Cholinoceptor Agonists and Anticholinesterase Agents

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Mageshwaran Lakshmanan

Abstract

The parasympathetic system (cholinergic system) is responsible for the conservation of energy, digestion, and growth. Acetylcholine is the major neurotransmitter of the parasympathetic system acting on muscarinic and nicotinic receptors. The cholinergic system can be directly stimulated by natural alkaloids like muscarine and nicotine, indirectly by inhibiting cholinesterase enzyme with carbamates. Toxins like bungarotoxins, conotoxin, and botulinum toxin inhibit cholinergic neurotransmission. Modulation of acetylcholine level by various acetylcholinesterase inhibitors plays a pivot role in the management of neurodegenerative disorders like Alzheimer's disease, glaucoma, snakebite envenomation, and others. Understanding the mechanism of inhibition of acetylcholinesterases by organophosphorus compounds, carbamates, and nerve gases has led to successful pharmacological management of poisoning by these compounds. This chapter will review the synthesis and secretion of ACh, its receptors and actions brought out by the cholinergic system with details about the drugs that modulate the cholinergic system for the benefit in various clinical conditions.

Keywords

Cholinergic system · Acetylcholine · ACh · Anticholinesterase · OPC poisoning

M. Lakshmanan (✉)
Deparment of Pharmacology, Thanjavur Medical College, Thanjavur, Tamil Nadu, India
e-mail: waranmagesh87@gmail.com

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1.1 Introduction

The term "cholinergic system" is an alternative notion for the parasympathetic nervous system. Anatomically referred to as "craniosacral outflow" and physiologically as "trophotropic," the parasympathetic nervous system is responsible for conservation of energy, which is active during the rest state and helps in digestion of food. Acetylcholine (ACh), the first neurotransmitter to be discovered, was named as "vagusstoff" (in reference to its release after stimulation of the vagus nerve) by Otto Loewi and "parasympathomimetic" by Dale, is the important and major neurotransmitter of parasympathetic nervous system. It is imprudent to represent ACh as the "lone neurotransmitter" of parasympathetic nervous system because neurotransmitters like vasoactive intestinal polypeptide (VIP) and ATP are also released by the parasympathetic neurons.

Moreover, as a neurotransmitter, ACh is not confined only to parasympathetic neurons. Various sites in brain, all preganglionic fibers of the sympathetic system, post-ganglionic fibers of sweat glands in sympathetic system, and somatic nervous system also utilize ACh as the neurotransmitter. The term cholinergic transmission is applied where ACh acts as neurotransmitter at the post-synaptic site.

1.2 Synthesis and Degradation of ACh

- The synthesis and degradation of ACh are summarized in Fig. 1.1. ACh is generated by combination of choline with acetyl CoA catalyzed by the enzyme choline acetyltransferase in the neuronal axoplasm.
- Acetyl CoA is synthesized from the neuronal mitochondria from pyruvate using pyruvate dehydrogenase. Acetyl CoA can also be generated from the reaction between acetate and ATP via acetate thiokinase with CoA.
- Choline is up taken actively from extracellular fluid by the Na^+-Choline cotransporter located in neuronal membrane. This is the rate-limiting step of ACh synthesis because "de novo" synthesis of choline by neurons is very limited and depends mainly on dietary source and choline recycling. Na^+-Choline cotransporter can be blocked by hemicholinium.
- The ACh synthesized in neuronal axoplasm is transported into vesicles for storage and for prompt release upon receiving signal via depolarization of neurons. Vesicular ACh Transporter (VAChT) is involved in storing the ACh inside the vesicle along with ATP and VIP (cotransmitters). VAChT is an ATPase dependent antiport that pumps out proton from the vesicle and moves ACh into it via proton electro-chemical gradient.
- ACh and ATP are stored inside the vesicle at the concentration of 10:1 along with vesiculin (negatively charged proteoglycan which anchors ACh), calmodulin, atractyloside binding protein (ATP carrier), synapsin (involved in exocytosis of ACh), and metal ions (Mg^{2+} and Ca^{2+}).
- In each motor neuronal terminal, around >3,00,000 vesicles are seen and the number of molecules of ACh stored in each vesicle ranges from 1000 to 50,000.

