheimer's disease. You are considering prescribing an anti-AChE drug to see if this will decrease his forgetfulness. Before making this prescription, you want to be sure that these drugs are suitable given the patient's medical history. Of the possible preexisting conditions listed below, you should be least concerned about
- (A) Asthma
 - (B) Weak atrioventricular conduction
 - (C) Glaucoma
 - (D) Obstruction of the GI tract
5. The choice of route of administration plays an important role in the actions of directly acting cholinomimetics. An adverse effect of choline esters that may be avoided by selection of an appropriate route of administration is:
- (A) Bradycardia
 - (B) Hypotension
 - (C) Delirium
 - (D) Sweating

ANSWERS

1. **C.** At an optimal dose of neostigmine, there should be no change in muscle strength with administration of edrophonium. If edrophonium increases muscle strength, the inhibition of AChE is insufficient and the maximum therapeutic benefit is not being achieved. If edrophonium decreases muscle strength, the dose of neostigmine is too high, bordering on the production of a depolarizing block of neuromuscular transmission.
2. **D.** Neostigmine will inhibit AChE and increase the ACh available to compete with the antagonist at the neuromuscular junction, overcoming the block of neurotransmission. Succinylcholine, a nicotinic agonist, will only very transiently increase the strength of the muscle, after which it will produce a depolarizing block. Carbachol is a nonselective cholinergic agonist that will stimulate nicotinic and muscarinic receptors without therapeutic benefit. Physostigmine will increase the strength of the muscle by the same mechanism as neostigmine, but it will also enter the CNS, producing undesirable side effects.
3. **A.** Excessive administration of pilocarpine can cause it to enter the circulatory system, activate endothelial muscarinic receptors, and produce a fall in blood pressure. This will activate sympathetic re-

flexes that increase the heart rate. Higher levels of pilocarpine would be required to stimulate muscarinic receptors on the heart that can decrease the heart rate. Although pilocarpine can enter the CNS and produce confusion in older patients, this also requires higher doses. Pilocarpine will constrict the pupil at therapeutically appropriate doses.

4. **C.** Glaucoma as a preexisting condition does not contraindicate an AChE inhibitor. The other preexisting conditions preclude the administration of AChE inhibitors. Potentiation of parasympathetic stimulation can constrict airway smooth muscle and aggravate asthma, further weaken A-V conduction, and risk perforation of the bowel if an obstruction is present.
5. **B.** Hypotension, which can be life threatening, can be avoided by preventing the entry of directly acting cholinomimetics into the circulatory system. Bradycardia and sweating are also avoided by the same precaution, but they are less significant. Delirium is not an issue for choline esters, since they do not enter the CNS.

SUPPLEMENTAL READING

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CASE Study Will you give 2-PAM to Pam?

A young woman named Pam has been brought to the emergency department. She is sweating profusely, vomiting, and having difficulty breathing. She cannot walk without assistance, and she has a pulse of 30. She is delirious and unable to explain her condition. The friend who brought her in said that the woman had threatened suicide 2 hours earlier. What should you do?

ANSWER: It is very likely that Pam has ingested an AChE inhibitor, most likely an insecticide. An additional diagnostic test would be to examine the size of the pupils and test for pupillary reflexes. If it is an anti-AChE overdose, the pupils will be constricted, and they will open only slightly (if at all) when the eye is darkened. The easy decision is to administer atropine, a treatment that typically presents relatively little risk. This will reduce or eliminate many symptoms, including the bradycardia, nausea, hypotension, sweating, and the component of the respiratory difficulty resulting from bronchoconstriction. A more difficult decision is whether to give an oxime (2-pralidoxime) to reactivate the AChE. It appears that the ingestion occurred in the past 2

hours, so reactivation by an oxime is still possible. The more difficult question is whether oxime treatment is necessary. Insecticides can include reversible carbamate AChE inhibitors or irreversible phosphorylating compounds. Unfortunately, you don't know which she has ingested. Certainly a quick inquiry to see if the product can be identified would be worth the effort. Oximes are effective in reactivating AChE inhibited by carbamates as well as phosphorylating inhibitors. However, oxime treatment does present some risk of its own, and it is not typically used for carbamate poisoning, since the life-threatening stage should pass within a few hours. You should immediately prepare for ventilatory support, as paralysis of the muscles of respiration is the primary cause of death. So there is no definitive answer to whether to administer an oxime. If there is reason to suspect a phosphorylating inhibitor was ingested or the patient is descending further into severe respiratory distress, treatment with an oxime might be warranted. However, if the patient's condition appears to be stable and adequate ventilatory support is available, it might be better to treat the patient symptomatically.