

Anticholinesterases and anticholinergic drugs

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Key points

There are two types of cholinesterase: acetylcholinesterase in the neuromuscular junction and erythrocytes; and butyrylcholinesterase in plasma (plasma cholinesterase).

Anticholinesterases inhibit all types of cholinesterase and are classified as prosthetic (e.g. edrophonium) and acid-transferring (e.g. neostigmine).

To counteract the muscarinic effects, anticholinesterases are given in combination with muscarinic antagonists such as atropine, glycopyrronium or hyoscine. Both groups of compounds have side-effects.

New compounds without the side-effects of the older agents are being developed to reverse neuromuscular block. They cause chemical encapsulation of the neuromuscular blocking agent in the plasma, preventing its access to the nicotinic receptor.

Non-depolarizing neuromuscular block is monitored throughout surgery and antagonized at the end of anaesthesia to restore muscle tone rapidly and completely, so that patients can maintain a patent airway and adequate pulmonary ventilation. Rapid, reliable antagonism of competitive neuromuscular block only occurs if spontaneous recovery from block has commenced before antagonism. The agents commonly used to effect it are the anticholinesterases. By their inhibitory effect on the enzyme acetylcholinesterase, these drugs increase the amount of acetylcholine at the neuromuscular junction, thus overcoming the effects of any residual neuromuscular blocking agent.

Cholinesterases

There are two types of cholinesterase enzyme, which are closely related in molecular structure but differ in distribution, substrate specificity, and function—acetylcholinesterase (true cholinesterase) and butyrylcholinesterase or plasma cholinesterase (pseudocholinesterase). In the soluble form, these enzymes consist of globular catalytic subunits. In their insoluble form, the subunits are linked to collagen-like tails or to glycolipids, which bind them to a basement membrane.

Acetylcholinesterase is present at all cholinergic junctions. It is bound to the basement membrane in the synaptic clefts where it hydrolyses released acetylcholine. The insoluble form is also found in the erythrocyte; its function is unknown. The soluble form of acetylcholinesterase is present in the cerebrospinal fluid (CSF) and cholinergic nerve terminals, where it is thought to regulate free acetylcholine concentration.

Each molecule of acetylcholinesterase consists of six active sites; each one comprises a peripheral anionic site and a central esteratic site (Fig. 1). The anionic site has a glutamate residue, and the esteratic site has a serine –OH group and an imidazole ring. In addition to the esteratic site, the active centre of the molecule contains an acyl packet and a choline subsite. The anionic site binds acetylcholine in such a way that the ester linkage of acetylcholine approximates to the esteratic site of acetylcholinesterase. Acetylcholine is hydrolysed and the acetyl group is transferred to the serine group at the esteratic site (Fig. 1). A free molecule of choline is then released. The acetylated enzyme is hydrolysed rapidly and free enzyme and acetic acid are formed. Approximately 10 000 molecules of acetylcholine are hydrolysed per second in each active site.

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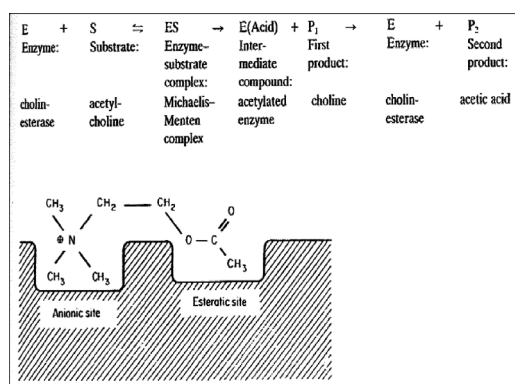


Fig. 1 Mechanism of acetylcholine hydrolysis by acetylcholinesterase. The acetylcholinesterase has two active sites, an anionic site and an esteratic site. Catalytic hydrolysis of acetylcholine occurs whereby the acetyl group is transferred to the esteratic site releasing a free choline molecule. Spontaneous hydrolysis of the enzyme follows and the acetylcholinesterase is reactivated. (Reprinted from: Parasympathomimetic agents and cholinergic agents: anticholinesterases. In: Vickers MD, Morgan M, Spencer PSJ, Read MS, eds. *Drugs in Anaesthetic & Intensive Care Practice*, 8th Edn., 1999; p. 301, with permission from Elsevier).