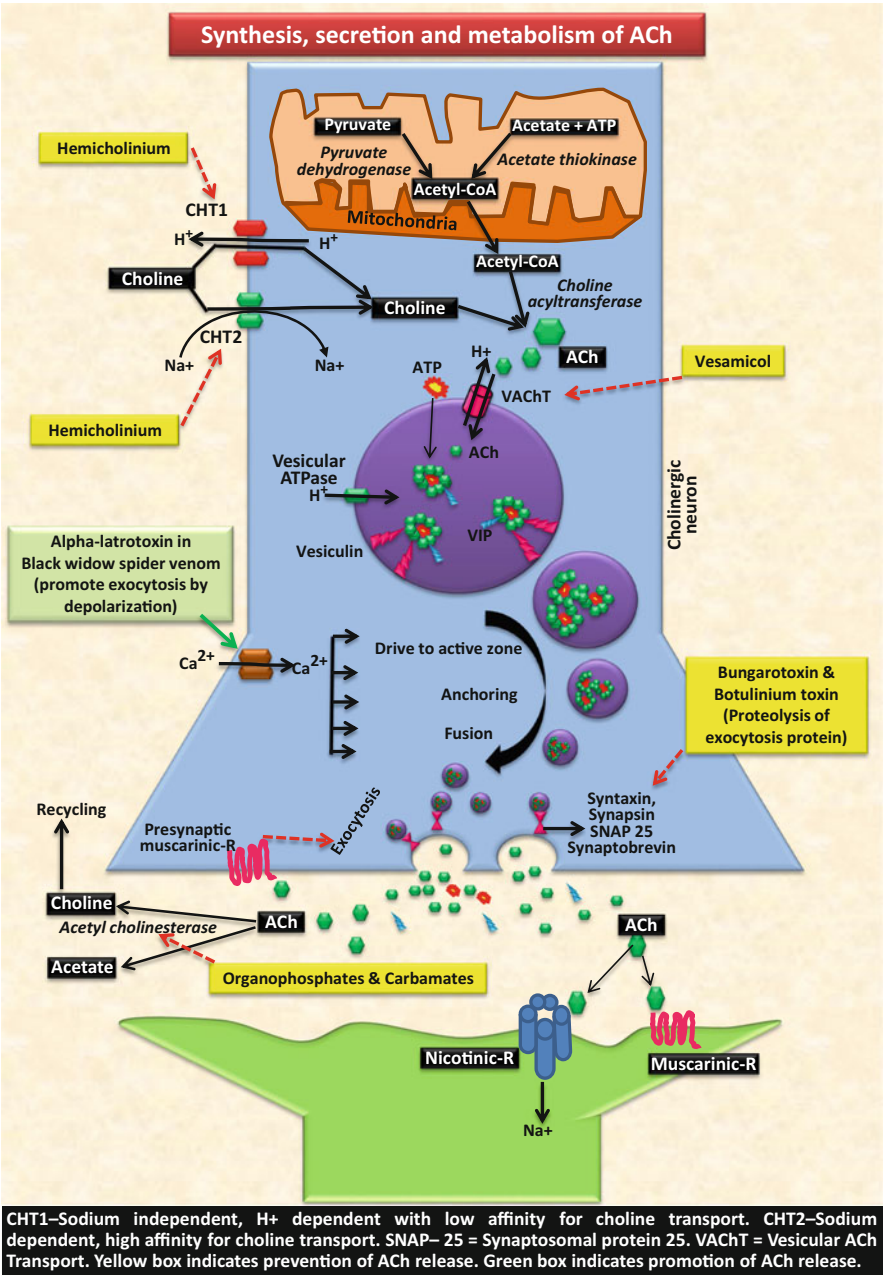

Fig. 1.1 Synthesis, storage, secretion, and metabolism of ACh

This enormous magnitude of ACh concentration achieved by storage in single neuron is responsible for the effective functioning of cholinergic transmission. Storage of ACh achieved by the VAChT can be blocked by the vesamicol.

- Upon neuronal depolarization and subsequent voltage gated channel mediated Ca^{2+} influx, the ACh vesicles undergo exocytosis by fusion of vesicular membrane with neuronal plasma membrane.
- Exocytosis of ACh vesicles requires the formation of multi-protein complex made by syntaxin, synaptosomal protein 25 (SNAP-25), and synaptobrevin which fuses vesicle to neuronal membrane. These three proteins are called together as SNAP regulators (SNARES). The SNARES are proteolysed by botulinum toxin and alpha-bungarotoxin and thereby prevent ACh release.
- After release, ACh acts on its respective receptors to bring the effect. ACh is terminated rapidly by the enzyme acetylcholinesterase (AChE) located in the neuronal synapse. A single molecule of AChE can hydrolyze 6,00,000 molecules of ACh per minute. Rapid destruction of ACh prevents its lateral diffusion and avoids unwanted effects.
- AChE is called as "true cholinesterase" as it is found abundantly in cholinergic neurons and concentrated highly in neuromuscular junction. Conversely, liver synthesizes "pseudo-cholinesterase" called as butyryl cholinesterase (BuChE) which is responsible for destruction of esters ingested from plants. BuChE is very limited in the central nervous system but abundant in plasma (hence, the name plasma cholinesterase).
- Both AChE and BuChE can be inhibited by various poisons like organophosphorus compounds (OPC), carbamates, and war gases. Hence, these agents are called anti-cholinesterase agents and they increase the cholinergic action by increasing the ACh level in synapse.

1.3 Cholinergic Receptors

- Sir Henry Dale observed that ACh produces action similar to crude extract obtained from the *Amanita muscaria* and *Nicotiana tabacum*. Consequently, the actions produced were named as "muscarinic" and "nicotinic" actions.
- Muscarinic actions of ACh are slower and it can be either inhibitory or excitatory. These actions are mediated via five subtypes of GPCRs called as "muscarinic receptors" (M_1, M_2, M_3, M_4, and M_5).
- Conversely, the nicotinic actions are very fast and are always excitatory. Nicotinic actions of ACh are mediated via ligand-gated ion channels, which permit the passage of Na^+, K^+, and Ca^{2+} upon activation. Based on the location, nicotinic receptors are classified into N_m (nicotinic muscle) and N_n (nicotinic neuronal). The location, action, stimulants, and blockers of the cholinergic receptors are summarized in Tables 1.1 and 1.2.

Table 1.1 Characteristics of nicotinic receptor subtypes in humans

Receptor subtype	Structure	Location	Molecular mechanism	Action	Stimulant	Blocker
Nicotinic muscular (N_m)	Pentameric (2α-β-γ-δ) ligand-gated ion channel	Neuromuscular junction (post-junctional)	Increases cation (Na^+ or K^+) permeability	Contraction of skeletal muscle	ACh Nicotine SCh	Curare α-Conotoxin α-bungarotoxin
Peripheral nicotinic neuronal (P-N_n)	Pentameric (2α-3β) ligand-gated ion channel	Autonomic ganglia and adrenal medulla	Increases cation (Na^+ or K^+) permeability	Excitation of post-ganglionic neuron and catecholamine secretion	ACh Nicotine DMPP	Trimethaphan Mecamylamine
Heterogenic central nicotinic neuronal (C-N_n)	Heterogenic pentameric (2α-3β) ligand-gated ion channel	Various areas of brain	Increases cation (Na^+ or K^+) permeability	Memory Co-ordination Learning	Cytosine Anatoxin A	Mecamylamine Lophotoxin Erysodine
Homogenic central nicotinic neuronal (C-N_n)	Homogenic pentameric (5α) ligand-gated ion channel	Various areas of brain	Increases Ca^{2+} permeability	Glutamatergic neurotransmission	Anatoxin A	α-Conotoxin α-bungarotoxin

