

CHOLINESTERASES, A TARGET OF PHARMACOLOGY AND TOXICOLOGY

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Background. Cholinesterases are a group of serine hydrolases that split the neurotransmitter acetylcholine (ACh) and terminate its action. Of the two types, butyrylcholinesterase and acetylcholinesterase (AChE), AChE plays the key role in ending cholinergic neurotransmission. Cholinesterase inhibitors are substances, either natural or man-made that interfere with the break-down of ACh and prolong its action. Hence their relevance to toxicology and pharmacology.

Methods and Results. The present review summarizes current knowledge of the cholinesterases and their inhibition. Particular attention is paid to the toxicology and pharmacology of cholinesterase-related inhibitors such as nerve agents (e.g. sarin, soman, tabun, VX), pesticides (e.g. paraoxon, parathion, malathion, malaoxon, carbofuran), selected plants and fungal secondary metabolites (e.g. aflatoxins), drugs for Alzheimer's disease (e.g. huperzine, metrifonate, tacrine, donepezil) and *Myasthenia gravis* (e.g. pyridostigmine) treatment and other compounds (propidium, ethidium, decamethonium).

Conclusions. The crucial role of the cholinesterases in neural transmission makes them a primary target of a large number of cholinesterase-inhibiting drugs and toxins. In pharmacology, this has relevance to the treatment of neurodegenerative disorders.

INTRODUCTION

The cholinergic system is based on the neurotransmitter acetylcholine (ACh), firstly recognized by Loewi in 1920s (ref.¹) and found widely distributed in both central and peripheral nervous systems. The two basic types of acetylcholine receptors in the nervous system and at neuromuscular junctions are: muscarinic acetylcholine receptors (mAChR) and nicotinic acetylcholine receptors (nAChR). Acetylcholine receptors are also found expressed in multiple cells including endothelial and immune system cells².

Cholinesterases are a family of enzymes that catalyse the hydrolysis of ACh into choline and acetic acid, an essential process allowing for the restoration of the cholinergic neuron. Cholinesterases are divided into two: acetylcholinesterase (AChE; EC 3.1.1.7.) and butyrylcholinesterase (BuChE; EC 3.1.1.8). AChE participates in cholinergic neurotransmission by hydrolyzing acetylcholine. It is expressed in nerve and blood cells. Compared to AChE, the importance of BuChE is not well understood. BuChE was known as plasmatic cholinesterase or pseudo-cholinesterase. Similarly, AChE was called blood, also erythrocytal cholinesterase as its activity remains in the cell mass after blood centrifugation. The name AChE derives from the natural substrate acetylcholine as opposed to BuChE that has no natural substrate. An absence or mutation of BuChE leads to a medical condition (see below) that shows itself only in the presence of some drugs (e.g. succinylcholine) and toxins (e.g. cocaine) (ref.³), due to its ability to split artificial substrates. This

review's main focus is on cholinesterases as targets of toxins and drugs. The biochemistry of AChE and BuChE is also discussed. Drugs and toxins are divided in chapters according to target sites.

BUTYRYLCHOLINESTERASE

Though BuChE activity is prevalent in the human body, its' physiological function is not completely understood. BuChE deficient individuals are generally healthy with no manifest signs of disease⁴. The case is similar for mice with a damaged BuChE gene^{5,6}. BuChE deficient individuals have increased sensitivity to muscle relaxants such as succinylcholine, resulting in lasting breath insufficiency⁷. Peoples with the deficient, K type BuChE have lower plasma activity as well as lower affinity for succinylcholine⁸. The K allele is widely-spread especially in the Caucasus area. In recent publications, a link between K type BuChE and lower incidence of Alzheimer's disease has been described⁹ However, more research is needed to examine this connection. Regular BuChE is sensitive to inhibition caused by dibucaine (or cinchocaine in some sources) whereas AChE and K type BuChE are relatively resistant to dibucaine. Biochemical examination of K type BuChE is based on serum/plasma BuChE assessment with and without dibucaine. The output is called a dibucaine number (DN). This represents the percentage of inhibited BuChE. People with regular BuChE have a high dibucaine number (DN \geq 75), heterozygotes have medial

inhibition of DN ~ 40–70, and K type homozygotes have nearly non-inhibited BuChE (DN < 20) (ref.¹⁰).

