College of pharmacy
3rd year stage, pharmacology lecture.

Cholinergic Agonists

Drugs affecting the autonomic nervous system are divided into two groups according to the type of neuron involved in their mechanism of action. The cholinergic drugs, act on receptors that are activated by acetylcholine. Cholinergic and adrenergic drugs both act by either stimulating or blocking receptors of the autonomic nervous system.

The Cholinergic Neuron

The preganglionic fibers terminating in the adrenal medulla, the autonomic ganglia (both parasympathetic and sympathetic), and the postganglionic fibers of the parasympathetic division use acetylcholine as a neurotransmitter . In addition, cholinergic neurons innervate the muscles of the somatic system and also play an important role in the central nervous system (CNS). [Note: Patients with Alzheimer's disease have a significant loss of cholinergic neurons in the temporal lobe and entorhinal cortex. Most of the drugs available to treat the disease are acetylcholinesterase inhibitors.

Neurotransmission at cholinergic neurons

Neurotransmission in cholinergic neurons involves sequential six steps. The first four-synthesis, storage, release, and binding of acetylcholine to a receptor-are followed by the fifth step, degradation of the neurotransmitter in the synaptic gap (that is, the space between the nerve endings and adjacent receptors located on nerves or effector organs), and the sixth step, the recycling of choline.

Synthesis of acetylcholine: Choline is transported from the extra-cellular fluid into the cytoplasm of the cholinergic neuron by an energy-dependent carrier system that cotransports sodium and that can be inhibited by the drug hemicholinium. [Note: Choline has a quaternary nitrogen and carries a permanent positive charge, and thus, cannot diffuse through the membrane.] The uptake of choline is the rate-limiting step in acetylcholine synthesis. Choline acetyltransferase catalyzes the reaction of choline with acetyl coenzyme A (CoA) to form acetylcholine-an ester-in the cytosol. Acetyl CoA is derived from the mitochondria and is produced by the Krebs cycle and fatty acid oxidation.

Storage of acetylcholine in vesicles: The acetylcholine is packaged into presynaptic vesicles by an active transport process coupled to the efflux of protons. The mature vesicle contains not only acetylcholine but also adenosine triphosphate (ATP) and proteoglycan. [Note: ATP has been suggested to be a cotransmitter acting at prejunctional purinergic receptors to inhibit the release of acetylcholine or norepinephrine.] most synaptic vesicles will contain the primary neurotransmitter, here acetylcholine, as well as a cotransmitter that will increase or decrease the effect of the primary neurotransmitter.

Release of acetylcholine: When an action potential propagated by the action of voltage-sensitive sodium channels arrives at a nerve ending, voltage-sensitive calcium channels on the presynaptic membrane open, causing an increase in the concentration of intracellular calcium. Elevated calcium levels promote the fusion of synaptic vesicles with the cell membrane and release of

their contents into the synaptic space. This release can be blocked by botulinum toxin. Incontrast, the toxin in black widow spider venom causes all the acetylcholine stored in synaptic vesicles to empty into the synaptic gap.

Binding to the receptor: Acetylcholine released from the synaptic vesicles diffuses across the synaptic space, and it binds to either of two postsynaptic receptors on the target cell or to presynaptic receptors in the membrane of the neuron that released the acetylcholine. The postsynaptic cholinergic receptors on the surface of the effector organs are divided into two classes-muscarinic and nicotinic. Binding to a receptor leads to a biologic response within the cell, such as the initiation of a nerve impulse in a postganglionic fiber or activation of specific enzymes in effector cells as mediated by second-messenger molecules .

Degradation of acetylcholine: The signal at the postjunctional effector site is rapidly terminated, because acetylcholinesterase cleaves acetylcholine to choline and acetate in the synaptic cleft [Note: Butyrylcholinesterase, sometimes called pseudocholinesterase, is found in the plasma, but it does not play a significant role in termination of acetylcholine's effect in the synapse.]

Recycling of choline: Choline may be recaptured by a sodium-coupled, high-affinity uptake system that transports the molecule back into the neuron, where it is acetylated into acetylcholine that is stored until released by a subsequent action potential.

Cholinergic Receptors (Cholinoceptors)

Two families of cholinoceptors, designated muscarinic and nicotinic receptors, can be distinguished from each other on the basis of their different affinities for agents that mimic the action of acetylcholine (cholinomimetic agents or parasympathomimetics).

