

Chapter 7 | Cholinergic Transmission and Cholinergic Drugs

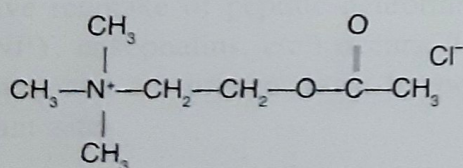
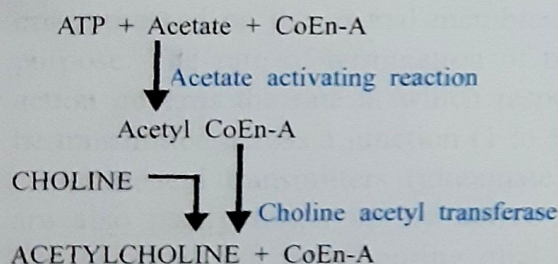
CHOLINERGIC TRANSMISSION

Acetylcholine (ACh) is a major neurohumoral transmitter at autonomic, somatic as well as central sites. These sites are listed in Table 7.1

Synthesis, storage and destruction of ACh

The cholinergic neuronal mechanisms are summarized in Fig. 7.1.

Acetylcholine is synthesized locally in the cholinergic nerve endings by the following pathway—



ACETYLCHOLINE CHLORIDE

Choline is actively taken up by the axonal membrane by a Na^+ : choline cotransporter (Na^+ChT) and acetylated with the help of ATP and coenzyme-A by the enzyme *choline acetyl transferase* (ChAT) present in the axoplasm. *Hemicholinium* (HC3) blocks choline uptake (the rate limiting step in ACh synthesis) and depletes ACh. Most of the ACh is stored within small clear synaptic vesicles which align mainly near the synaptic membrane. Active transport

of ACh into synaptic vesicles is effected by a vesicle associated transporter (VAT), which is blocked by *vesamicol*. The cholinergic axon also contains some large dense-core vesicles located relatively away from the synaptic membrane. These vesicles contain peptide cotransmitters, such as VIP.

Release of ACh from nerve terminals occurs in small quanta. The amount contained in individual vesicles is extruded in pulses by exocytosis. In response to a nerve AP synchronous release of multiple quanta triggers postjunctional events.

Two toxins interfere with cholinergic transmission by affecting release: *botulinum toxin* inhibits release, while *black widow spider toxin* induces massive release and depletion.

Botulinum toxin

Botulinum toxin A and B are highly potent exotoxins produced by *Clostridium botulinum* that are responsible for 'botulism' (a type of food poisoning). These neurotoxic proteins cause long-lasting loss of cholinergic transmission by interacting with axonal proteins involved in exocytotic release of ACh. Localized injection of minute quantity of botulinum toxin A (**BOTOX**) or its haemagglutinin complex (**DYSPORT**) can be used in the treatment of a number of spastic and other neurological conditions like blepharospasm, spastic cerebral palsy, strabismus, spasmodic torticollis, nystagmus, hemifacial spasm, post stroke spasticity, spasmodic dysphonia, axillary hyperhidrosis, etc. which are due to overactivity of cholinergic nerves. It is being employed popularly as beauty treatment for removal of age-related facial wrinkles. However, incorrect injection or overdose can be dangerous; ptosis, diplopia, facial swelling, dry mouth.

Table 7.1: Sites of cholinergic transmission and type of receptor involved

Site	Type of receptor	Selective agonist	Selective antagonist
1. a. All postganglionic parasymp. b. Few postganglionic symp. (sweat glands, some blood vessels)	Muscarinic	Muscarine	Atropine
2. a. Ganglia (both symp. and parasymp). b. Adrenal medulla	Nicotinic (N_N)	DMPP*	Hexamethonium
3. Skeletal muscles	Nicotinic (N_M)	PTMA**	d-tubocurarine
4. CNS (cortex, basal ganglia, spinal cord and other sites)	Muscarinic	Muscarine/ Oxotremorine	Atropine
	Nicotinic	Carbachol	d-tubocurarine

* DMPP—Dimethyl phenyl piperazinium

** PTMA—Phenyl trimethyl ammonium

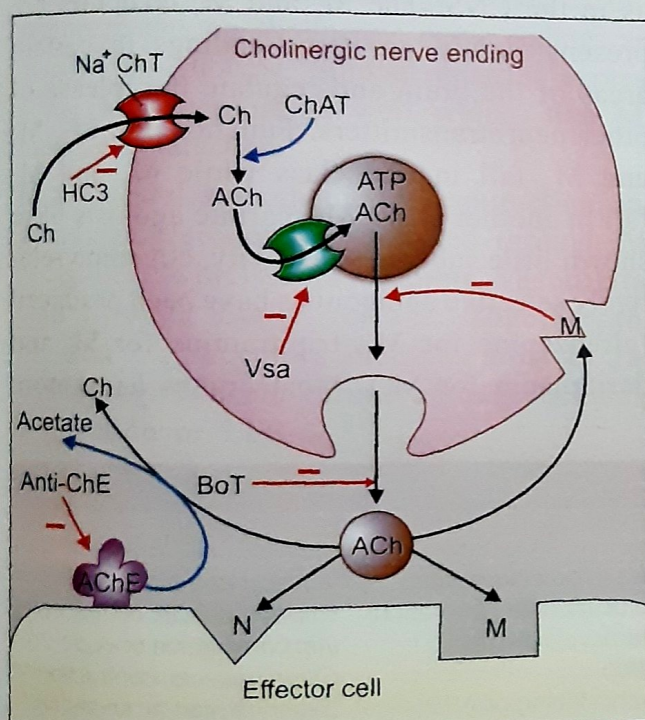
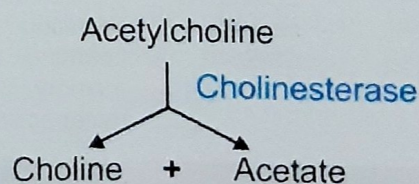


Fig. 7.1: Cholinergic neuronal mechanisms
Minus sign indicates inhibition, while bold arrow indicates active transport
Ch—Choline, ACh—Acetylcholine, ChAT—Choline acetyltransferase, AChE—Acetylcholinesterase, Anti-ChE—Anticholinesterase, M—Muscarinic receptor, N—Nicotinic receptor, HC3—Hemicholinium, BoT—Botulinum toxin, Vsa—Vesamicol, Na⁺ChT—Na⁺-Choline Cotransporter.

dysphagia, dysarthria, muscular weakness and even respiratory paralysis has occurred.