SCh succinylcholine, *DMPP* dimethylphenylpiperazinium

Table 1.2 Characteristics of muscarinic receptor subtypes in humans

Sub type	Location	Action	Molecular mechanism	Relatively specific stimulant	Specific blocker
M_1	1. CNS (hippocampus, thalamus, and striatum) 2. Salivary glands 3. Gastric glands 4. Enteric nerves 5. Autonomic ganglia	1. Learning, cognition, and locomotion 2. Salivary secretion 3. Acid secretion 4. Intestinal contraction 5. Ganglion stimulation	GPCR with $G_{q/11}$ type of α subunit (activates PLC and increases IP_3, DAG, and Ca^{2+} levels)	Oxotremorine Sabcomeline	Pirenzepine Telenzepine
M_2	1. CNS (hindbrain, thalamus, and cortex) 2. SA node of heart 3. AV node of heart 4. Atrial muscles 5. Presynaptic in lungs 6. Smooth muscle 7. Autonomic ganglion	1. Produces tremors and hypothermia 2. ↓ HR (↓ automaticity) 3. ↑ AV nodal delay 4. ↓ ERP of atria 5. Bronchoconstriction 6. ↑ smooth muscle contraction 7. ↓ ganglionic transmission	GPCR with $G_{i/o}$ type of α subunit (inhibits AC and ↓cAMP level and opens K^+ channels)	Methacholine	Methoctramine Tripitramine Himbacine
M_3	1. Lungs smooth muscle 2. Bronchial glands 3. Salivary glands 4. Iris 5. Ciliary body 6. Vascular endothelium 7. Urinary bladder 8. Intestinal smooth muscles and glands	1. Bronchoconstriction 2. Airway mucus secretion 3. Saliva secretion 4. Miosis (contraction of sphincter pupillae) 5. Ciliary body contraction 6. NO release—relaxation 7. Detrusor contraction 8. Propulsive movement and enzyme secretion	GPCR with $G_{q/11}$ type of α subunit (activates PLC and increases IP_3, DAG, and Ca^{2+} levels)	Bethanechol	Darifenacin Solifenacin Trospium
M_4	1. Various parts of CNS	1. Auto receptor	Same as M_2	–	PD-0298029
M_5	1. Substantia nigra 2. Ventral tegmentum 3. Cerebral blood vessels	1. Facilitates DA release 2. Reward and drug seeking 3. Mediates cerebral blood vessels dilatation	Same as M_1	–	ML-381

1.4 Action of ACh in Different Organ Systems

1.4.1 Central Nervous System

- All five muscarinic receptors and central nicotinic neuronal receptors are present in brain. ACh cannot cross BBB and hence no response will be noted if injected i.v. Moreover, it will be rapidly degraded by plasma esterases.
- Direct injection of ACh into brain produces stimulant effect mediated via nicotinic receptors. This is followed by depressor response mediated via muscarinic and nicotinic receptors.
- As a neurotransmitter, ACh in brain (via M_1, M_2, and M_5) is responsible for learning, memory, cognition, and co-ordination of locomotion. Hence, cholinomimetics are useful in Alzheimer's diseases and in treating various dementias.

1.4.2 Heart

- Cardiac effects of ACh are mediated mainly via M_2 receptors. ACh produces negative chronotropic, dromotropic, and inotropic effect in heart (i.e., HR, rate of conduction, and force of contraction are decreased by ACh).

1.4.3 Blood Vessels

- Blood vessels lack cholinergic nerve supply and hence, endogenous ACh does not have significant role in physiological maintenance of vascular tone. However, the endothelium of blood vessels has M_3 receptor and upon stimulation by i.v. ACh, it releases NO leading to vasodilation.
- In a similar way, penile erection is brought upon by the action of ACh on M_3 receptors in penile arteries leading to penile vasodilatation via NO release.
- This vasodilatory effect by ACh is observed only in blood vessels with intact endothelium. Vasoconstriction by ACh is noted in endothelium-damaged blood vessels due to activation of smooth muscle contraction via M_3—due to lack of NO to counteract this effect.

1.4.4 Lungs

- ACh increases bronchial secretion from respiratory tract glands via stimulation of M_3 receptors in airway. In COPD, the bronchial secretion is persistently increased due to increased vagal tone because of inflammatory process. Hence, antimuscarinic drugs are useful in COPD.
- Moreover, M_3 receptors are present in the smooth muscles post-junctionally. Stimulation of M_3 receptors by ACh causes bronchoconstriction.

- Vagal cholinergic neurons in lungs have presynaptic M_2 receptors and stimulation of these receptors by ACh causes a decrease in release of ACh. This is the reason for paradoxical bronchoconstriction observed during antimuscarinic therapy (ipratropium) in COPD.

1.4.5 Gastrointestinal Tract

- ACh causes contraction of intestinal smooth muscles and secretes digestive enzymes. Increased tone and peristalsis of intestine are observed with ACh.
- Although the number of M_2 receptors is more in GI tract, the contraction of smooth muscles and other actions of ACh in GI tract are mediated via M_3 receptor stimulation.
- ACh released from the vagus nerve stimulates acid secretion via both M_1 and M_3 receptors in the gastric glands.

1.4.6 Urinary Tract

- Detrusor muscle contraction, ureteral peristalsis, and increased voiding pressure are exerted by ACh via stimulation of M_3 receptors (10–20%) in the urinary bladder.
- Similar to GI tract, urinary bladder also has high concentration of M_2 receptors (80%). However, M_2 receptors only aid M_3 receptor indirectly in detrusor contraction by inhibiting the beta-adrenergic mediated detrusor relaxation.
- Endogenous action of ACh on M_2 receptor resulting in significant detrusor contraction is observed in certain disease states of bladder like spinal cord injury and bladder denervation.

1.4.7 Eye

- ACh stimulates M_3 receptors in the sphincter pupillae of iris and causes miosis.
- It also contracts the ciliary body and thereby opens the "angle" and increases trabecular outflow. These actions are beneficial in glaucoma and explain the rationale for using cholinomimetics (physostigmine) in glaucoma.