Muscarinic receptors

These receptors, in addition to binding acetylcholine, also recognize muscarine, an alkaloid that is present in certain poisonous mushrooms. By contrast, the muscarinic receptors show only a weak affinity for nicotine. There are five subclasses of muscarinic receptors: M_1, M_2, M_3, M_4, and M_5. Although five muscarinic receptors have been identified by gene cloning, only M_1, M_2 and M_3, receptors have been functionally characterized.

Locations of muscarinic receptors: These receptors have been found on ganglia of the peripheral nervous system and on the autonomic effector organs, such as the heart, smooth muscle, brain, and exocrine glands . Specifically, although all five subtypes have been found on neurons, M_1 receptors are also found on gastric parietal cells, M_2 receptors on cardiac cells and smooth muscle, and M_3 receptors on the bladder, exocrine glands, and smooth muscle. [Note: Drugs with muscarinic actions preferentially stimulate muscarinic receptors on these tissues, but at high concentration they may show some activity at nicotinic receptors.]

Mechanisms of acetylcholine signal transduction: A number of different molecular mechanisms transmit the signal generated by acetylcholine occupation of the receptor. For example, when the M_1 or M_3 receptors are activated, the receptor undergoes a conformational change and interacts with a G protein, designated G_q, which in turn activates phospholipase C.

This leads to the hydrolysis of phosphatidylinositol-(4,5)-bisphosphate-P_2 to yield diacylglycerol and inositol (1,4,5)-trisphosphate (formerly called inositol (1,4,5)-triphosphate), which cause an increase in intracellular Ca^{2+}. This cation can then interact to stimulate or inhibit enzymes, or cause hyperpolarization, secretion, or contraction. In contrast, activation of the M_2 subtype on the cardiac muscle stimulates a G protein, designated G_i, that inhibits adenylyl cyclase and increases K^+ conductance, to which the heart responds with a decrease in rate and force of contraction.

Muscarinic agonists and antagonists: Attempts are currently underway to develop muscarinic agonists and antagonists that are directed against specific receptor subtypes. For example, pirenzepine, a tricyclic anticholinergic drug, has a greater selectivity for inhibiting M_1 muscarinic receptors, such as in the gastric mucosa. At therapeutic doses, pirenzepine does not cause many of the side effects seen with the non-subtype-specific drugs; however, it does produce a reflex tachycardia on rapid infusion due to blockade of M_2 receptors in the heart. Therefore, the usefulness of pirenzepine as an alternative to proton pump inhibitors in the treatment of gastric and duodenal ulcers is questionable. Darifenacin is a competitive muscarinic receptor antagonist with a greater affinity for the M_3 receptor than for the other muscarinic receptors. The drug is used in the treatment of overactive bladder. [Note: At present, no clinically important agents interact solely with the M_4 and M_5 receptors.]

Nicotinic receptors: These receptors, in addition to binding acetylcholine, also recognize nicotine but show only a weak affinity for muscarine . The nicotinic receptor is composed of five subunits, and it functions as a ligand-gated ion channel . Binding of two acetylcholine molecules elicits a conformational change that allows the entry of sodium ions, resulting in the depolarization of the effector cell. Nicotine (or acetylcholine) initially stimulates and then blocks the receptor. Nicotinic receptors are located in the CNS, adrenal medulla, autonomic ganglia, and the neuromuscular junction. Those at the neuromuscular junction are sometimes designated N_M and the others N_N. The nicotinic receptors of autonomic ganglia differ from those of the neuromuscular junction. For example, ganglionic receptors are selectively blocked by hexamethonium, whereas neuromuscular junction receptors are specifically blocked by tubocurarine.

Direct-Acting Cholinergic Agonists

Cholinergic agonists (also known as parasympathomimetics) mimic the effects of acetylcholine by binding directly to cholinoceptors. These agents may be broadly classified into two groups:
1- choline esters, which include acetylcholine and **2- synthetic esters of choline**, such as **carbachol and bethanechol**.Naturally occurring alkaloids, such as **pilocarpine** constitue the second group. All of the direct-acting cholinergic drugs have longer durations of action than acetylcholine. Some of the more therapeutically useful drugs (pilocarpine and bethanechol) preferentially bind to muscarinic receptors and are sometimes referred to as muscarinic agents. [Note: Muscarinic receptors are located primarily, but not exclusively, at the neuroeffector junction of the parasympathetic nervous system.] However, as a group, the direct-acting agonists show little specificity in their actions, which limits their clinical usefulness.