Butyrylcholinesterase is found in the liver, skin, gastrointestinal smooth muscle, kidneys and brain, as well as in its soluble form in plasma (plasma cholinesterase). The circulating enzyme hydrolyses butyrylcholine more rapidly than it does acetylcholine. It also inactivates other esters on which acetylcholinesterase has little or no effect, for example succinylcholine, mivacurium, ester local anaesthetics, diamorphine and aspirin. (Butyrylcholinesterase does not play an important part in the hydrolysis of esmolol and remifentanyl; they are metabolized mainly by non-specific cholinesterases.) Fresh frozen plasma is rich in the enzyme and has been used with limited success to antagonize neuromuscular block. The physiological function of butyrylcholinesterase is unknown.

Anticholinesterases

Anticholinesterases are drugs that prolong the existence of acetylcholine after it is released from cholinergic nerve endings by inhibiting both acetylcholinesterase and butyrylcholinesterase. They are two types: prosthetic and acid-transferring. The prosthetic inhibitors have an affinity for the anionic site of acetylcholinesterase and prevent acetylcholine from accessing it. They act as reversible, competitive inhibitors of the enzyme and are short-acting. In contrast, acid-transferring inhibitors react with the enzyme and form an intermediate compound. This intermediate compound cannot be hydrolysed as rapidly as the acetylated enzyme formed from acetylcholine. Depending on the stability of this intermediate compound, the duration of action of these anticholinesterases vary; they are divided into medium duration (reversible) and long-acting (irreversible) anticholinesterases.

Pharmacological properties

The anticholinesterases produce effects equivalent to excessive stimulation of the cholinergic system, i.e. stimulation of muscarinic receptor responses at the autonomic effector organs, stimulation and then depression of the autonomic ganglia and skeletal muscle, and stimulation of cholinergic receptors in the CNS. It is advisable to co-administer muscarinic antagonists such as atropine or glycopyrronium to counter the effects of the excess acetylcholine that accumulates in the muscarinic synapses of the gut, bronchi and cardiovascular system. Compounds containing a quaternary ammonium group such as neostigmine and pyridostigmine cannot penetrate the cell membrane or the blood–brain barrier, so exert their action predominantly on peripheral nicotinic and muscarinic receptors. Lipid soluble agents such as physostigmine and organophosphate compounds can also act on the central cholinergic receptors within the brain.

Neuromuscular junction

Anticholinesterases increase the residence time of acetylcholine in the synapse. This allows rebinding of the transmitter to nicotinic receptors. It thus gives acetylcholine the competitive advantage over the neuromuscular blocking agent. In addition, neostigmine and other quaternary ammonium anticholinesterases have a direct

action on skeletal muscle. Anticholinesterases increase the amount of acetylcholine released, by their effect on presynaptic receptors. In overdose, depolarization of the endplate caused by excess acetylcholine predominates and leads to depolarization block. The excess acetylcholine at the synapse also causes repeated stimulation of the receptors resulting in the decay time of the endplate potential being prolonged. This destroys the synchrony between endplate depolarization and the development of action potentials, leading to asynchronous excitation, and fibrillation and fasciculation of the muscle.

Cardiovascular system

Vagal influences on the heart are augmented by anticholinesterases. The effective refractory period of atrial muscle is shortened and the refractory period and conduction time at the sino-atrial (SA) and atrio-ventricular (AV) nodes are prolonged. The predominant effect on the heart is bradycardia caused by the accumulation of acetylcholine. This can result in a decrease in cardiac output and blood pressure. Centrally-acting agents may cause these effects by action on the vasomotor centre.