In comparison with AChE, BuChE is not constituted *in situ* but in different organs, mainly in the liver¹¹. BuChE reaches serum levels of 5 mg/ml with a half time of 12 days¹². Assay of BuChE activity in plasma can also serve as a liver function test. BuChE activity decreases until complex liver necrosis occurs. However, the importance of BuChE as a liver function marker is limited by low sensitivity. Genetic aspects (see above) or intoxication with some compounds such as organophosphate pesticides and/or organophosphonate nerve agents (see below) are sources of false positive findings. BuChE is capable of detoxifying a large number of exogenous substances: procaine¹³, succinylcholine¹⁴, cocaine¹⁵, heroin, acetylsalicylic acid¹⁶, and it can also protect the body from the impact of organophosphorus AChE inhibitors¹⁷. However the primary reason for the existence of BuChE is still unknown.

As mentioned, BuChE, is named according to its preference for the artificial substrate butyrylcholine. BuChE can split butyrylcholine with higher turnover number than AChE. BuChE is also able to hydrolyze much slower than AChE, indole derivatives¹⁸, adipoylcholine¹⁹, benzoylcholine²⁰, acetylcholine/acetylthiocholine^{21,22}, butyrylcholine/butyrylthiocholine^{23,24} and propionylcholine/propionylthiocholine^{25,26}. On the other hand, BuChE is not able to split acetyl- β -methyl-thiocholine or acetyl- β -methyl-choline^{27,28} whereas AChE can. A summary of substrates and reaction products is depicted below (Table 1).

The fact that BuChE has wider substrate specificity than AChE is structurally determined. BuChE is a tetrameric glycoprotein composed of four subunits. Both dimeric and monomeric forms are stable and ubiquitous in the body²⁹. All subunits are identical and composed of 574 amino acids with an overall molecular weight close to 85 kDa. As described by Lockridge et al.³⁰ the structural similarity to AChE is 54% and to bovine thyroglobuline 28%, which leaves ample differences: BuChE is not in-

hibited by substrate excess as is typical for AChE (ref.³¹), the active site is wider for BuChE (ref.³²), BuChE is sensitive to inhibition by tetraisopropyl pyrophosphoramidate (iso-OMPA; see text about AChE), and the inhibition is considered to be a fast proof whether a sample contains BuChE or AChE.

ACETYLCHOLINESTERASE

AChE and BuChE are similar, resembling each other by more than 50% but their significance and localization in the body are very different. AChE is expressed in cholinergic neurons. Relatively high AChE activity can also be found in blood cells responsible for the degradation of plasma acetylcholine³³. The primary function of AChE is rapid splitting of acetylcholine and terminating cholinergic neurotransmission. Individuals with inhibited AChE or knock out AChE mice have over-stimulated acetylcholine receptors³⁴. Although, AChE deficient mice are viable, they have reduced musculature with changed morphology³⁵ and levels of extracellular acetylcholine nearly sixty times higher than normal. It seems that BuChE is able to partially recover the AChE missing activity in the deficient animals³⁶.

The structure of AChE has been extensively investigated since the 1990s. The first experiments were conducted on AChE in the electric eel (*Torpedo californica*) due to its availability³⁷. This was also considered an informal model until the commercialization of human recombinant AChEs. The AChE active site as well as the whole AChE structure is evolutionary conservative and it contains common regions similar to the other serine hydrolases. Cholinesterases are a type α/β hydrolase folded with an α helix bound with β sheet that contains a catalytic domain³⁸ with catalytic triad Ser - His - Glu, the same as in AChE, BuChE, and lipases. A similar structure can be also found in carboxyesterases where glutamate is re-

Table 1. Selected substrates and products of AChE respectively BuChE catalyzed hydrolysis.

Substrate	Product	Enzyme	Reference
Succinylcholine + water	succinate + choline	BuChE	14
Adipoylcholine + water	adipoate + choline	BuChE	19
Benzoylcholine + water	benzoiate + choline	BuChE	20
Acetylcholine + water	acetate + choline	AChE > BuChE	21
Acetylthiocholine + water	acetate + thiocholine	AChE > BuChE	22, 67
Butyrylcholine + water	butyrate + choline	BuChE > AChE	23
Butyrylthiocholine + water	butyrate + thiocholine	BuChE > AChE	24, 61, 67
Propionylcholine + water	propionate + choline	BuChE, AChE	25
Propionylthiocholine + water	propionate + thiocholine	BuChE, AChE	26, 67
Acetyl- β -methyl-thiocholine + water	β -methyl-thiocholine + acetate	AChE	27
Acetyl- β -methyl-choline + water	β -methyl-choline + acetate	AChE	28

placed by aspartate³⁹. Serine is a part of a stable sequence Gly-Glu(His)-Ser-Gly-Ala/Gly (ref.⁴⁰).