***Acetylcholine**

Acetylcholine is a quaternary ammonium compound that cannot penetrate membranes. Although it is the neurotransmitter of parasympathetic and somatic nerves as well as autonomic ganglia, it is therapeutically of no importance because of its multiplicity of actions and its rapid inactivation by the cholinesterases. Acetylcholine has both muscarinic and nicotinic activity.

Its actions include:

Decrease in heart rate and cardiac output: The actions of acetylcholine on the heart mimic the effects of vagal stimulation. For example, acetylcholine, if injected intravenously, produces a brief decrease in cardiac rate (negative chronotropy) and stroke volume as a result of a reduction in the rate of firing at the sinoatrial (SA) node. [Note: It should be remembered that normal vagal activity regulates the heart by the release of acetylcholine at the SA node.]

Decrease in blood pressure: Injection of acetylcholine causes vasodilation and lowering of blood pressure by an indirect mechanism of action. Acetylcholine activates M_3 receptors found on endothelial cells lining the smooth muscles of blood vessels. This results in the production of nitric oxide from arginine. [Note: nitric oxide is also known as endothelium-derived relaxing factor.] . Nitric oxide then diffuses to vascular smooth muscle cells to stimulate protein kinase G production, leading to hyperpolarization and smooth muscle relaxation. In the absence of administered cholinergic agents, the vascular receptors have no known function, because acetylcholine is never released into the blood in any significant quantities. Atropine blocks these muscarinic receptors and prevents acetylcholine from producing vasodilation.

Other actions: In the gastrointestinal tract, acetylcholine increases salivary secretion and stimulates intestinal secretions and motility. Bronchiolar secretions are also enhanced. In the genitourinary tract, the tone of the detrusor urinae muscle is increased, causing expulsion of urine. In the eye, acetylcholine is involved in stimulating ciliary muscle contraction for near vision and in the constriction of the pupillae sphincter muscle, causing miosis (marked constriction of the pupil). Acetylcholine (1% solution) is instilled into the anterior chamber of the eye to produce miosis during ophthalmic surgery.

***Bethanechol**

Bethanechol is structurally related to acetylcholine, in which the acetate is replaced by carbamate and the choline is methylated. Hence, it is not hydrolyzed by acetylcholinesterase (due to the addition of carbonic acid), although it is inactivated through hydrolysis by other esterases. It lacks nicotinic actions (due to the addition of the methyl group) but does have strong muscarinic activity. Its major actions are on the smooth musculature of the bladder and gastrointestinal tract. It has a duration of action of about 1 hour.

Actions: Bethanechol directly stimulates muscarinic receptors, causing increased intestinal motility and tone. It also stimulates the detrusor muscles of the bladder whereas the trigone and sphincter are relaxed, causing expulsion of urine.

Therapeutic applications: In urologic treatment, bethanechol is used to stimulate the atonic bladder, particularly in postpartum or postoperative, nonobstructive urinary retention. Bethanechol may also be used to treat neurogenic atony as well as megacolon.

Adverse effects: Bethanechol causes the effects of generalized cholinergic stimulation. These include sweating, salivation, flushing, decreased blood pressure, nausea, abdominal pain, diarrhea, and bronchospasm.

***Carbachol (carbamylcholine)**
Carbachol has both muscarinic as well as nicotinic actions (lacks a methyl group present in bethanechol; . Like bethanechol, carbachol is an ester of carbamic acid and a poor substrate for acetylcholinesterase . It is biotransformed by other esterases, but at a much slower rate. A single administration can last as long as 1 hour.

Actions: Carbachol has profound effects on both the cardiovascular system and the gastrointestinal system because of its ganglion-stimulating activity, and it may first stimulate and then depress these systems. It can cause release of epinephrine from the adrenal medulla by its nicotinic action. Locally instilled into the eye, it mimics the effects of acetylcholine, causing miosis and a spasm of accommodation in which the ciliary muscle of the eye remains in a constant state of contraction

Therapeutic uses: Because of its high potency, receptor nonselectivity, and relatively long duration of action, carbachol is rarely used therapeutically except in the eye as a miotic agent to treat glaucoma by causing pupillary contraction and a decrease in intraocular pressure.

Adverse effects: At doses used ophthalmologically, little or no side effects occur due to lack of systemic penetration (quaternary amine).

***Pilocarpine**
The alkaloid pilocarpine is a tertiary amine and is stable to hydrolysis by acetylcholinesterase. Compared with acetylcholine and its derivatives, it is far less potent, but it is uncharged and will penetrate the CNS at therapeutic doses. Pilocarpine exhibits muscarinic activity and is used primarily in ophthalmology.