Cholinesterase Immediately after release, ACh is hydrolyzed by the enzyme cholinesterase and choline is recycled. A specific (*Acetylcholinesterase*—AChE or true cholinesterase) and a nonspecific (*Butyrylcholinesterase*—BuChE or pseudocholinesterase) type of enzyme occurs in the body; important differences between these two types of the enzyme are given in Table 7.2. While AChE is strategically located at all cholinergic sites and serves to inactivate ACh instantaneously, BuChE present in plasma and elsewhere probably serves to metabolize ingested esters.

**Table 7.2:** Differences between the two types of cholinesterases

	<i>Acetylcholinesterase (True)</i>	<i>Butyrylcholinesterase (Pseudo)</i>
1. Distribution	All cholinergic sites, RBC, gray matter	Plasma, liver, intestine, white matter
2. Hydrolysis		
ACh	Very fast (in μ s)	Slow
Methacholine	Slower than ACh	Not hydrolysed
Benzoylcholine	Not hydrolysed	Hydrolysed
Butyrylcholine	Not hydrolysed	Hydrolysed
3. Function	Termination of ACh action	Hydrolysis of ingested esters

Cholinoceptors

Two classes of receptors for ACh are recognised—*muscarinic* and *nicotinic*; the former is a G protein coupled receptor, while the latter is a ligand gated cation channel.

Muscarinic These receptors are selectively stimulated by muscarine and blocked by atropine. They are located primarily on autonomic effector cells in heart, blood vessels, eye, smooth muscles and glands of gastrointestinal, respiratory and urinary tracts, sweat glands, etc. and in the CNS. While autonomic ganglia primarily function through nicotinic (N_N) receptors, subsidiary muscarinic receptors are also present, which appear to play a modulatory role by inducing a long-lasting late EPSP.

Muscarinic autoreceptors are present prejunctionally on postganglionic cholinergic nerve endings. Activation of these receptors inhibits further ACh release. Similar ones have been demonstrated on adrenergic terminals: their activation inhibits NA release (may contribute to vasodilator action of injected ACh). All blood vessels have muscarinic recep-

tors (though most of them lack cholinergic innervation) located on endothelial cells whose activation releases EDRF (nitric oxide) which diffuses to the smooth muscle to cause relaxation.

Subtypes of muscarinic receptor By pharmacological as well as molecular cloning techniques, muscarinic receptors have been divided into 5 subtypes M_1 , M_2 , M_3 , M_4 and M_5 . The first 3 are the major subtypes (Table 7.3) that are present on effector cells as well as on prejunctional nerve endings, and are expressed both in peripheral organs as well as in the CNS. The M_4 and M_5 receptors are present mainly on nerve endings in certain areas of the brain and regulate the release of other neurotransmitters. Functionally, M_1 , M_3 and M_5 fall in one class while M_2 and M_4 fall in another class. Muscarinic agonists have shown little subtype selectivity, but some relatively selective antagonists have been produced (pirenzepine for M_1 , triptiramine for M_2 and darifenacin for M_3). Most organs have more

Table 7.3: Characteristics of important subtypes of muscarinic receptor

	M_1	M_2	M_3	
1. Location and function subserved	<i>Autonomic ganglia:</i> <i>Gastric glands:</i> <i>Enteric neurones</i> <i>CNS:</i>	Depolarization (late EPSP) Hist. release, acid secretion ↑ intestinal secretion Learning, memory, motor activity	SA node: Hyperpolarization, ↓ rate of impulse generation AV node: ↓ velocity of conduction Atrium: shortening of APD, ↓ contractility Ventricle: ↓ contractility (slight) (receptors sparse) Cholinergic nerve endings: ↓ ACh release CNS: tremor, analgesia Visceral smooth muscle: contraction	<i>Visceral and bronchial smooth muscle:</i> contraction <i>Iris:</i> constriction of pupil <i>Ciliary muscle:</i> contraction <i>Exocrine glands:</i> secretion <i>Vascular endothelium:</i> release of NO → vasodilatation
2. Nature	Gq-protein coupled	Gi/Go-protein coupled	Gq-protein coupled	
3. Transducer mechanism	$IP_3/DAG \rightarrow \uparrow$ cytosolic Ca^{2+} , $PLA_2 \uparrow \rightarrow$ PG synthesis	K^+ channel opening, ↓ cAMP	$IP_3/DAG \rightarrow \uparrow$ cytosolic Ca^{2+} , $PLA_2 \uparrow \rightarrow$ PG synthesis	
4. Agonists*	MCN-343A, Oxotremorine	Methacholine	Bethanechol	
5. Antagonists*	Pirenzepine, Telenzepine	Methoctramine, Triptiramine	Solifenacin, Darifenacin	

*Relatively selective

- ACh activates and atropine blocks all 3 subtypes of muscarinic receptors.
- The CNS contains all subtypes of muscarinic receptors, but M_1 appear to predominate.
- Most smooth muscles and glands have both M_2 and M_3 subtypes; M_3 predominates.

than one subtype, but usually one subtype predominates in a given tissue.

M₁: The M₁ is primarily a neuronal receptor located on ganglion cells, enteric and central neurones, especially in cortex, hippocampus and corpus striatum. It plays a major role in mediating gastric secretion, relaxation of lower esophageal sphincter (LES) caused by vagal stimulation, intestinal secretion and in learning, memory, motor functions, etc.

M₂: Cardiac muscarinic receptors are predominantly M₂ and mediate vagal bradycardia. Autoreceptors on cholinergic nerve endings are also of M₂ subtype. Smooth muscles express some M₂ receptors as well which, like M₃, mediate contraction.

M₃: Visceral smooth muscle contraction and glandular secretions are elicited through M₃ receptors, which also mediate vasodilatation through EDRF release. Together the M₂ and M₃ receptors mediate most of the well-recognized muscarinic actions including contraction of LES.

The muscarinic receptors are G-protein coupled receptors having the characteristic 7 membrane traversing amino acid sequences. The M₁ and M₃ (also M₅) subtypes function through G_q protein (see Fig. 4.7) and activate membrane bound phospholipase C (PLC)—generating inositol trisphosphate (IP₃) and diacylglycerol (DAG) which in turn release Ca²⁺ intracellularly—cause depolarization, glandular secretion, raise smooth muscle tone and release NO (from endothelium). They also activate phospholipase A₂ resulting in enhanced synthesis and release of prostaglandins and leucotrienes in certain tissues. The M₂ (and M₄) receptor opens

K⁺ channels (through $\beta\gamma$ subunits of regulatory protein Gi) and inhibits adenylyl cyclase (through α subunit of Gi, as depicted in Fig. 4.6), resulting in hyperpolarization, reduced pacemaker activity, slowing of conduction and decreased force of contraction in the heart. The M₄ receptor has been implicated in facilitation/inhibition of transmitter release in certain areas of the brain, while M₅ has been found to dilate cerebral arterioles, facilitate dopamine release and mediate reward behaviour.