1.4.8 Glands

- ACh increases secretion from salivary, respiratory, lacrimal, and sweat glands. Stimulation of M_1 and M_3 receptors by ACh results in active secretion and increased peristalsis of glandular ducts.
  Sweat glands though receive sympathetic innervation, ACh acts as a neurotransmitter in both pre- and post-ganglionic fibers. This fact explains the failure of

adrenergic blockers to "mask" sweating during adrenergic overactivity (hypoglycemia or anxiety).

1.4.9 Skeletal Muscles

- ACh causes contraction of skeletal muscle by stimulation of N_m receptors and virtually no muscarinic receptors are present in the adult skeletal muscles. Though in vitro studies have demonstrated the presence of muscarinic receptors in skeletal muscle cell culture, clinically it is not relevant.
- ACh produces inotropism of the skeletal muscle fiber and causes twitching and fasciculations.

1.4.10 Ganglion

- ACh stimulates both sympathetic and parasympathetic ganglion via peripheral N_n receptors. In both the systems, the preganglionic fibers release ACh as the neurotransmitter and the stimulation of post-ganglionic fibers is mediated via N_n receptors. Generally high dose of ACh is required in in vivo experiments to stimulate nicotinic receptors in the ganglion than the muscarinic receptors.
- Since a common receptor (N_n) is involved in both sympathetic and parasympathetic ganglion, stimulants (high dose ACh, nicotine, lobeline) and blockers (trimethaphan, hexamethonium) of autonomic ganglion produce unpredictable effects.

1.5 Cholinomimetic Drugs

ACh is not generally used for therapeutic purposes (except, rarely in certain ocular surgery) due to the following reasons:

- Wide, non-specific effects.
- Has to be administered i.v.
- Extremely short acting.
- Unstable.
- Availability of better and more specific agents.

1.5.1 Classification

- Various drugs facilitate the action of ACh on its receptors by blocking AChE and few drugs act as direct agonist at the cholinergic receptors. These are collectively called as cholinomimetic agents or "parasympathomimetics."
- Direct Muscarinic Agonists

  - *Choline esters*: Acetylcholine, methacholine, bethanechol, and carbachol.
  - *Alkaloids*: Muscarine, pilocarpine, and arecoline.
  - *Synthetic*: Cevimeline.
- Reversible Cholinesterase Inhibitors (Anticholinesterases)
  - Carbamates:
    Lipid insoluble: Neostigmine, edrophonium, pyridostigmine, and ambenonium.
    Lipid soluble: Physostigmine, rivastigmine, donepezil, and galantamine.
  - Acridine: Tacrine.
- Irreversible Cholinesterase Inhibitors (Poisons)
  - Carbamates: Carbaryl and propoxur.
  - Organophosphates: Malathion, parathion, diazinon, malaoxon, paraoxon, isoflurophate, echothiophate, and chlorpyrifos.
  - Nerve gases: Tabun, sarin, and soman.

1.5.2 Direct Muscarinic Agonists

- In the clinical setting, direct muscarinic agonists have limited role except cevimeline and pilocarpine.
- Currently, the only major indication of muscarinic agonist is in Sjogren syndrome, radiation-induced sialadenitis, xerostomia, and glaucoma. Both pilocarpine and cevimeline (synthetic M_3 receptor agonists) stimulate salivary and lacrimal secretion and are beneficial in xerostomia and Sjogren syndrome. Pilocarpine induces miosis, opens "angle of iris," facilitates trabecular outflow of aqueous humor, and thereby reduces intraocular tension (beneficial in glaucoma).
- In case of choline esters, bethanechol, an obsolete drug, was used in the management of post-operative ileus and abdominal distension, gastroparesis, and GERD. Methacholine was employed for confirmation of asthma by inhalational methacholine-induced bronchoconstriction called "methacholine challenge test." The comparative features of various muscarinic agonists are mentioned in Tables 1.3 and 1.4.
- The important contraindications of muscarinic agonists are patients with COPD, asthma, sick sinus syndrome, acid-peptic disease, and urinary or GI obstruction. Muscarinic agonists can convert partial heart block to complete heart block. In patients with hyperthyroidism, muscarinic agonists can cause fatal arrhythmias.

1.5.3 Toxicology of Muscarinic Alkaloids

Due to availability of muscarinic alkaloids in natural sources, it is very common to encounter poisoning by such alkaloids in clinical practice. Muscarine and pilocarpine are commonly encountered than arecoline.

Table 1.3 Features of various muscarinic agonists (choline esters)

S. No.	Parameter	Acetylcholine	Methacholine	Bethanechol	Carbachol
1	AChE susceptibility	High	Low	Nil	Nil
2	BuChE susceptibility	High	Nil	Nil	Nil
3	Nicotinic action	High	Very minimal	Nil	Highest
4	Atropine antagonism	High	High	High	Minimal
5	Crossing BBB	Nil	Nil	Minimal	Minimal
6	Molecular weight (g/mol)	146.21	160.1	161.22	182.6
7	Duration of action	1–2 min	Not known	1–6 h	4–8 h
8	Major clinical indication	Induction of miosis in surgery	Methacholine challenge test	Post-operative ileus, urinary retention	Glaucoma and induction of miosis in surgery
9	Route of administration	Eye drop	Inhalation (nebulization)	Oral tablets	Eye drops
10	Dose and strength	1% solution (as required)	0.025, 0.25, 2.5, 10, and 25 mg/ml	10–50 mg in divided doses (TDS)	0.03–1% solution (as required)

- Mushroom species like *Amanita species, Inocybe species,* and *Clitocybe species* contain muscarine and related alkaloid in large quantity. Based upon the type of mushroom consumed, nature of alkaloid, and clinical features, mushroom poisoning (mycetism) is classified into three types (Table 1.5).