Actions: Applied topically to the cornea, pilocarpine produces a rapid miosis and contraction of the ciliary muscle. The eye undergoes miosis and a spasm of accommodation; the vision is fixed at some particular distance, making it impossible to focus .[Note the opposing effects of atropine, a muscarinic blocker, on the eye . Pilocarpine is one of the most potent stimulators of secretions (secretagogue) such as sweat, tears, and saliva, but its use for producing these effects has been limited due to its lack of selectivity. The drug is beneficial in promoting salivation in patients with xerostomia resulting from irradiation of the head and neck. Sj¡¶gren's syndrome, which is characterized by dry mouth and lack of tears, is treated with oral pilocarpime tablets and cevimeline, a cholinergic drug that also has the drawback of being nonspecific.

Therapeutic use in glaucoma: Pilocarpine is the drug of choice in the emergency lowering of intraocular pressure of both narrow-angle (also called closed-angle) and wide-angle (also called open-angle) glaucoma. Pilocarpine is extremely effective in opening the trabecular meshwork around Schlemm's canal, causing an immediate drop in intraocular pressure as a result of the increased drainage of aqueous humor. This action lasts up to 8 hours and can be repeated. The organophosphate echothiophate inhibits acetylcholinesterase and exerts the same effect for a

longer duration. [Note: Carbonic anhydrase inhibitors, such as acetazolamide, as well as the β-adrenergic blocker timolol, are effective in treating glaucoma chronically but are not used for emergency lowering of intraocular pressure.]

Adverse effects: Pilocarpine can enter the brain and cause CNS disturbances. It stimulates profuse sweating and salivation.

Indirect-Acting Cholinergic Agonsists: Anticholinesterases (Reversible)

Acetylcholinesterase is an enzyme that specifically cleaves acetylcholine to acetate and choline and, thus, terminates its actions. It is located both pre- and postsynaptically in the nerve terminal, where it is membrane bound. Inhibitors of acetylcholinesterase indirectly provide a cholinergic action by prolonging the lifetime of acetylcholine produced endogenously at the cholinergic nerve endings. This results in the accumulation of acetylcholine in the synaptic space. These drugs can thus provoke a response at all cholinoceptors in the body, including both muscarinic and nicotinic receptors of the autonomic nervous system, as well as at neuromuscular junctions and in the brain.

***Physostigmine**

Physostigmine is a nitrogenous carbamic acid ester found naturally in plants and is a tertiary amine. It is a substrate for acetylcholinesterase, and it forms a relatively stable intermediate with the enzyme, which then becomes reversibly inactivated. The result is potentiation of cholinergic activity throughout the body.

Actions: Physostigmine has a wide range of effects as a result of its action, and not only the muscarinic and nicotinic sites of the autonomic nervous system but also the nicotinic receptors of the neuromuscular junction are stimulated. Its duration of action is about 2 to 4 hours, and it is considered to be an intermediate-acting agent. Physostigmine can enter and stimulate the cholinergic sites in the CNS.

Therapeutic uses: The drug increases intestinal and bladder motility, which serve as its therapeutic action in atony of either organ . Placed topically in the eye, it produces miosis and spasm of accommodation, as well as a lowering of intraocular pressure. It is used to treat glaucoma, but pilocarpine is more effective. Physostigmine is also used in the treatment of overdoses of drugs with anticholinergic actions, such as atropine, phenothiazines, and tricyclic antidepressants.

Adverse effects: The effects of physostigmine on the CNS may lead to convulsions when high doses are used. Bradycardia and a fall in cardiac output may also occur. Inhibition of acetylcholinesterase at the skeletal neuromuscular junction causes the accumulation of acetylcholine and, ultimately, results in paralysis of skeletal muscle. However, these effects are rarely seen with therapeutic doses.

***Neostigmine**

Neostigmine is a synthetic compound that is also a carbamic acid ester, and it reversibly inhibits acetylcholinesterase in a manner similar to that of physostigmine. Unlike physostigmine, neostigmine has a quaternary nitrogen; hence, it is more polar and does not enter the CNS. Its

effect on skeletal muscle is greater than that of physostigmine, and it can stimulate contractility before it paralyzes. Neostigmine has a moderate duration of action, usually 30 minutes to 2 hours. It is used to stimulate the bladder and GI tract, and it is also used as an antidote for tubocurarine and other competitive neuromuscular blocking agents . Neostigmine has found use in symptomatic treatment of myasthenia gravis, an autoimmune disease caused by antibodies to the nicotinic receptor at neuromuscular junctions. This causes their degradation and, thus, makes fewer receptors available for interaction with the neurotransmitter.