The electric eel AChE's **active site** lies on a bottom of long and narrow cavity 20 Å deep. The active site contains a catalytic triad within an **esteratic site** with amino acid positions for the electric eel AChE: Ser 200, His 440 and Glu 327 (ref.⁴¹). The **anionic site** (also α -anionic site) is another part of the active site and it is close to the esteratic site. The anionic site is composed of the amino acids Trp 86, Tyr 337 and Phe 338 for murine AChE (ref.⁴²) or Trp 84, Tyr 121 and Phe 330 for the electric eel AChE (ref.^{43,44}). While the esteratic site hydrolyzes the ester bond, the anionic site interacts with the acetylcholine quaternary ammonium atom and is responsible for its correct orientation. Entry into the active site through the cavity composed of aromatic amino acids, i.e. **aromatic gorge**, enables higher selectivity for acetylcholine. Substrate penetration is allowed by cation - π interactions between acetylcholine quaternary ammonium atom and π electrons of phenylalanine, tryptophan and tyrosine aromatic cores^{45,46}.

The **peripheral anionic site** (also β -anionic site) is localized on the AChE surface around the cavity entrance. This site was recognized as a target for multiple AChE activity modulators and the first experiments began in the 1960s (ref.⁴⁷). The aromatic site contains loops and it has good conformational flexibility. Tyr 70, Asp 72, Tyr 121, Trp 279 and Tyr 334 amino acids residues are the most significant residues in the peripheral anionic site⁴⁸. As described in the following chapters, the peripheral anionic site is a target for a number of toxins and also promising drugs^{49,50}. It probably plays an important role in the development of Alzheimer's disease. Amyloid β peptide interacts with the peripheral anionic site resulting in the formation of amyloid plaques and consequent damage to cholinergic neurons⁵¹.

Both AChE and BuChE form mainly tetramer G4 but they can also form dimer G2 that can be secreted as a water soluble molecule⁵². Monomeric AChE has molecular weight of 69 kDa (ref.⁵³). The predominant part of AChE localized in the central nervous system contains both hydrophilic and hydrophobic regions i.e. it is

amphiphilic. There are differences between amphiphilic and non-amphiphilic cholinesterases. The amphiphilic cholinesterase contains G4 catalytic tetramer and one non-catalytic subunit P (ref.⁵⁴). The P subunit has a molecular weight 20 kDa and it is asymmetrically bound to two G subunits. From a chemical point of view, it is glycosphosphatidylinositol (GPI) called **GPI anchor** (ref.^{55,56}). In the human AChE, the lipophilic part of the GPI anchor is palmitate⁵⁷. The biological role of individual AChE forms can be ascertained from an experiment done on monkey brains⁵⁸. 85% of AChE are tetramers with a sedimentation constant 9.7 S, 10% is dimeric (5.7 S) and 5% monomeric (3.2 S). In total, 83% AChE molecules are amphiphilic and only 17% hydrophilic. Besides free and membrane bound AChE, there is also collagen bound AChE. From the symbols introduced by Massoulie and Bon, bound AChE is abbreviated AChE_T and one collagen oligomer is connected with one (A₄)_T, two (A₈) or three (A₁₂) tetrameric AChE molecules^{59,60}.

AChE and BuChE have different abilities to split substrates. Compared to BuChE, AChE is not able to hydrolyze high molecular weight esters but AChE has higher affinity for acetylcholine and BuChE for butyrylcholine. The differences in affinity to substrate are probably caused by changes in the aromatic gorge disposition. Substitution of two phenylalanines to leucine and valine in the electric eel AChE aromatic gorge possessed butyrylcholine turnover rate at a similar level to BuChE. Further, mutated AChE was sensitive to inhibition by iso-OMPA and was not inhibited by propidium, a peripheral anionic site inhibitor⁶¹. Differences in AChE and BuChE structures are revealed by huperzine A. This Alzheimer's disease drug is a strong AChE inhibitor binding to the peripheral anionic site; however, BuChE is intact in the presence of huperzine⁶². Another Alzheimer's disease drug, tacrine, binds into the α -anionic site. It inhibits AChE as well as BuChE to a comparable degree⁶³. A similar situation to tacrine is common for other drugs containing quaternary ammonium, nerve agents, and neurotoxic pesticides⁶⁴⁻⁶⁷. The basic parameters for AChE and BuChE are summarized below (Table 2).