1.5.4 AChE and Anticholinesterases

- Anticholinesterase reacts with AChE similar to ACh. The AChE has two active sites for cleavage of ACh into acetate and choline, namely the *anionic site* (glutamate residue) and the *esteratic site* (serine and histidine residue).
- Choline being a positively charged molecule binds to the negatively charged glutamate residue (anionic site). Acetate being a negatively charged molecule binds to the positively charged serine-OH/histidine residue in AChE.
- After successful binding, serine moiety is acetylated forming the acetylated AChE and choline is released. Subsequent hydration of acetylated AChE results in release of acetate from AChE. This hydration reaction is very rapid and occurs in milliseconds.

Table 1.4 Features of various muscarinic agonists (natural alkaloid and synthetic derivative)

S. No.	Parameter	Muscarine	Arecoline	Pilocarpine	Cevimeline
1	Source	*Amanita muscaria*	*Areca catechu*	*Pilocarpus microphyllus*	Synthetic
2	Serum esterase action	Nil	Nil	Converted to pilocarpic acid	Nil
3	Nicotinic action	Nil	Present	Nil	Very minimal
4	Structure	Quaternary amine	Tertiary amine	Tertiary amine	Quinuclidine derivative
5	Crossing BBB	Poor	High	High	Minimal
6	Duration of action	Not determined	Not determined	1–2 h	6–8 h
7	Metabolism	Not metabolized	Liver (CYP2A6 and CYP2E1)	Serum esterase and liver (CYP2D6)	Liver (CYP2D6 and CYP3A4)
8	Elimination	Renal	Renal	Renal	Renal (>80%) and fecal
9	Clinical use	Nil	Nil	Sjogren syndrome Radiation sialadenitis Xerostomia Glaucoma	Sjogren syndrome Xerostomia
10	Route of administration with dose	–	–	Oral tablet (5–10 mg TDS) 0.5–6% eye drop (sixth hourly)	30 mg capsule TDS

- Based on the type of mechanism of inhibition of AChE, the anticholinesterases can be divided into three groups:
  - Anticholinesterase with quaternary alcohol structure (Edrophonium) binds with AChE electrostatically (non-covalent) bond with anionic site and prevents the ACh interaction with AChE. Tacrine also follows the same pathway. Since the bond formed is non-covalent type, the reaction is short-lived (2–10 min).
  - Carbamates like neostigmine and physostigmine take part in carbamylation of serine residue in AChE. The carbamylated serine residue is less stable and the carbamoyl moiety can be split from the enzyme by spontaneous hydrolysis. Thus, the carbamylated AChE undergoes slow hydration reaction to release acetate from the serine-OH/histidine residue (0.5–6 h). Hence, the carbamylated AChEs are unable to bind ACh and thus, carbamates are considered as reversible competitive inhibitors of ACh.

Table 1.5 Types of mushroom poisoning and its management

S. No	Parameter	Early mushroom poisoning	Hallucinogenic mushroom poisoning	Late mushroom poisoning
1	Mushroom	*Amanita muscaria* *Inocybe lacera* *Clitocybe dealbata*	*Amanita muscaria* *Psilocybe Mexicana* *Panaeolus cyanescens*	*Amanita phalloides* *Galerina marginata*
2	Toxic compound	Muscarine	Muscimol Ibotenic acid Psilocybin Tryptamine	α-amanitin β-amanitin
3	Onset of illness	Within 30–60 min of consumption	Within 15–30 min of consumption	1–2 days
4	Symptoms	Symptoms of muscarinic overactivity (bradycardia, miosis, sweating, vomiting, diarrhea, salivation, bronchospasm)	Irritability Restlessness Hallucination Seizures Delirium and drowsiness	Initial symptom free period of 1 day followed by diarrhea, cramps, renal, hepatic failure, and death
5	Mechanism of action of toxin	Muscarinic receptor agonism	$GABA_A$ agonism (muscimol) NMDA agonism (ibotenic acid) 5-HT_{2B} and 5-HT_{2C} agonism (psilocybin)	Cellular death by inhibition of RNA polymerase II and blockade of mRNA synthesis
6	Role of atropine	Very useful (reverses all muscarinic overactivity)	Contraindicated (aggravates delirium)	No role
7	Management	Symptomatic Antidote: Atropine Fast recovery	Symptomatic (no antidote) Diazepam for seizures	Antidote: Penicillin, thioctic acid (displaces toxin from albumin and promotes excretion) Silibinin Poor prognosis
8	Mortality	Moderate, if not treated immediately	Mild to moderate	Very high even with supportive management

– Organophosphorus compounds (OPC) and nerve gases bind only to the *esteratic site* (serine-OH residue) with very strong covalent bond. Hydration reaction to free serine residue of AChE is extremely slow (>100 h) resulting in irreversible inhibition of AChE.

- Moreover, when R_2 alkyl group in the OPCs is lost in due course after binding, then the covalent bond between OPC and AChE serine site is strengthened further and hydration reaction becomes impossible. This is called as "aging of enzyme."
- Oximes like pralidoxime and diacetyl monoxime donate the nucleophilic OH group to OPC and break the covalent bond between phosphate of OPC and serine of AChE.
- Hence, oximes are called as the *enzyme regenerators*. However, oximes are ineffective for "aged" enzymes. The inhibition of AChE by edrophonium, carbamates, and OPC is illustrated in Figs. 1.2 and 1.3.

1.5.5 Pharmacological Actions of Anticholinesterases

- Majority of actions produced by the anticholinesterases are due to amplification of endogenous ACh in the organs.
- Variable effects are noted in CVS. Depressive actions in heart are due to direct ACh mediated action. In later course, ganglionic stimulation by anticholinesterase leads to stimulant effects on the heart. To make the effect more complex, high dose of anticholinesterase causes persistent depolarization of ganglion leading to ganglionic blockade and depressive effect on heart is noted.
- In skeletal muscles, low to moderate dose produces twitching and fasciculations due to increased synaptic ACh level. Partially curarized and myasthenic muscle fibers exhibit improvement in contractility. However, high dose of anticholinesterase produces persistent depolarization resulting in paralysis of muscles.