Nicotinic These receptors are selectively activated by nicotine and blocked by tubocurarine or hexamethonium. They are rosette-like pentameric structures (see Fig. 4.4) which enclose a ligand gated cation channel: their activation causes opening of the channel and rapid flow of cations resulting in depolarization and an action potential. On the basis of location and selective agonists and antagonists, two subtypes N_M and N_N (previously labelled N₁ and N₂) are recognized (Table 7.4).

N_M: These are present at skeletal muscle endplate: are selectively stimulated by phenyl trimethyl ammonium (PTMA) and blocked by tubocurarine. They mediate skeletal muscle contraction.

N_N: These are present on ganglionic cells (sympathetic as well as parasympathetic), adrenal medullary cells (embryologically derived from the same site as ganglionic cells), in spinal cord and certain areas of brain. They are selectively stimulated by dimethyl phenyl piperazinium (DMPP), blocked by hexamethonium, and constitute the primary pathway of transmission in ganglia.

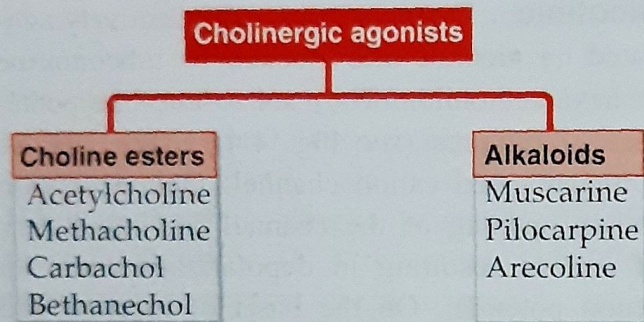
Table 7.4: Characteristics of subtypes of nicotinic receptor

	N _M	N _N
1. Location and function subserved	Neuromuscular junction: depolarization of muscle end plate —contraction of skeletal muscle	Autonomic ganglia: depolarization —postganglionic impulse Adrenal medulla: catecholamine release CNS: site specific excitation or inhibition
2. Nature	Has intrinsic ion channel, pentamer of $\alpha 2$ β ϵ or γ and δ subunits, each subunit has 4 TM segments	Has intrinsic ion channel, pentamer of only α or α, β subunits, each subunit has 4 TM segments
3. Transducer mechanism	Opening of cation (Na ⁺ , K ⁺) channels	Opening of cation (Na ⁺ , K ⁺ , Ca ²⁺) channels
4. Agonists	PTMA, Nicotine	DMPP, Nicotine
5. Antagonists	Tubocurarine, α -Bungarotoxin	Hexamethonium, Trimethaphan

CHOLINERGIC DRUGS

(Cholinomimetic, Parasympathomimetic)

These are drugs which produce actions similar to that of ACh, either by directly interacting with cholinergic receptors (cholinergic agonists) or by increasing availability of ACh at these sites (*anticholinesterases*).



ACTIONS (of ACh as Prototype)

Depending on the type of receptor through which it is mediated, the peripheral actions of ACh are classified as muscarinic or nicotinic. The central actions are not so classifiable and are described separately.

A. Muscarinic actions

1. Heart ACh hyperpolarizes the SA nodal cells and decreases their rate of diastolic depolarization. As a result, rate of impulse generation is reduced—*bradycardia* or even cardiac arrest may occur.

At the A-V node and His-Purkinje fibres refractory period (RP) is increased and *conduction is slowed*: P-R interval increases and partial to complete A-V block may be produced. The *force of atrial contraction is markedly reduced* and RP of atrial fibres is abbreviated. Due to nonuniform vagal innervation, the intensity of effect on RP and conduction of different atrial fibres varies—inducing inhomogeneity and predisposing to atrial fibrillation or flutter.

Ventricular contractility is also decreased but the effect is not marked. The cardiac muscarinic receptors are of the M_2 subtype.

2. Blood vessels All blood vessels are dilated, though only few (skin of face, neck, salivary glands) receive cholinergic innervation. Fall in

BP and flushing, especially in the blush area occurs. Muscarinic (M_3) receptors are present on vascular endothelial cells: vasodilatation is primarily mediated through the release of an *endothelium dependent relaxing factor* (EDRF) which is nitric oxide (NO). The $PLA_2/PP_3/DAG$ pathway activates endothelial NO synthase through the Ca^{2+} -Calmodulin mechanism. When the endothelium is damaged by disease, ACh can diffuse to the vascular smooth muscle and cause vasoconstriction *via* M_3 receptors located on their plasma membrane.

Stimulation of cholinergic nerves to the penis causes erection by releasing NO and dilating cavernosal vessels through M_3 receptors. However, this response is minimal with injected cholinomimetic drugs.

3. Smooth muscle Smooth muscle in most organs is contracted (mainly through M_3 receptors). Tone and peristalsis in the gastrointestinal tract is increased and sphincters relax → abdominal cramps and evacuation of bowel.

Peristalsis in ureter is increased. The detrusor muscle contracts while the bladder trigone and sphincter relaxes → voiding of bladder.

Bronchial muscles constrict, asthmatics are highly sensitive → bronchospasm, dyspnoea, precipitation of an attack of bronchial asthma.

4. Glands Secretion from all parasympathetically innervated glands is increased *via* M_3 and some M_2 receptors: sweating, salivation, lacrimation, increased tracheobronchial and gastric secretion. The effect on pancreatic and intestinal glands is not marked. Secretion of milk and bile is not affected.

5. Eye Contraction of circular muscle of iris resulting in miosis.

Contraction of ciliary muscle causing spasm of accommodation, increased aqueous outflow facility, reduction in intraocular tension (especially in glaucomatous patients).

B. Nicotinic actions

1. Autonomic ganglia Both sympathetic and parasympathetic ganglia are stimulated. This

effect is manifested at higher doses. High dose of ACh given after atropine causes tachycardia and rise in BP due to stimulation of sympathetic ganglia and release of catecholamines.

2. Skeletal muscles Ionophoretic application of ACh to muscle endplate causes contraction of the fibre. Intraarterial injection of high dose can cause twitching and fasciculations, but i.v. injection is generally without any effect (due to rapid hydrolysis of ACh).

C. CNS actions

ACh injected i.v. does not penetrate blood-brain barrier and no central effects are seen. However, direct injection into the brain produces arousal response followed by depression. Cholinergic drugs which enter brain produce complex behavioral and neurological effects.

The important features of other choline esters are summarised in Table 7.5.

Interactions Anticholinesterases potentiate ACh markedly, methacholine to less extent and have only additive action with carbachol or bethanechol, depending upon the role of ChE in the termination of action of the particular choline ester. Atropine and its congeners competitively antagonize muscarinic actions.