Table 2. Basic parameters of AChE and BuChE.

	AChE	BuChE	References
Subunit	69 kDa	85 kDa	29, 30, 53
Quaternary structure	Tetramer predominate, mono-, di- and trimeric forms can also occur		29, 54, 55, 66, 68, 69
Conversion of acetyl- β -methyl-(thio)choline	High	low	27, 28, 71, 72
Inhibition by excess of substrate	Yes	no	68, 69, 70
Inhibition by iso-OMPA*	No	yes	61, 75
Inhibition by nerve agents	Yes	yes	65, 66
Inhibition by huperzine	Yes	no	62

*Iso-OMPA: tetraisopropyl pyrophosphoramidate

Apropos AChE enzymology, there are differences between AChE and BuChE. Inhibition by excess of substrate is probably the most important fact^{68,69}. The biological significance of AChE inhibition by substrate excess has not yet been proven. One theory however looks feasible⁷⁰. After acetylcholine vesicles are released into the neuro-synaptic cleft, AChE is inhibited and the receptors are stimulated. The termination signal, which has a square plot in cholinergic nerves, is fast once the ACh level drops below threshold concentration. The basic biochemical test for estimating whether a sample contains AChE and not BuChE is based on assessment of acetylthiocholine and acetyl- β -methyl-thiocholine as fast conversion in low concentrations of substrate and slow conversion in high concentration^{71,72}. Specific inhibitors introduced later are also applicable. Non-enzymatic functions of AChE are probable and the research is on-going. Interestingly, body growth and cell adhesion are probably partially connected with AChE (ref.^{73,74}).

CHOLINESTERASE INHIBITORS

Cholinesterase inhibitors (e.g. drugs, natural toxins, pesticides, chemical warfare agents) are a wide group of chemical compounds with different physico-chemical properties. AChE inhibitors play a significant role in the biochemical processes of the human body due to the physiological importance of AChE. Specific BuChE inhibitors, such as iso-OMPA (Fig. 1), have mainly diagnostic importance⁷⁵. Lower interest in BuChE inhibitors can be explained by probable BuChE physiological redundancy. For example, drugs suppressing the manifestation of Alzheimer's disease through impact on the cholinergic system are predominantly selective inhibitors of AChE (ref.⁷⁶). Selective inhibitors of BuChE have also been investigated as potential drugs for Alzheimer's disease⁷⁷; but to a lesser degree than AChE. Inhibition of AChE also plays an important role in nerve agent toxicology. Intact BuChE however can temporarily substitute inhibited AChE and is able to slowly hydrolyze accumulated acetylcholine⁷⁸.

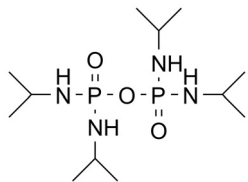


Fig. 1. Tetraisopropyl pyrophosphoramidate (iso-OMPA).

Compounds inhibiting AChE can be divided into three basic groups⁷⁹:

1. Compounds binding at the **active site** interact with either **esteratic** (e.g. nerve agents) or **anionic site** (e.g. tacrine).
2. Compounds interacting with the **aromatic gorge** (e.g. decamethonium).

3. Compounds bound at the **peripheral (β) anionic site** (e.g. huperzine, propidium).

Inhibitors binding at the esteratic part of active site

Inhibitors of the esteratic part found on the active site are compounds with the chemical structure of organophosphorus or carbamate derivatives. Inhibitors of esteratic subsite of the active centre are mainly toxins, chemical warfare agents or pesticides. Of course some are used as drugs. These compounds interact with serine in the catalytical triad of active site, providing stable esters. Organophosphorus compounds create stable, covalently bound adducts with spontaneous dissociation once covalently connected with serine hydroxyl. Some drugs containing the oxime group are able to split organophosphorus moiety from the active site resulting in liberation and enzyme reactivation. Obidoxime, trimedoxime, pralidoxime (2-PAM) and asoxime (HI-6) can be mentioned as commercially available drugs³. After a specific time interval (from minutes up hours) for each organophosphorus inhibitor, the bound inhibitor undergoes dealkylation called "aging" (ref.⁸⁰). The aging has no beneficial effect on the enzyme as it remains inactive. In comparison with organophosphorus inhibitors, the carbamate moiety is spontaneously hydrolyzed and liberated. AChE becomes active again. Carbamates are probably able to bind through non-covalent interactions⁸¹. The mechanism of AChE inhibition by an organophosphonate is depicted below (Fig. 2).