Respiratory system

Anticholinesterases cause bronchial smooth muscle contraction leading to bronchospasm and hypoxia, which is aggravated by an increase in secretions.

Gastrointestinal system

Oesophageal motility, gastric motility and production of gastric secretions are enhanced. Also, anticholinesterases augment the motor activity of the small and large bowel. In high doses, they can lead to vomiting, diarrhoea and incontinence.

Eye

On local application, anticholinesterases cause constriction of the sphincter pupillae and ciliary muscles leading to miosis and blocking of the accommodation reflex. Intraocular pressure, if elevated, usually decreases as a result of facilitation of the outflow of aqueous humour.

Secretory glands

Anticholinesterases increase the activity of all secretory glands innervated by postganglionic cholinergic fibres, i.e. bronchial, salivary, sweat, lacrimal, gastric, intestinal and pancreatic glands.

Short-acting anticholinesterases

Edrophonium

Edrophonium is the only short-acting anticholinesterase available; it is a synthetic quaternary ammonium compound. The drug competes with acetylcholine and binds by a non-covalent bond to acetylcholinesterase at the anionic site. The recommended dose for antagonism of neuromuscular block is 0.5–1 mg kg⁻¹. When edrophonium is administered intravenously, the peak effect is attained within 0.8–2.0 min and its duration of action is only 10 min (Table 1). This brief duration of action is caused by the

Table 1 Comparison of anticholinesterases

Drug	Neostigmine	Edrophonium	Pyridostigmine
Dose	0.05 mg kg ⁻¹	1 mg kg ⁻¹	0.1 mg kg ⁻¹ (for myasthenia gravis)
Onset of action (min)	1	1–2	>16
Duration of action (min)	20–30	10	360
Distribution half-life (min)	3.4	7.2	6.7
Elimination half-life (min)	77	110	113
Total plasma clearance (ml min ⁻¹ kg ⁻¹)	9.1	9.5	8.6
Recommended anticholinergic	Glycopyrronium	Atropine	Glycopyrronium

reversibility of its binding to acetylcholinesterase, and rapid renal elimination. Its potency is 12 to 16 times less than that of neostigmine. When used to antagonize non-depolarizing neuromuscular block, edrophonium's effects are less predictable than those of neostigmine. For edrophonium to be effective, there should be significant spontaneous recovery. The duration of action of edrophonium makes it unsuitable for antagonizing long-acting agents. In equipotent doses, the muscarinic effects of edrophonium are less pronounced than those of neostigmine. Its onset of action is similar to atropine. If glycopyrronium is used in combination with edrophonium, it has to be given several minutes earlier to ensure a simultaneous onset of action.

Tacrine (tetrahydroaminacrine hydrochloride)

Tacrine is a short-acting anticholinesterase that can cross the blood–brain barrier producing central effects. It has been used in the past to extend the duration of action of succinylcholine. Currently, it is used in the USA in the management of Alzheimer's disease.

Medium duration anticholinesterases

Neostigmine

Neostigmine is a quaternary ammonium compound that possesses a strongly basic carbamyl group, which binds to the anionic site of acetylcholinesterase (Fig. 1). It is then transferred to the esteratic subsite and hydrolysed. However, the rate of hydrolysis is much slower than acetylcholine (minutes rather than microseconds). The quaternary ammonium group (N⁺(CH₃)₃) renders neostigmine lipid insoluble, preventing it from crossing the blood–brain barrier.

Neostigmine is used mainly for antagonism of neuromuscular blocking agents; the i.v. dose is 0.05–0.07 mg kg⁻¹ (Table 1). Onset of action is within 1 min, peak effect occurs in 10 min and duration of action is 20–30 min. The elimination half-life is approximately 77 min. Neostigmine is metabolized by plasma esterases to a quaternary alcohol; 60% of the drug is excreted in urine. In the presence of renal impairment, plasma clearance is reduced and the elimination half-life prolonged.