Adrenaline is a physiological antagonist.

Uses Choline esters are rarely, if ever, used clinically. ACh is not used because of evanescent and nonselective action.

Bethanechol It has been used in postoperative/postpartum nonobstructive urinary retention, neurogenic bladder to promote urination. It can afford symptomatic relief in congenital megacolon and gastroesophageal reflux, but is rarely used for these. Side effects are prominent: belching, colic, involuntary urination/defecation, flushing, sweating, fall in BP, bronchospasm.

Dose: 10–40 mg oral, 2.5–5 mg s.c.;
UROTONIN, BETHACOL 25 mg tab.

CHOLINOMIMETIC ALKALOIDS

Pilocarpine It is obtained from the leaves of *Pilocarpus microphyllus* and other species. It has prominent muscarinic actions and also stimulates ganglia.

Pilocarpine causes marked sweating, salivation and increase in other secretions. The cardiovascular effects are complex. Small doses generally cause fall in BP (muscarinic), but higher doses elicit rise in BP and tachycardia which is probably due to ganglionic stimulation (through ganglionic muscarinic receptors). Applied to the eye, it penetrates cornea and promptly causes miosis, ciliary muscle contraction and fall in intraocular tension lasting 4–8 hours.

Pilocarpine is used only in the eye as 0.5–4% drops. It is a third-line drug in open angle glaucoma. An initial stinging sensation in the eye and painful spasm of accommodation are frequent side effects. Other uses as a miotic are—to counteract mydriatics after they have been used for testing refraction and to prevent/break adhesions of iris with lens or cornea by alternating it with mydriatics.

Though it can be used as a sialogogue, no oral preparation is available.

PILOCAR 1%, 2%, 4% eye drops, CARPINE 0.5% eye drops, PILODROPS 2% eye drops.

Muscarine It occurs in poisonous mushrooms *Amanita muscaria* and *Inocybe* species and has only muscarinic actions. It is not used therapeutically but is of toxicological importance.

Mushroom poisoning Depending on the toxic principle present in the particular species, at least 3 types of mushroom poisoning is known.

Table 7.5: Properties of choline esters

Choline ester	Hydrolysis by		Actions		Selective action on
	ACh E	BuChE	Musc.	Nico.	
Acetylcholine	+++	++	++	+	Non-selective
Methacholine	+	–	++	±	CVS
Carbachol	–	–	+	++	g.i.t., bladder
Bethanechol	–	–	+	–	g.i.t., bladder

Muscarine type (Early mushroom poisoning) due to *Inocybe* and related species. Symptoms characteristic of muscarinic actions appear within an hour of eating the mushroom, and are promptly reversed by atropine.

Hallucinogenic type It is due to muscimol and other isoxazole compounds which are present in *A. muscaria* and related mushrooms in much larger quantities than is muscarine. These compounds activate amino acid receptors, and block muscarinic receptors in the brain; have hallucinogenic property. Manifestations of poisoning are primarily central. There is no specific treatment and atropine is contraindicated. Another hallucinogenic mushroom is *Psilocybe mexicana* whose active principle psilocybine is a tryptaminergic (5-HT related) compound.

Phalloidin type (Late mushroom poisoning) It is due to peptide toxins found in *A. phalloides*, *Galerina* and related species. These inhibit RNA and protein synthesis. The symptoms start after many hours and are due to damage to the gastrointestinal mucosa, liver and kidney. Treatment consists of supportive measures. Thiocetic acid may have some antidotal effect.

Arecoline It is found in betel nut *Areca catechu* and has muscarinic as well as nicotinic actions, including those on skeletal muscle endplate. It also has prominent CNS effect: has been tried in dementia as an enhancer of cognitive functions, but not found useful—has no therapeutic use.

ANTICHOLINESTERASES

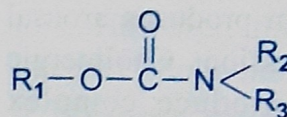
Anticholinesterases (anti-ChEs) are agents which inhibit ChE, protect ACh from hydrolysis—

produce cholinergic effects *in vivo* and potentiate ACh both *in vivo* and *in vitro*. Quaternary ammonium anti-ChEs like neostigmine have additional direct action on nicotinic cholinergic receptors.

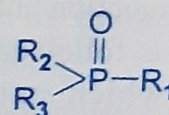
CHEMISTRY

Anti-ChEs are mostly esters of carbamic acid or derivatives of phosphoric acid. Some other anti-ChEs like edrophonium, tacrine, donepezil and galantamine have carbamate like action but are chemically non-carbamates.

The generic formula of carbamates and organophosphates is shown below:

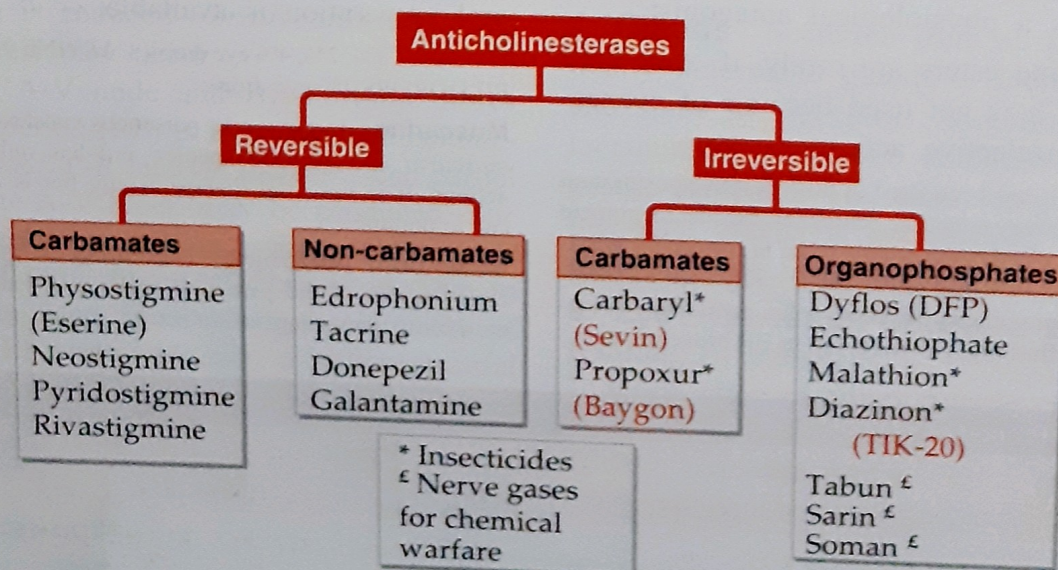


CARBAMATES



ORGANOPHOSPHATES

The R_1 in carbamates may have a nonpolar tertiary amino N, e.g. in physostigmine, rendering the compound lipid soluble. In others, e.g. neostigmine, R_1 has a quaternary N^+ —rendering it lipid insoluble. All organophosphates are highly lipid soluble except echothiophate which is water soluble.