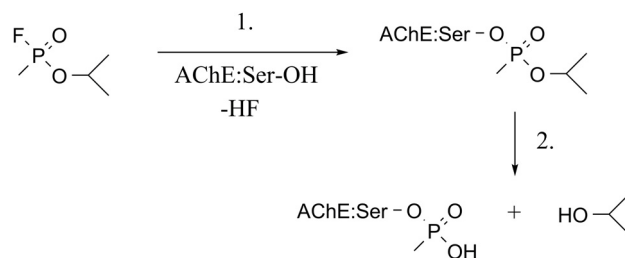


Fig. 2. Inhibition of AChE by nerve agent sarin (reaction 1) and the consequent aging (reaction 2). Serine hydroxyl is indicated in AChE molecule by an abbreviation (Ser-OH).

Nerve agents are organophosphonate compounds used in chemical warfare. The older group of nerve agents called G series was discovered before World War II. Tabun was the first known nerve agent first synthesized in 1936 by professor Gerhard Schrader. After World War II, the most toxic nerve agents called the V series were extensively investigated⁸². Tabun (abbreviated GA according to NATO), sarin (GB), soman (GD) and cyclosarin (GF) are representatives of the G series nerve agents. Among VX nerve agents are the Russian VX (VR) and Chinese (VC) variants⁸³. The chemical structures of selected nerve agents are depicted in (Fig. 3).

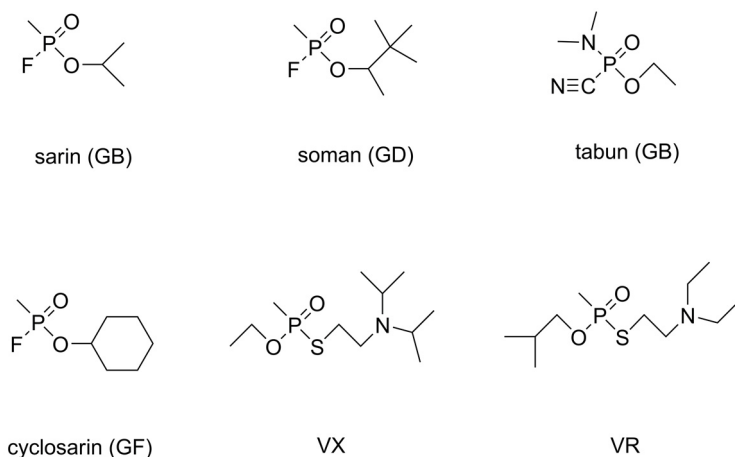


Fig. 3. Selected nerve agents.

Nerve agents are extremely hazardous due to superior penetration ability into the human body by all routes and their high toxicity. Nerve agents differ from pesticides in their toxicity and rapid bodily dissemination. The median lethal dose (LD_{50}) is different for individual nerve agents: e.g. for subcutaneous administrations to rat, the LD_{50} s are 193 $\mu\text{g}/\text{kg}$ for tabun, 103 $\mu\text{g}/\text{kg}$ for sarin, 75 $\mu\text{g}/\text{kg}$ for soman, and 12 $\mu\text{g}/\text{kg}$ for VX (ref.⁸⁴⁻⁸⁶). Median lethal concentration and time (LCt_{50}) in rats for sarin is 150 mg/m^3 for ten minutes lasting inhalation⁸⁷. The toxicity of nerve agents is much more apparent than standard pesticides: e.g. the commercially available organophosphate pesticide primiphos-methyl (e.g. preparation Actellic 50EC) has a declared LD_{50} for rat males and per oral administration $\geq 1,500 \text{ mg}/\text{kg}$.