Adverse effects of neostigmine include those of generalized cholinergic stimulation, such as salivation, flushing, decreased blood pressure, nausea, abdominal pain, diarrhea, and bronchospasm. Neostigmine does not cause CNS side effects and is not used to overcome toxicity of central-acting antimuscarinic agents such as atropine.

Pyridostigmine and Ambenomium

Pyridostigmine and Ambenomium are other cholinesterase inhibitors that are used in the chronic management of myasthenia gravis. Their durations of action are intermediate (3 to 6 hours and 4 to 8 hours, respectively), but longer than that of neostigmine. Adverse effects of these agents are similar to those of neostigmine.

***Demecarium**

Demecarium is another cholinesterase inhibitor used to treat chronic open-angle glaucoma (primarily in patients refractory to other agents) closed-angle glaucoma after irredectomy. It is also used for the diagnosis and treatment of accommodative esotropia. Demecarium is a quaternary amine that is structurally related to neostigmine. Mechanisms of actions and side effects are similar to those of neostigmine.

***Edrophonium**

The actions of edrophonium are similar to those of neostigmine, except that it is more rapidly absorbed and has a short duration of action of 10 to 20 minutes (prototype short-acting agent). Edrophonium is a quaternary amine and is used in the diagnosis of myasthenia gravis. Intravenous injection of edrophonium leads to a rapid increase in muscle strength. Care must be taken, because excess drug may provoke a cholinergic crisis. Atropine is the antidote.

***Tacrine, donepezil, rivastigmine, and galantamine**

As mentioned above, patients with Alzheimer's disease have a deficiency of cholinergic neurons in the CNS. This observation led to the development of anticholinesterases as possible remedies for the loss of cognitive function. Tacrine was the first to become available, but it has been replaced by the others because of its hepatotoxicity. Despite the ability of donepezil ,rivastigmine , and galantamine to delay the progression of the disease, none can stop its progression. Gastrointestinal distress is their primary adverse effect .

Indirect-Acting Cholinergic Agonsists: Anticholinesterases (Irreversible)

A number of synthetic organophosphate compounds have the capacity to bind covalently to acetylcholinesterase. The result is a long-lasting increase in acetylcholine at all sites where it is released. Many of these drugs are extremely toxic and were developed by the military as nerve agents. Related compounds, such as **parathion**, are employed as insecticides.

***Echothiophate**

Mechanism of action: Echothiophate is an organophosphate that covalently binds via its phosphate group to the serine-OH group at the active site of acetylcholinesterase . Once this occurs, the enzyme is permanently inactivated, and restoration of acetylcholinesterase activity requires the synthesis of new enzyme molecules. Following covalent modification of acetylcholinesterase, the phosphorylated enzyme slowly releases one of its ethyl . The loss of an alkyl group, which is called aging, makes it impossible for chemical reactivators, such as pralidoxime to break the bond between the remaining drug and the enzyme.

Actions: Actions include generalized cholinergic stimulation, paralysis of motor function (causing breathing difficulties), and convulsions. Echothiophate produces intense miosis and, thus, has found therapeutic use. Atropine in high dosage can reverse many of the muscarinic and some of the central effects of echothiophate.

Therapeutic uses: An ophthalmic solution of the drug is used directly in the eye for the chronic treatment of open-angle glaucoma. The effects may last for up to one week after a single administration. Echothiophate is not a first-line agent in the treatment of glaucoma. In addition to its other side effects, the potential risk for causing cataracts limits the use of echothiophate.

Reactivation of acetylcholinesterase: Pralidoxime can reactivate inhibited acetylcholinesterase. However, it is unable to penetrate into the CNS. The presence of a charged group allows it to approach an anionic site on the enzyme, where it essentially displaces the phosphate group of the organophosphate and regenerates the enzyme. If given before aging of the alkylated enzyme occurs, it can reverse the effects of echothiophate, except for those in the CNS. With the newer nerve agents, which produce aging of the enzyme complex within seconds, pralidoxime is less effective. Pralidoxime is a weak acetylcholinesterase inhibitor and, at higher doses, may cause side effects similar to other acetylcholinsterase inhibitors .