MECHANISM OF ACTION

The anti-ChEs react with the enzyme essentially in the same way as ACh. The carbamates and phosphates respectively carbamylate and phosphorylate the esteratic site of the enzyme.

The mammalian AChE has been cloned and details of its structure as well as mode of interaction with ACh and various anti-ChEs has been worked out.

The active region of AChE forms a gorge which contains an *anionic site* (near glutamate 334) and an *esteratic site* formed by serine 203, and histidine 447 (Fig. 7.2A). Hydrolysis of ACh involves electrostatic attraction of positively charged N^+ of ACh to the anionic site (Fig. 7.2B) and nucleophilic attack by serine-OH which is activated by the adjacent histidine leading to acetylation of serine (Fig. 7.2C). The acetylated enzyme reacts with water to produce acetic acid and choline (Fig. 7.2D).

Whereas the acetylated enzyme reacts with water extremely rapidly and the esteratic site is freed in a fraction of a millisecond, the carbamylated enzyme (reversible inhibitors) reacts slowly (Fig. 7.2E, F) and the phosphorylated enzyme (irreversible inhibitors) reacts extremely slowly or not at all (Fig. 7.2G). It is noteworthy that edrophonium and tacrine attach only to the anionic site and do not form covalent bonds with the enzyme, while organophosphates attach only to the esteratic site forming strong covalent bonds. Reactivation of edrophonium-inhibited enzyme occurs in < 10 min, because the interaction is by formation of weak hydrogen bonds, and does not involve hydrolysis of the inhibitor, but only its diffusion; therefore action is brief. The half-life of reactivation of carbamylated enzyme (1/2 to 6 hours) is less than that of synthesis of fresh enzyme protein, while that of phosphorylated enzyme (in days) is more than the regeneration time. The phosphorylated enzyme may also undergo 'aging' by the loss of one of the alkyl groups and become totally resistant to hydrolysis. Thus, apparently reversible and irreversible enzyme inhibition is obtained, though the basic pattern of inhibitor-enzyme interaction remains the same.

PHARMACOLOGICAL ACTIONS

The actions of anti-ChEs are due to amplification of endogenous ACh. As such they are qualitatively

similar to those of directly acting cholinceptor stimulants. However, relative intensity of action on muscarinic, ganglionic, skeletal muscle and CNS sites varies among the different agents.

Lipid-soluble agents (physostigmine and organophosphates) have more marked muscarinic and CNS effects; stimulate ganglia but action on skeletal muscles is less prominent.

Lipid-insoluble agents (neostigmine and other quaternary ammonium compounds) produce more marked effect on the skeletal muscles (they have direct action on muscle endplate N_M receptors as well), stimulate ganglia, but muscarinic effects are less prominent. They do not penetrate CNS and have no central effects.

Ganglia Local hydrolysis of ACh is less important in ganglia: inactivation occurs partly by diffusion and hydrolysis in plasma. Anti-ChEs stimulate ganglia primarily through muscarinic receptors present there. High doses cause persistent depolarization of the ganglionic nicotinic receptors and blockade of transmission.

CVS Cardiovascular effects of anti-ChEs are complex. Whereas muscarinic action would produce bradycardia and hypotension (fall in BP is mainly due to bradycardia), ganglionic stimulation would tend to increase heart rate and BP. Action on medullary centres (stimulation followed by depression) further complicates the picture, so does ganglionic blockade with high doses. Thus, the overall effects are often unpredictable and depend on the agent and its dose. Bradycardia and a small rise in BP are common with moderate doses of many anti-ChEs.

Skeletal muscles After treatment with anti-ChEs, the ACh released by a single nerve impulse is not immediately destroyed—rebinds to the same receptor, diffuses to act on neighbouring receptors and causes retrograde activation of prejunctional fibres. The resulting repetitive firing produces twitching and fasciculations. Force of contraction in partially curarized and myasthenic muscles is increased, but muscle twitching is not prominent. Higher doses cause