The G series of nerve agents penetrate the body by all routes and spread quickly through the organism. In comparison with G series, the V series of nerve agents are able to penetrate via the lungs and skin with ease; however, V agents create sub-epithelial reservoirs and the agent is slowly released from the reservoirs⁸⁸. It should be emphasized that nerve agents as well as organophosphate and carbamate pesticides are quite reactive. Apart from binding to AChE and BuChE, they can bind to multiple organs and tissues in the body. Inhibition of AChE is the most crucial from the toxicological point of view whereas from the therapeutic point of view the most significant fact is the inhibition of carboxylesterase 1. Carboxylesterase 1 is able to recover its activity even after sarin inhibition. This fact encourages scientists in the search for an effective scavenger that would serve as a prophylactic against nerve agent intoxication⁸⁹. The other effective enzyme is serum paraoxonase (PON). The PON is able to split organophosphate and thereby detoxify the poisoned person. On the other hand, PON activity is quite low and fluctuates greatly in the general population⁹⁰.

The less toxic variant of organophosphonate nerve agents are organophosphate pesticides. Highly toxic organophosphate pesticides are e.g. paraoxon ethyl, paraoxon methyl, and malaoxon. These compounds are approximately equally toxic to warm-blooded as well as cold-blooded organisms. Due to the effort to enhance pes-

ticide specificity, numerous derivatives of highly toxic pesticides have been prepared to reduce the toxicity towards warm-blooded organisms and retain toxicity to insects. Thioforms of organophosphates such as parathion ethyl, parathion methyl and malathion are some relevant examples. The thioforms of organophosphate pesticides are converted into the above mentioned oxoforms by mixed function oxidases (MFO). The activation proceeds in cold-blooded organisms but this is not common in warm-blooded organisms where no metabolizing or dealkylation into non toxic compound take place⁹¹.

Carbamates are the second group of pesticides inhibiting cholinesterases. From the chemical point of view, they are N-alkyl and N,N-dialkyl carbamates. The natural derivate of carbamate is physostigmine. It is produced as a secondary metabolite in the African plant *Physostigma venenosum* (*Fabaceae*). Physostigmine is a strong reversible inhibitor of AChE. It has broad use in *Myasthenia gravis* treatment as it increases acetylcholine levels in the damaged neurosynaptic clefts and also as a prophylactic to nerve agent exposure as it blocks the irreversible binding of nerve agents^{92,93}. Carbamates are pseudo-irreversible inhibitors of cholinesterases; the carbamoyl moiety can be split from cholinesterase by spontaneous hydrolysis⁹⁴. Carbamates cannot penetrate the blood brain barrier in the healthy body; however, stress conditions can enhance diffusion into the central nervous system⁹⁵. Organophosphate and carbamate compounds are not only used in agriculture but for medical purposes too. Rivastigmine is a drug available for the symptomatic treatment of Parkinson's as well as Alzheimer's disease⁹⁶. Trichlorfon (metrifonate) has similar application in medicine to rivastigmine though it was used as a pesticide in the past⁹⁷.

The majority of countries have strong regulations on the application of pesticides; e.g. in the European Union it is regulated by the directive 91/41/EHS. Individual preparations are approved for commercialization and the list is regularly updated. Commonly used and relatively safe for warm-blooded organisms, are mainly pesticides: organophosphates – chlorpyrifos, fenitrothion, pirimiphos-methyl, dimethoate, phosalone and carbamates – pirimicarb,