Glycopyrronium is better matched to the time course of action of neostigmine than atropine. Neostigmine causes bradycardia

and there are reports of cardiac arrest after high doses. It has been shown to cause an increase in postoperative nausea and vomiting. Neostigmine has no effect on Phase I block caused by depolarizing neuromuscular blocking agents; it does antagonize Phase II block, albeit transiently.

Pyridostigmine

Pyridostigmine is an analogue of neostigmine with one quarter of its potency. It is similar to neostigmine in that it binds to acetylcholinesterase via a covalent bond and is lipid insoluble. Pyridostigmine is not used for antagonism of neuromuscular block owing to its slow onset time (>16 min). It also has a long duration of action (6 h) (Table 1). Its elimination half-life is 113 min, which makes pyridostigmine the anticholinesterase of choice for myasthenia gravis.

Physostigmine

Physostigmine was the first anticholinesterase to be used for the treatment of glaucoma (1897). It is a natural alkaloid derived from the Calabar bean. It has a carbamate but no quaternary ammonium group and crosses the blood–brain barrier. It is used to antagonize the central anticholinergic toxicity caused by anticholinergic drug overdose. Physostigmine is metabolized by plasma esterases; elimination does not depend on renal excretion, unlike the other anticholinesterases.

Clinical applications

Antagonism of residual neuromuscular block

Residual muscle weakness is common after the use of long-acting neuromuscular blocking agents. Patients who have been given non-depolarizing neuromuscular blocking drugs should be monitored using a nerve stimulator throughout anaesthesia and recovery to ensure that antagonism is complete. Antagonism of residual block should not be attempted unless the twitch height has recovered to more than 20% of control, or two twitches are detectable on train-of-four stimulation. The deeper the block on antagonism, the longer the time required for a standard dose of anticholinesterase to restore the twitch height or train-of-four response to control values.

Myasthenia gravis

This autoimmune disease is characterized by weakness and fatigability of skeletal muscle with frequently occurring exacerbations and partial remissions. Over 90% of patients are positive for an antibody to the acetylcholine receptor. Neostigmine, pyridostigmine or ambenonium are used in the treatment of myasthenia gravis. Edrophonium is used mainly to diagnose myasthenia gravis. A test dose of 2 mg followed 30 s later by 8 mg i.v. causes transient improvement in muscle power. It is also used to determine whether a patient with myasthenia is receiving inadequate or excessive treatment with cholinergic drugs. If inadequately treated and a myasthenic crisis occurs, transient improvement is noticed, whereas if treatment is excessive (cholinergic crisis) the symptoms intensify.

Alzheimer's disease

A deficiency of structurally intact cholinergic neurones leads to progressive dementia in patients with Alzheimer's disease. Anticholinesterases enhance the concentration of cholinergic neurotransmitter and can slow the degenerative process; they do not reverse it. Donepezil is a reversible anticholinesterase given in a once daily dose and rivastigmine is a non-competitive reversible anticholinesterase that is given twice daily. Treatment is commenced at a low dose and slowly increased depending on response and side-effects.

Paralytic ileus

Neostigmine has been used to relieve the intestinal dilatation caused by paralytic ileus. It is contraindicated in cases of intestinal obstruction, peritonitis, and when the viability of the bowel is in doubt.

Glaucoma

Anticholinesterases have been used for the treatment of primary and secondary glaucoma; they facilitate the drainage of aqueous humour, thus lowering intraocular pressure. Prolonged use of ecothiopate and physostigmine eye drops can lead to acquired cholinesterase deficiency and prolonged block from neuromuscular blocking drugs that are metabolized by this enzyme.

Reversal of intoxication caused by central anticholinergic drugs

Anticholinergics that cross the blood–brain barrier (e.g. atropine, hyoscine) can give rise to central excitation or depression. This is known as the central anticholinergic syndrome. Patients may suffer thought impairment, hallucinations, ataxia, recent memory loss, and behavioural abnormalities. It can be reversed by intravenous physostigmine 2 mg followed by additional doses as required.