1.5.6 Therapeutic Uses of Anticholinesterases

- Reversible anticholinesterases like carbamates (except, propoxur and carbaryl which are used as insecticides against mosquitoes, house flies) and tacrine can be used as therapeutic agents. This is because the inhibition of AChE is reversible and occurs only for 0.5–6 h. The anticholinesterase used as drugs and their characteristics are summarized in Table 1.6.
- In Acute Angle Closure Glaucoma
  - Anticholinesterases cause miosis, ciliary body contraction, widening of corneal angle, and increased trabecular outflow of aqueous humor. This results in lowering of intraocular tension.
  - Being highly lipophilic agent, physostigmine eye drops (0.025–1% every 4 h) is used for treating acute angle closure glaucoma. However, physostigmine solutions are unstable and decompose with pH changes or after exposure to light; hence, pilocarpine is more commonly used. Echothiophate (0.125%) is an alternative.

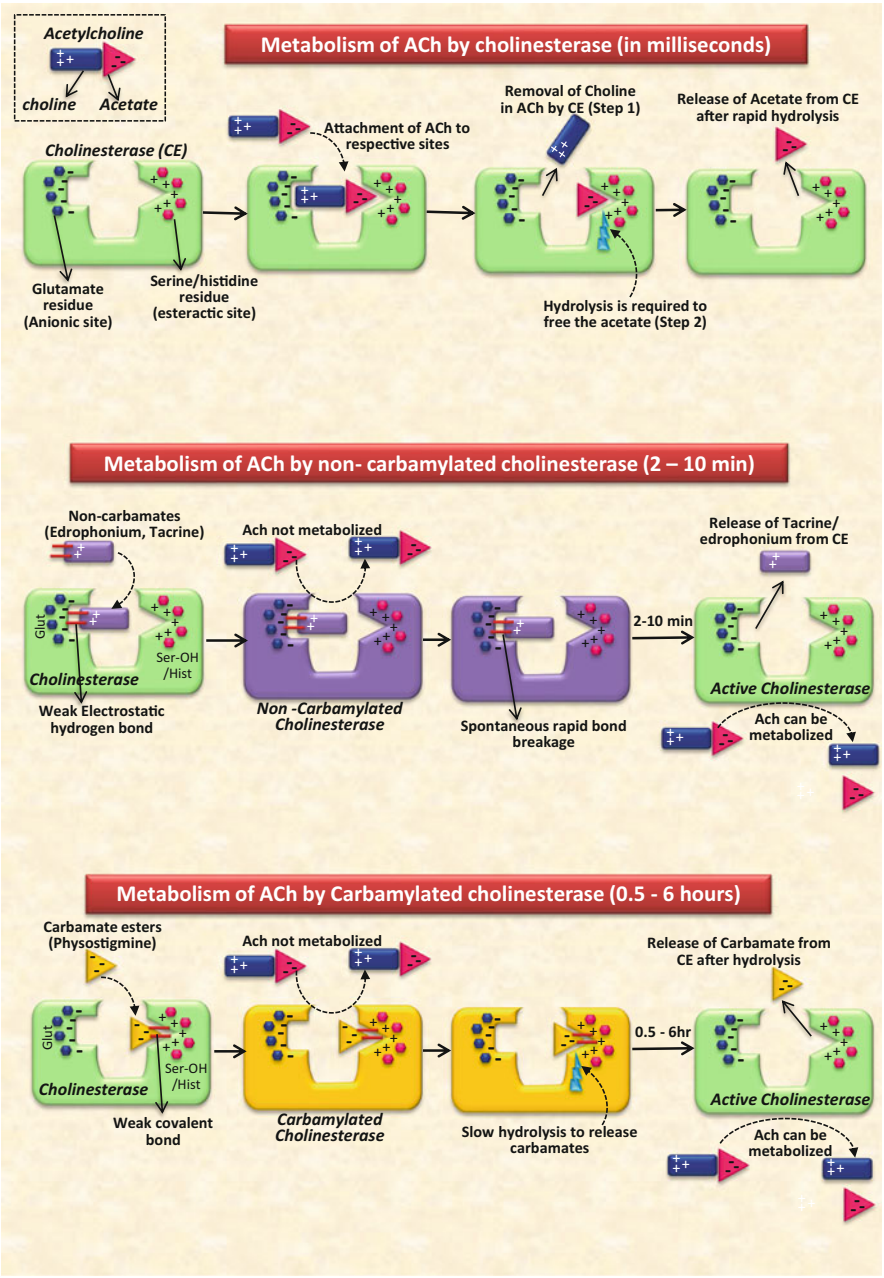

Fig. 1.2 Inhibition of cholinesterase by edrophonium, tacrine, and carbamates

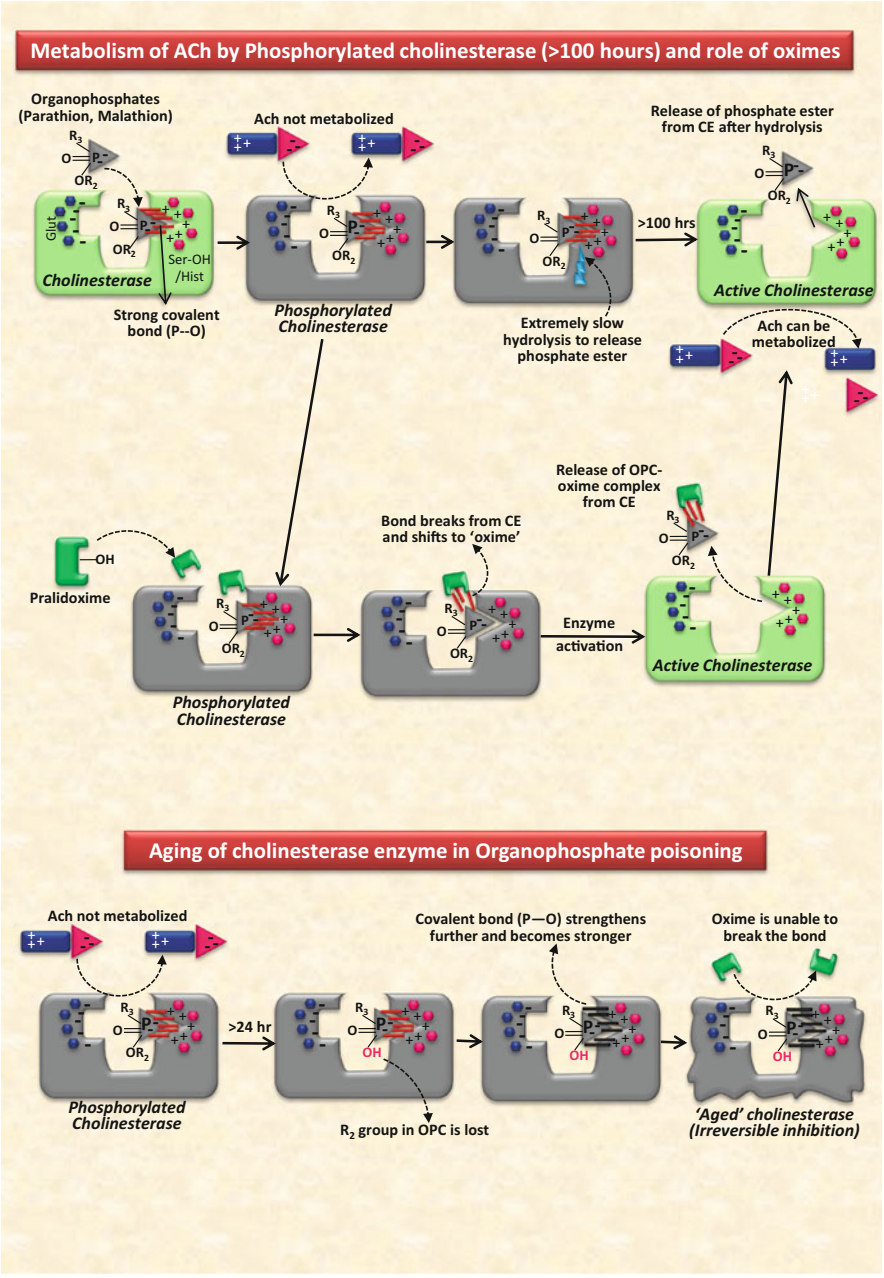

Fig. 1.3 Inhibition of cholinesterase by the organophosphate compounds

Table 1.6 Comparison of reversible inhibitors of AChE

S. No.	Drug	Source	Lipid solubility	Structure	Derivative	Major indication
1	Physostigmine	Natural [*Physostigma venenosum* (Calabar beans)]	Soluble	Tertiary amine	Carbamate	1. Glaucoma 2. Atropine poisoning
2	Neostigmine	Synthetic	Insoluble	Quaternary amine	Carbamate	1. Post-operative ileus and urinary retention 2. Cobra bite 3. Decurarization 4. Myasthenia gravis
3	Pyridostigmine	Synthetic	Insoluble	Quaternary amine	Carbamate	Myasthenia gravis
4	Edrophonium	Synthetic	Insoluble	Quaternary amine	Noncarbamate	Edrophonium challenge test
5	Rivastigmine	Synthetic	Soluble	Tertiary amine	Carbamate	Alzheimer's disease
6	Donepezil	Synthetic	Soluble	Tertiary amine	Piperidine	Alzheimer's disease
7	Tacrine	Synthetic	Soluble	Tertiary amine	Acridine	Obsolete (Alzheimer's)
8	Galantamine	Natural (*Galanthus nivalis*)	Soluble	Tertiary amine	Carbamate	Alzheimer's disease

  - Systemic absorption of anticholinesterase eyedrops causes bronchospasm, diarrhea, hypotension, and bradycardia may occur. Brow pain and headache are the frequent side effects.
  - Echothiophate may cause cataract on long-term administration.

- Other Ophthalmic Uses