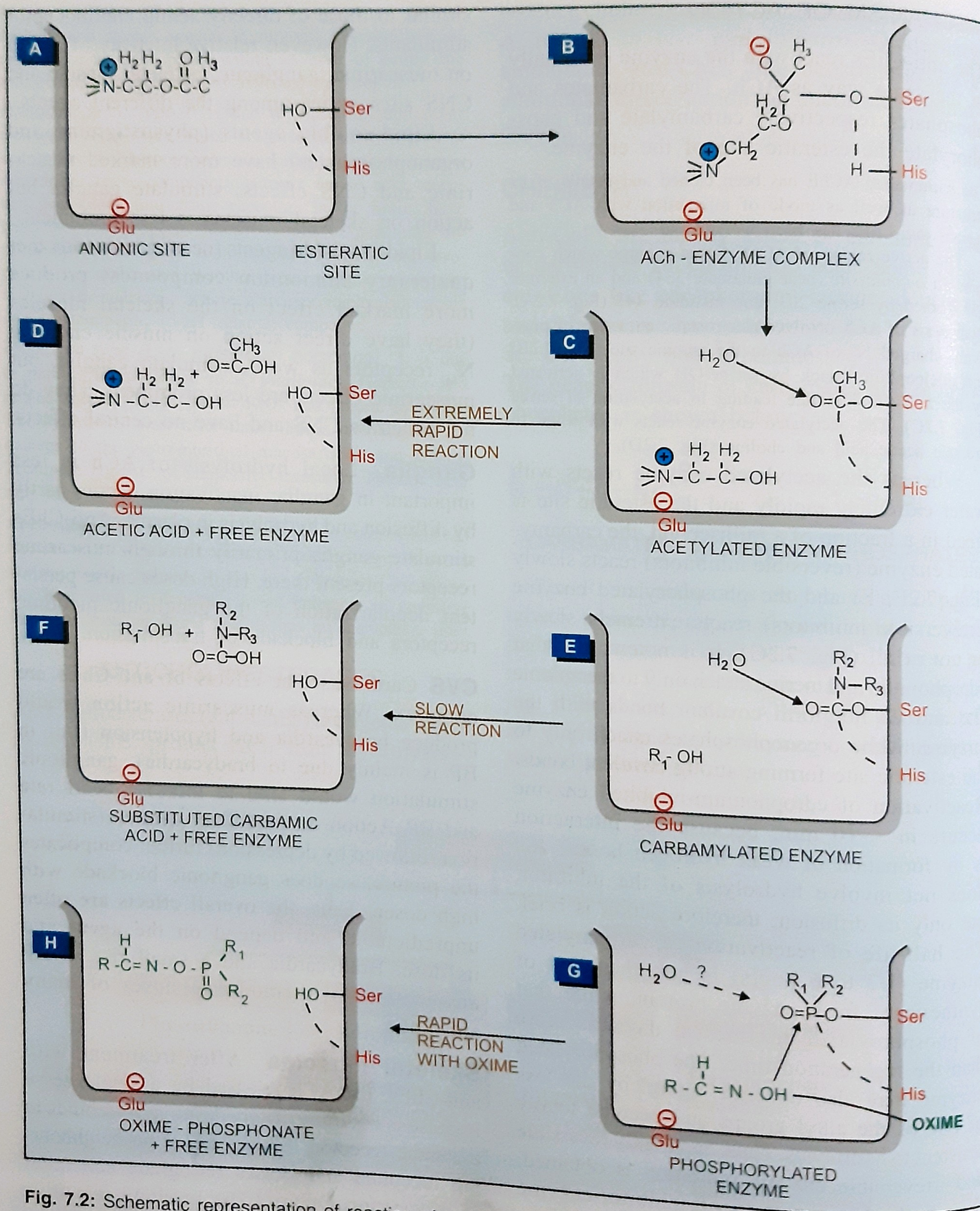


Fig. 7.2: Schematic representation of reaction of acetylcholine (A-D), or carbamate anticholinesterase (E, F), or organophosphate anticholinesterase (G) with cholinesterase enzyme; and reactivation of phosphorylated enzyme by oxime (G, H)
 Ser—Serine; His—Histidine; Glu—Glutamic acid.

persistent depolarization of endplates resulting in blockade of neuromuscular transmission giving rise to weakness and paralysis. Direct action of neostigmine and its congeners at the muscle endplates results in augmentation of these features.

CNS Lipophilic anti-ChEs which penetrate into brain produce a generalised alerting response. Cognitive function may be improved in Alzheimer's disease. However, higher doses produce excitement, mental confusion, disorientation, tremors and convulsions followed by coma.

Other effects These result from stimulation of smooth muscles and glands of the gastrointestinal, respiratory, urinary tracts and in the eye as described for ACh: viz. enhanced g.i. motility and secretion, bronchoconstriction and increased airway secretion, voiding of bladder due to detrusor muscle contraction with trigone and sphincter relaxation, miosis and ciliary muscle contraction → lowering of intraocular tension in glaucoma.

PHARMACOKINETICS

Physostigmine It is rapidly absorbed from g.i.t. and parenteral sites. Applied to the eye, it penetrates cornea freely. It crosses blood-brain barrier and is disposed after hydrolysis by ChE.

Neostigmine and congeners These are poorly absorbed orally; oral dose is 20–30 times higher than parenteral dose. They do not effectively penetrate cornea or cross blood-brain barrier. They are partially hydrolysed and partially excreted unchanged in urine.

Organophosphates These are absorbed from all sites including intact skin and lungs. They are hydrolysed as well as oxidised in the body and little is excreted unchanged.

INDIVIDUAL COMPOUNDS

The important features of physostigmine and neostigmine are presented in Table 7.6.

Physostigmine eye drops are usually prepared freshly by ophthalmology departments.

BI-MIOTIC 0.25% eye drops with 2% pilocarpine nitrate.

Neostigmine PROSTIGMIN, MYOSTIGMIN, TILSTIGMIN 15 mg tab, 0.5 mg/ml in 1 ml and 5 ml inj.

Pyridostigmine Resembles neostigmine in all respects but is dose to dose less potent and longer acting, less frequent dosing is required in myasthenia gravis.

DISTINON, MYESTIN 60 mg tab; 1–3 tab TDS.

Ambenonium is another longacting congener used in myasthenia.

Edrophonium Resembles neostigmine in action, has a brief duration (10–30 min), suitable only as a diagnostic agent for myasthenia gravis.

Dose: 2–10 mg i.v.

Tacrine It is a lipophilic acridine compound which interacts with ChE in a manner analogous to edrophonium. It crosses blood-brain barrier and has a longer duration of action. By increasing brain ACh levels it was found to produce some symptomatic improvement in Alzheimer's disease, but has gone into disuse due to hepatotoxicity (see Ch. 35).

Rivastigmine This lipophilic relatively cerebroselective ChE inhibitor has been introduced for Alzheimer's disease (AD), see Ch. 35.

Donepezil Another centrally acting anti-AChE that has produced cognitive and behavioural improvement in AD. It is long-acting and suitable for once daily administration (see Ch. 35).

Galantamine This natural alkaloid inhibitor of cerebral AChE has in addition weak agonistic action on nicotinic receptors. It is being used to afford symptomatic relief in AD (see Ch. 35).

Dyflus It is Diisopropyl-fluoro-phosphate (DFP), a very potent and long-acting anti-ChE. It is now obsolete as a miotic.

Echothiophate It is an organophosphate with quaternary structure. It is water soluble; and was used as a long acting miotic.

Precautions Anti-ChEs are contraindicated in sick sinus, A-V conduction defects and hypotensive states. They are to be used cautiously in peptic ulcer, hypertension, asthma, COPD and seizure patients.

USES

1. As miotic

(a) In glaucoma: Miotics increase the tone of ciliary muscle (attached to scleral spur) as well as that of sphincter pupillae which pull on and somehow improve alignment of the trabeculae

Table 7.6: Comparative features of physostigmine and neostigmine

	<i>Physostigmine</i>	<i>Neostigmine</i>
1. Source	Natural alkaloid from <i>Physostigma venenosum</i> (Calabar bean)	Synthetic
2. Chemistry	Tertiary amine derivative	Quaternary ammonium compound
3. Oral absorption	Good	Poor
4. CNS actions	Present	Absent
5. Applied to eye	Penetrates cornea	Poor penetration
6. Direct action on N_M cholinceptors	Absent	Present
7. Prominent effect on	Autonomic effectors	Skeletal muscles
8. Important use	Miotic (glaucoma)	Myasthenia gravis
9. Dose	0.1–1.0% eye drops	0.5–2.5 mg i.m./s.c. 15–30 mg orally
10. Duration of action	Systemic 4–6 hrs In eye 6 to 24 hrs	3–4 hrs.

so that outflow facility is increased. As a result i.o.t. falls in open angle glaucoma.

Pilocarpine is the preferred miotic. The action is rapid and short lasting (4–6 hr); 6–8 hourly instillation is required and even then i.o.t. may fluctuate inbetween. Diminution of vision, especially in dim light (due to constricted pupil), spasm of accommodation and brow pain are frequent side effects. Systemic effects—nausea, diarrhoea, sweating and bronchospasm may occur with higher concentration eye drops.