Long-acting 'irreversible' anticholinesterases

These are pentavalent phosphorous compounds containing a labile or an organic group, which is released when the drug attaches to the esteratic site of acetylcholinesterase. The inactive phosphorylated enzyme is very stable and the stability is enhanced by a process known as ageing, which results from the loss of one of the alkyl groups. Recovery of anticholinesterase activity depends mainly on synthesis of new enzyme. High lipid solubility, low molecular weight and volatility are features of this group of drugs facilitating rapid and effective absorption via inhalation and the transdermal route. They also readily penetrate the central nervous system.

Organophosphate compounds

Organophosphate compounds such as parathion (*O,O*-diethyl *O*-(4-nitrophenyl)-phosphorothioate) and malathion (*O,O*-dimethyl *S*-(1,2-dicarbethoxy-ethyl) phosphorodithioate) are used as insecticides. The drugs can be detoxified by *in vivo* hydrolysis of the acetylcholinesterase linkage. Malathion is the main ingredient

in dermatological preparations used in the treatment of pediculosis.

Nerve agents used in chemical warfare such as tabun, sarin, VX, and soman are anticholinesterases of high potency. They produce virtually irreversible inactivation of acetylcholinesterase by alkylphosphorylation. The nerve agents also have direct action on the nicotinic, cardiac muscarinic, and glutamate NMDA receptors. The newer agents can even penetrate gas protection suits. After exposure, a triphasic clinical syndrome develops consisting of an initial cholinergic phase (24–48 h), followed by an intermediate phase (4–18 days). A third phase of delayed polyneuropathy occurs 7–14 days after exposure.

Patients require intensive care and organ support. The high concentrations of acetylcholine in the circulation can cause death from bradycardia, bronchoconstriction, vocal cord paralysis, or convulsions. In addition to supportive treatment, two antidotes are of benefit, atropine and oximes. Atropine is given in boluses until the full antimuscarinic effect is obtained (i.e. red dry skin, dilated pupils, heart rate >80 beats min^{-1}). An infusion of atropine might be required for resistant bradycardia. Oximes act by reactivating acetylcholinesterase, by competing with anticholinesterases for binding onto its active sites. They also detoxify unbonded nerve agent and have an endogenous anticholinergic effect. Early administration is necessary. They should be given before the formation of irreversible bonds between the anticholinesterase and acetylcholinesterase. Pralidoxime is given intramuscularly or slowly i.v. over 20 min in a dose of 15–30 mg kg^{-1} . The dose may be repeated after 4 h. People at risk (soldiers and other emergency personnel) may be pretreated with pyridostigmine, which acts as an antidote enhancer. By combining with acetylcholinesterase it makes available a store of the enzyme, which in association with atropine and pralidoxime reduces the incidence of a cholinergic crisis.

Anticholinergic agents

The cholinesterase inhibitors preferentially act at the neuromuscular junction, but acetylcholinesterase in other synapses is also inhibited. A muscarinic antagonist is given to counter the effects of excess acetylcholine in the muscarinic synapses. Muscarinic antagonists used commonly are atropine and the quaternary ammonium compound, glycopyrronium. The muscarinic effects of acetylcholine are prevented by blocking its binding to muscarinic receptor sites. The nicotinic receptor sites are relatively spared but, at very high doses, a partial block is also produced.

Pharmacological properties

The anticholinergics compete with acetylcholine for a common binding site on the muscarinic receptor. This binding site is in a cleft formed by the muscarinic receptor's transmembrane helices. The competitive antagonism by atropine can be antagonized if the concentration of acetylcholine at the receptor site increases.

Atropine causes a transient bradycardia followed by a tachycardia. The bradycardia was thought to be caused by central vagal

stimulation. However, recent data suggest that it is caused by blocking of muscarinic M_2 receptors on the postganglionic parasympathetic neurones, transiently increasing the amount of acetylcholine in the synapse. Blocking the vagal influence on the M_2 receptors at the SA node produces an increase in heart rate. It decreases AV conduction time and can cause dysrhythmias.