  - Anticholinesterases can be tried but are preferred the least for the management of open angle closure glaucoma due to availability of better drugs like PG analogues and beta blockers.
  - Along with atropine, anticholinesterases are used to break adhesions by producing alternate mydriasis when used with miotics.
  - Physostigmine at low concentration (0.025%) is proven to be effective in reducing the pain and blurred vision associated with tonic (Adie) pupil.
  - Anticholinesterases are also used to prevent drooping of eyelids in myasthenia gravis and accommodative esotropia.
- Management of Myasthenia Gravis
  - Anticholinesterase with poor lipophilic property like neostigmine, pyridostigmine, and ambenonium is preferred for myasthenia gravis. Due to lipid insoluble nature these drugs have poor CNS penetration and exert maximal peripheral action (i.e., on skeletal muscles).
  - Anticholinesterases increase ACh concentration in NMJ and thereby displace the autoimmune antibodies bound with N_m receptors.
  - Neostigmine (7.5–15 mg), pyridostigmine (30–60 mg), and ambenonium (2.5–5 mg) are given orally twice or thrice a day.
  - Excessive muscarinic action of neostigmine on heart can be prevented with atropine. Tolerance develops over a period due to increased turnover of muscarinic receptor population with agonistic therapy.
  - Glucocorticoid, immunosuppressant, and thymectomy should also be added in successful management of myasthenia gravis.
- Diagnosis of Myasthenia Gravis
  - A short acting anticholinesterase (edrophonium) is used to diagnose myasthenia gravis and differentiate it from cholinergic crisis.
  - Initially, 2 mg of edrophonium is given as rapid i.v. injection which improves the muscle strength. If no response is noted, then after 45 s 8 mg of edrophonium is repeated. In case of cholinergic crisis, the muscle paralysis is worsened.
  - Patient should be atropinized prior to the edrophonium test to avoid muscarinic overactivity.
- Post-Operative Paralytic Ileus, Abdominal Distension, and Bladder Atony
  - Neostigmine improves the contraction of detrusor and intestinal smooth muscles and hence used in post-operative ileus and bladder atony.
  - After an oral dose of 15–30 mg of neostigmine, peristaltic activity is observed in 20–24 h. Conversely, rapid response can be generated by subcutaneous administration of neostigmine methylsulfate (0.5–1 mg). Peristaltic activity is observed within 30 min.