Physostigmine (0.1%) is used only to supplement pilocarpine. Miotics are now 3rd choice drugs, used only as add on therapy in advanced cases. However, they are effective in aphakic glaucoma. Pilocarpine (along with other drugs) is used in angle closure glaucoma as well.

(b) To reverse the effect of mydriatics after refraction testing.

(c) To prevent formation of adhesions between iris and lens or iris and cornea, and even to break those which have formed due to iritis, corneal ulcer, etc.—a miotic is alternated with a mydriatic.

2. Myasthenia gravis

Myasthenia gravis is an autoimmune disorder affecting about 1 in 10,000 population, due to development of antibodies directed to the

nicotinic receptors (NR) at the muscle endplate. The number of free N_M cholinceptors may be reduced to 1/3 of normal or less (Fig. 7.3) and structural damage to the neuromuscular junction. This results in weakness and easy fatigability on repeated activity, with recovery after rest. The eyelid, external ocular, facial and pharyngeal muscles are generally involved first. Later, limb and respiratory muscles get affected. Neostigmine and its congeners are the first line drugs used to restore muscle strength. They improve muscle contraction by allowing ACh released from prejunctional endings to accumulate and act on the receptors over a larger area, as well as by directly depolarizing the endplate.

Treatment is usually started with *neostigmine* 15 mg orally 6 hourly; dose and frequency is then adjusted to obtain optimum relief from weakness. However, the dosage requirement may fluctuate from time to time and there are often unpredictable periods of remission and exacerbation. Pyridostigmine is an alternative which needs less frequent dosing. If intolerable muscarinic side effects are produced, atropine can be added to block them. These drugs have no effect on the basic disorder which often progresses; ultimately it may not be possible

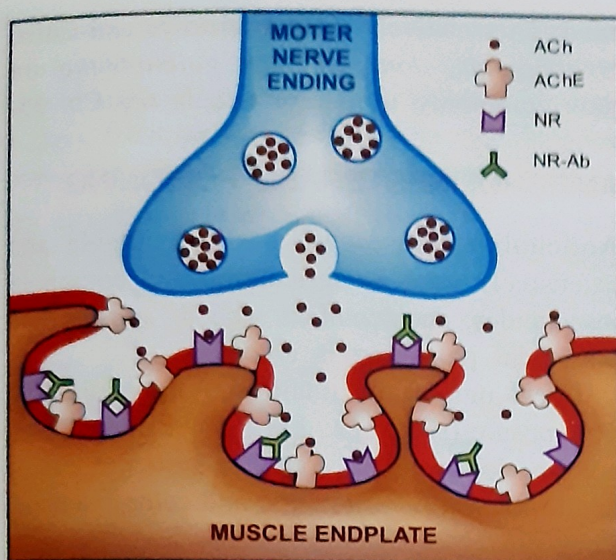


Fig. 7.3: Neuromuscular junction of myasthenic muscle. In myasthenia gravis the population of nicotinic receptors (NR) available at muscle endplate for binding acetylcholine (ACh) is markedly reduced due to their obliteration by nicotinic receptor antibodies (NR-Ab). Acetylcholinesterase (AChE) molecules located strategically at the muscle endplate rapidly hydrolyse ACh. Anticholinesterases inhibit AChE, allowing the same ACh molecules to repeatedly interact with the available NRs; frequency of ACh-NR interaction is increased.

to restore muscle strength adequately with anti-ChEs alone.

Thymectomy is now generally advised for patients with generalised weakness, particularly for those with a thymoma and for younger patients. It is effective in majority of the cases. Thymectomy produces gradual improvement, and even complete remission has been obtained. Thymus may contain modified muscle cells with NRs on their surface, which may be the source of the antigen for production of anti-NR antibodies in myasthenic patients.

Corticosteroids afford improvement in myasthenia gravis by their immunosuppressant action. They inhibit production of antibodies to NR, and may increase synthesis of new NRs. However, they are generally reserved for patients who do not respond adequately to anti-ChEs and have failed after thymectomy or are unsuitable for it. Prednisolone 30–60 mg/day induces remission in about 80% of advanced cases. After tapering the dose, 10 mg daily or

on alternate days can be used for maintenance therapy. However, long term use of corticosteroids has problems of its own (see Ch. 20).

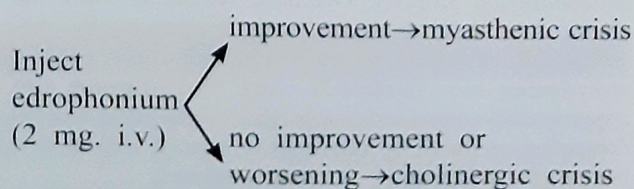
Immunosuppressants like azathioprine and cyclosporine are also beneficial in advanced cases. Both inhibit NR-antibody synthesis by affecting T-cells. Azathioprine is generally used first but has a slow onset of effect; may take months to induce a remission. However, it is good for maintaining remission.

Removal of antibodies by *plasmapheresis* (plasma exchange) is another approach. It may produce dramatic but often short-lived improvement in myasthenic crisis.

Myasthenic crisis is characterized by acute weakness of respiratory muscles. It is managed by tracheal intubation and mechanical ventilation. Generally, i.v. methylprednisolone pulse therapy is given while anti-ChEs are withheld for 2–3 days followed by their gradual reintroduction. Most patients can be weaned off the ventilator in 1–3 weeks. Plasmapheresis hastens recovery.

Overtreatment with anti-ChEs If the dose of the antiChE is not adjusted according to the fluctuating requirement, relative overdose may occur from time-to-time. Overdose also produces weakness by causing persistent depolarization of muscle endplate, and is called *cholinergic weakness*. Late cases with high anti-ChE dose requirements often alternately experience myasthenic and cholinergic weakness and these may assume crisis proportions.

The two types of weakness require opposite treatments. They can be differentiated by *edrophonium test*—



Diagnostic tests for myasthenia gravis

(a) **Ameliorative test:** Initially edrophonium 2 mg is injected i.v. as a test dose. If nothing untoward happens, the remaining 8 mg is injected after 30–60 sec. Reversal of weakness

and short-lasting improvement in the strength of affected muscles occurs only in myasthenia gravis and not in other muscular dystrophies.

In case edrophonium is not available, the test can be performed with 1.5 mg i.v. neostigmine. Atropine pretreatment may be given to block the muscarinic effects of neostigmine.

(b) **Provocative test:** myasthenics are highly sensitive to d-tubocurarine; 0.5 mg i.v. causes marked weakness in them but is ineffective in non-myasthenics. This test is hazardous.