Anticholinergics cause bronchodilatation and a decrease in bronchial secretions; respiratory rate may be increased. In therapeutic doses, mild vagal excitatory effects may be noticed. In high doses, central excitation is prominent manifesting as restlessness, delirium, disorientation and hallucination. This is followed by generalized CNS depression. Anticholinergics reduce the tone of the lower oesophageal sphincter. Salivary and gastric secretions are inhibited and gastric emptying is delayed. The normal pupillary reflex is abolished. They may cause mydriasis and cycloplegia, which manifest as photophobia and paralysis of accommodation. They normally have minimal effect on intraocular pressure but in patients with narrow angle glaucoma, dangerous rises in intraocular pressure have been reported.

Typical anticholinergic side-effects include dry mouth, inhibition of sweating, urinary retention and the central anticholinergic syndrome. The inhibitory effect on the sweat glands and increase in basal metabolic rate can lead to a rise in body temperature.

Atropine

Atropine is an organic ester formed by combination of an aromatic acid, tropic acid and a complex organic base, tropine. The intact ester is required for it to act, as is the presence of a free hydroxyl group in the acid portion of the ester. The drug is given i.v. in a dose of 5 to 20 $\mu\text{g kg}^{-1}$. Peak effect is reached 1 min after i.v. administration.

Atropine is absorbed from the gastrointestinal tract and metabolized in the liver to tropine and tropic acid. Over 90% of the drug and its metabolites are excreted in the urine within 24 h.

Glycopyrronium

Glycopyrronium is a quaternary ammonium derivative, which is more potent than atropine at most muscarinic and nicotinic receptors. It lacks central anticholinergic effects because of poor penetration through the blood-brain barrier. The i.v. dose is 3–10 $\mu\text{g kg}^{-1}$. Although onset of action is within 1 min of i.v. administration, peak effect is at 3 min (Table 2). Glycopyrronium is used as a premedicant for its powerful antisialogogue effect. It is five times more potent than atropine in this respect and the effect lasts for 8 h. The vagolytic effect on the heart lasts ~2–3 h and is less potent than atropine. Oral absorption is very poor. The drug is metabolized in the liver by hydroxylation and oxidation, and excreted in urine and bile.

Hyoscine

This is an ester of tropic acid and scopine. It is used in the prophylaxis of motion sickness and as an antispasmodic. It has also

Table 2 Comparison of anticholinergic drugs

Drug	Atropine	Glycopyrronium	Hyoscine
I.V. dose ($\mu\text{g kg}^{-1}$)	5–20	3–10	5–10
Duration of action (h)	3	6	2
Volume of distribution (litre kg^{-1})	2–4	0.2–0.64	2
Elimination half-life (h)	2.5	0.6–1.1	2.5
Inhibition of salivation	++	++++	+++
Effect on heart rate	++++	++	+++
Bronchodilatation	++	++	+
Sedation	+	–	+++
Central anticholinergic syndrome	++	–	++

been used as a premedicant for its antisialogogue and sedative actions. The drug may be administered orally or transdermally. It has less effect on the cardiovascular system than atropine, but a greater effect on the CNS, on the sweat glands and on the eye (Table 2).

New developments

Cyclodextrin-based synthetic hosts

In order to circumvent the side-effects of the anticholinesterases and the anticholinergic agents, a new approach has been developed to reverse residual neuromuscular block. Chemical encapsulation or chelation of the neuromuscular blocking agent in the plasma by an exogenous host molecule, which would promote dissociation of the agent from its site of action thus antagonizing neuromuscular block, has been attempted. Cyclodextrins have been used as the host molecule as they have a well defined lipophilic cavity for host–guest complex formation, are water soluble, very stable, and have few side-effects. The complex formed is excreted in the urine. Org 25969 has been shown to antagonize 90% rocuronium-induced neuromuscular block within 3 min, producing 90% recovery of muscle function.

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See multiple choice questions 123–127.