  - Anticholinesterases are contraindicated when peritonitis or bowel obstruction and viability of bowel are suspected.
  - The dose regimen of neostigmine for bladder atony is same as that for paralytic ileus.
- Reversal of Paralysis of Competitive Neuromuscular Blocking Drugs
  - Anticholinesterase increases the ACh concentration in NMJ and thereby displaces the curare drugs from the Nm receptor (called decurarization).
  - Neostigmine at the dose of 0.5–2 mg i.v. produces rapid reversal of muscle paralysis. Atropine or glycopyrrolate should be added to prevent muscarinic overactivity.
- Cobra Bite
  - Respiratory and muscle paralysis observed in cobra venom are due to blockade of N_m and N_n receptors by the toxins in snake venom.
  - Anticholinesterase displaces the toxins from the receptors by increasing the ACh level at synapse and thereby prevents respiratory paralysis.
  - Neostigmine at the dose of 0.5–1 mg is given subcutaneously with atropine (i.v.). Anti-snake venom should be used as a specific antidote always.
- Alzheimer's Disease
  - Centrally acting anticholinesterases (with lipid soluble property) like rivastigmine, galantamine, donepezil, and tacrine are used in Alzheimer's disease.
  - Anticholinesterases increase endogenous ACh level in nucleus basalis of Myernet, hippocampus, and striatum. Increased cholinergic neurotransmission in these areas improves memory and learning. Dementia is improved and decline of cognitive function is slowed in Alzheimer's disease.
  - Tacrine use has declined due to occurrence of fatal hepatotoxicity. Currently donepezil (5–10 mg at night) and rivastigmine (1.5–6 mg in two divided doses) are used. Anticholinesterases are not disease modifying agents and hence, tolerance develops eventually.
- Other Uses
  - Prophylaxis against cholinesterase poisoning (pretreatment with pyridostigmine lessens the toxicity of nerve gases during attack in wars but soldiers later developed nervous disorders on chronic use called *Persian Gulf War Syndrome*).
  - To counteract anticholinergic actions in tricyclic antidepressant (TCA) and antihistaminic poisoning, anticholinesterases are used with extreme caution.
  - Physostigmine 0.5–2 mg i.v. is used as "antidote" in atropine poisoning with limited efficacy. Neostigmine is less efficacious in this situation than physostigmine as it does not cross the BBB.

1.6 Toxicology of Anticholinesterases: Management of OPC and Carbamate Poisoning

- Organophosphates and nerve gases produce irreversible inhibition and aging of AChE. Hence, these compounds are not used therapeutically but have toxicological importance.

- Majority of agricultural pesticides are OPCs and hence, prevalence of OPC poisoning is higher in developing nations like India and various Southeast Asian countries.

1.6.1 Acute Organophosphorus Poisoning

- Symptoms of muscarinic overactivity (miosis, bradycardia, lacrimation, diarrhea, salivation, bronchorrhea, and sweating) and nicotinic overactivity (fasciculations, muscle spasms, and paralysis) are observed in acute OPC poisoning.
- General Measures
  - Removal of clothes and washing the skin (to prevent further absorption of OPCs via skin because OPCs are highly lipophilic).
  - Monitoring of vital signs (HR, BP, and RR) and maintenance of airway and oxygenation.
  - Control of seizures with diazepam with caution of respiratory paralysis.
- Specific Measures
- Atropinization
  - Atropine is the specific antidote to reverse the muscarinic overactivity like miosis, bradycardia, lacrimation, diarrhea, salivation, bronchorrhea, and sweating.
  - Atropine should be given 2 mg i.v. and repeated every 10 min till full atropinization occurs. Atropinization is indicated by maximal mydriasis, HR $>$100 bpm, and absence of secretions in respiratory tract.
  - Among all signs of atropinization, absence of secretion in respiratory tract is taken as a clinical endpoint of atropinization owing to maximal receptor concentration in respiratory tract.
  - Patient should be monitored for atropine toxicity which may be observed if higher doses ($>$200 mg of atropine) are administered.
- AChE Enzyme Reactivators
  - Pralidoxime and diacetyl monoxime (DAM) are called as enzyme reactivators as they donate the nucleophilic group to break the strong covalent bond between phosphate of OPC and serine-OH of AChE. Breakage of bond results in regeneration of enzymatic activity of AChE.
  - Pralidoxime and DAM should be started within 24 h of poisoning. This is because "aged" AChE enzymes cannot be reactivated with oximes.
  - High dose of oximes is often administered with enthusiasm to treat OPC poisoning but it should be noted that high dose oximes itself can cause muscle paralysis by AChE inhibition and worsen the situation.
  - As different OPCs cause "aging" of AChE at different time points, the outcome of oximes use also depends upon the type of OPC consumed.
  - Pralidoxime is given at a loading dose of 30 mg/kg followed by 10 mg/kg of continuous infusion.

1.6.2 Acute Carbamate Poisoning

- The management is the same as that of acute OPC poisoning except that oximes are not indicated in carbamate poisoning.
- This is because oximes have no role in carbamate poisoning (mechanism explained in Fig. 1.2) and oximes can also cause AChE inhibition.

1.6.3 Intermediate Syndrome in OPC Poisoning

- Intermediate syndrome occurs in approximately 20% of patients with acute OPC poisoning.
- Usually develops after 2–5 days of exposure to the OPC and patients present with neck, proximal muscles, and respiratory paralysis.
- Close monitoring for respiratory muscle paralysis and early ventilator support reduces mortality.

1.6.4 Delayed Neuropathy in OPC Poisoning

- OPC compounds with fluorine like isoflurophate, sarin, soman, and mipafox cause delayed neurotoxicity due to inhibition of specific esterase called "neurotoxic esterase."
- Severe polyneuropathy with symptoms like mild sensory abnormalities, ataxia, muscle weakness, reduced tendon reflexes is observed after weeks to months of exposure to OPC.
- The symptoms may persist even for years in some patients. The management is symptomatic with no proven efficacy.

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