(c) Demonstration of anti-NR antibodies in plasma or muscle biopsy specimen is a more reliable test.

3. Postoperative paralytic ileus/urinary retention This may be relieved by 0.5–1 mg s.c. neostigmine, provided no organic obstruction is present; but is seldom employed.

4. Postoperative decurarization Neostigmine 0.5–2.0 mg (30–50 µg/kg) i.v., preceded by atropine or glycopyrrolate 10 µg/kg to block muscarinic effects, rapidly reverses muscle paralysis induced by competitive neuromuscular blockers.

5. Cobra bite Cobra venom has a curare like neurotoxin. Though specific antivenom serum is the primary treatment, neostigmine + atropine prevent respiratory paralysis.

6. Belladonna poisoning Physostigmine 0.5–2 mg i.v. repeated as required is the specific antidote for poisoning with belladonna or other anticholinergics. It penetrates blood-brain barrier and antagonizes both central and peripheral actions. However, physostigmine often itself induces hypotension, arrhythmias and undesirable central effects. It is therefore employed only as a last resort. Neostigmine does not block the central effect, but is less risky.

7. Other drug overdosages Tricyclic antidepressants, phenothiazines and many antihistaminics have additional anticholinergic property. Overdose symptoms and coma produced by these drugs are partly antagonized by physostigmine. However, it may worsen the fall in BP and arrhythmias; use therefore is risky. Physostigmine also appears to have a modest nonspecific arousal effect in CNS depression produced by diazepam or general anaesthetics, but use for this purpose is rarely warranted.

8. Alzheimer's disease (AD) Characterized by progressive dementia, AD is a neurodegenerative disorder, primarily affecting cholinergic neurones in the brain. Various measures to augment cholinergic transmission in the brain have been

tried. The relatively cerebroselective anti-ChEs, *rivastigmine*, *donepezil* and *galantamine* are now commonly used. For details see Ch. 35.

ANTICHOLINESTERASE POISONING

Anticholinesterases are easily available and extensively used as agricultural and household insecticides; accidental as well as suicidal and homicidal poisoning is common.

Local muscarinic manifestations at the site of exposure (skin, eye, g.i.t.) occur immediately and are followed by complex systemic effects due to muscarinic, nicotinic and central actions. They are—

- Irritation of eye, lacrimation, salivation, sweating, copious tracheo-bronchial secretions, miosis, blurring of vision, bronchospasm, breathlessness, colic, involuntary defecation and urination.
- Fall in BP, bradycardia or tachycardia, cardiac arrhythmias, vascular collapse.
- Muscular fasciculations, weakness, respiratory paralysis (central as well as peripheral).
- Irritability, disorientation, unsteadiness, tremor, ataxia, convulsions, coma and death.
- Death is generally due to respiratory failure.

Treatment

1. Termination of further exposure to the poison—fresh air, wash the skin and mucous membranes with soap and water, gastric lavage according to need.
2. Maintain patent airway, positive pressure respiration if it is failing.
3. Supportive measures—maintain BP, hydration, control of convulsions with judicious use of diazepam.
4. Specific antidotes—

(a) **Atropine** It is highly effective in counteracting the muscarinic symptoms, but higher doses are required to antagonize the central effects. It does not reverse peripheral muscular paralysis which is a nicotinic action. All cases of anti-ChE (carbamate or organophosphate) poisoning must be promptly given atropine

2 mg i.v. repeated every 10 min till dryness of mouth or other signs of atropinization appear (upto 200 mg has been administered in a day). Continued treatment with maintenance doses may be required for 1–2 weeks.

(b) Cholinesterase reactivators Oximes are used to restore neuromuscular transmission only in case of organophosphate anti-ChE poisoning. The phosphorylated ChE reacts very slowly or not at all with water. However, if more reactive OH groups in the form of oximes (generic formula $R-CH=N-OH$) are provided, reactivation occurs more than a million times faster (see Fig. 7.2G and H).

Pralidoxime (2-PAM) has a positively charged quaternary nitrogen: attaches to the anionic site of the enzyme which remains unoccupied in the presence of organophosphate inhibitors. Its oxime end reacts with the phosphorus atom attached to the esteratic site: the oxime-phosphonate so formed diffuses away leaving the reactivated ChE. Pralidoxime is ineffective as an antidote to carbamate anti-ChEs (physostigmine, neostigmine, carbaryl, propoxur) in which case the anionic site of the enzyme is not free to provide attachment to it. It is rather contraindicated in carbamate poisoning, because not only it does not reactivate carbamylated enzyme, it has weak anti-ChE activity of its own.

Pralidoxime (NEOPAM, PAM-A INJ. 500 mg/20 ml infusion, LYPHE 1 g/vial for inj.) is injected i.v. slowly in a dose of 1–2 g (children 20–40 mg/kg). Another regimen is 30 mg/kg i.v.

loading dose, followed by 8–10 mg/kg/hour continuous infusion till recovery. Pralidoxime causes more marked reactivation of skeletal muscle ChE than at autonomic sites and not at all in the CNS, because it does not penetrate into brain. Treatment should be started as early as possible (within few hours), before the phosphorylated enzyme has undergone 'aging' and become resistant to hydrolysis. Doses may be repeated according to need (max. 12 g in first 24 hrs. Lower doses according to symptoms are continued for 1–2 weeks). The role of oximes in organophosphate poisoning is only adjunctive to that of atropine, which remains the primary antidote. Moreover, the clinical benefit of oxime therapy is highly variable depending upon the compound involved (different organophosphates 'age' at different rates), the amount of poison that has entered the body, time lapse before therapy is started and dose of the oxime.

Other oximes are obidoxime (more potent than pralidoxime) and diacetyl-monoxime (DAM), which is lipophilic.

Chronic organophosphate poisoning Repeated exposure to certain fluorine containing and triaryl organophosphates results in polyneuritis and demyelination after a latent period of days to weeks. Sensory disturbances occur first followed by muscle weakness, tenderness and depressed tendon reflexes—lower motor neurone paralysis. In the second phase, spasticity and upper motor neurone paralysis gradually supervenes. Recovery may take years. The mechanism of this toxicity is not known, but it is not due to inhibition of ChE; there is no specific treatment.

PROBLEM DIRECTED STUDY

7.1 A man aged 45 years presented with gradual onset complaints of double vision, drooping eyelids, difficulty in chewing food and weakness of limbs which is accentuated by exercise. The symptoms fluctuate in intensity over time. A provisional diagnosis of myasthenia gravis is made.

- Can a pharmacological test be performed to confirm/refute the diagnosis?
- In case the diagnosis is confirmed, can this disease be cured by medication?
- Is there a surgical solution for this illness?

(see Appendix-1 for solution)