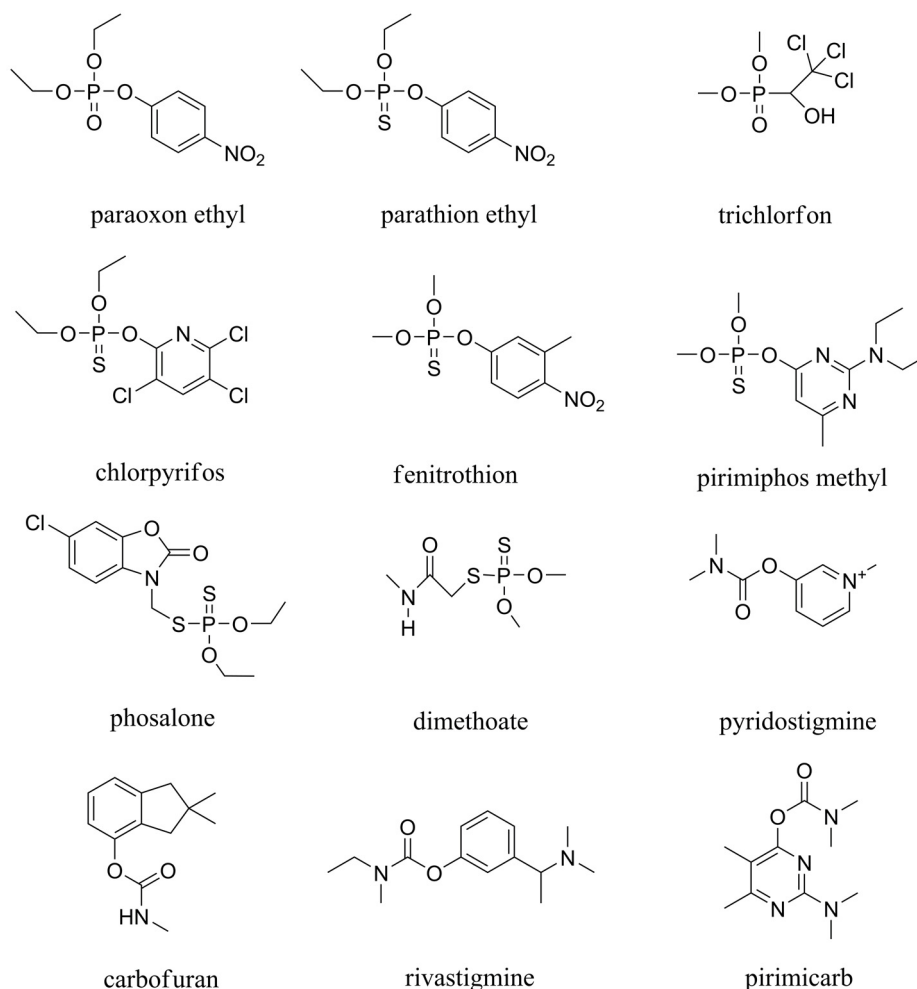


Fig. 4. Selected organophosphate and carbamate inhibitors of cholinesterases.

carbofuran, carbosulfan, methiocarb, fenoxycarb. The structures of selected organophosphate and carbamate compounds are shown below (Fig. 4).

Inhibitors of the α -anionic site

Cholinesterase inhibitors binding to the α -anionic site are a group of chemical compounds containing certain common motives. Firstly, these compounds typically contain condensed aromatic cores. Secondly, there should be quarternary ammonium or nitrogen included as a heteroatom. Acridines and tetrahydroacridines can be mentioned as examples. Quinolines and isoquinolines are other common structures interacting with the α -anionic site of cholinesterases. In comparison with the esteratic site inhibitors, compounds interacting with the α -anionic site are reversible inhibitors. 9-amino-1,2,3,4-tetrahydroacridine known as tacrine, which is also considered one of the most important inhibitors of the α -anionic site able to suppress Alzheimer's disease manifestation. It is marketed worldwide under the trade name Cognex. The main disadvantage of tacrine is its relatively high hepatotoxicity⁹⁸. There is an effort underway to find less toxic derivatives of tacrine⁹⁹. In the past, 7-methoxytacrine was extensively investigated as a promising substitute to tacrine. This compound is less toxic than tacrine and

some *in vitro* as well as *in vivo* tests proved superior to tacrine^{100,101}. Protoberberine alkaloids are strong natural inhibitors of AChE. Berberine, palmatine, jatrorrhizine and epiberberine are examples. These substances are considered promising drugs for Alzheimer's disease symptomatic treatment¹⁰².

Galantamine (Nivalin) is another well known drug interacting with the α -anionic site. It is an alkaloid from the Caucasian snowdrop (*Galanthus woronowii*, Amaryllidaceae). The properties of galantamine were firstly recognized by Mashkovsky and Kruglikova-Lvova in the 1950s (ref.¹⁰³). Beside the α -anionic site, galantamine also binds at another important part of the AChE active site including aromatic gorge^{104,105}. The formulas of these compounds are shown in (Fig. 5).

Inhibitors binding into aromatic gorge

The aromatic gorge is not a typical target for cholinesterase inhibitors. On the other hand, inhibitors interacting with the α -anionic site will probably also interact with the aromatic gorge. Galantamine can be mentioned as an example (see above). *In silico* methods and structural analyses have shown that some bisquarternary compounds such as the depolarizing muscle relaxant decamethonium (Fig. 6) provide a stable complex with

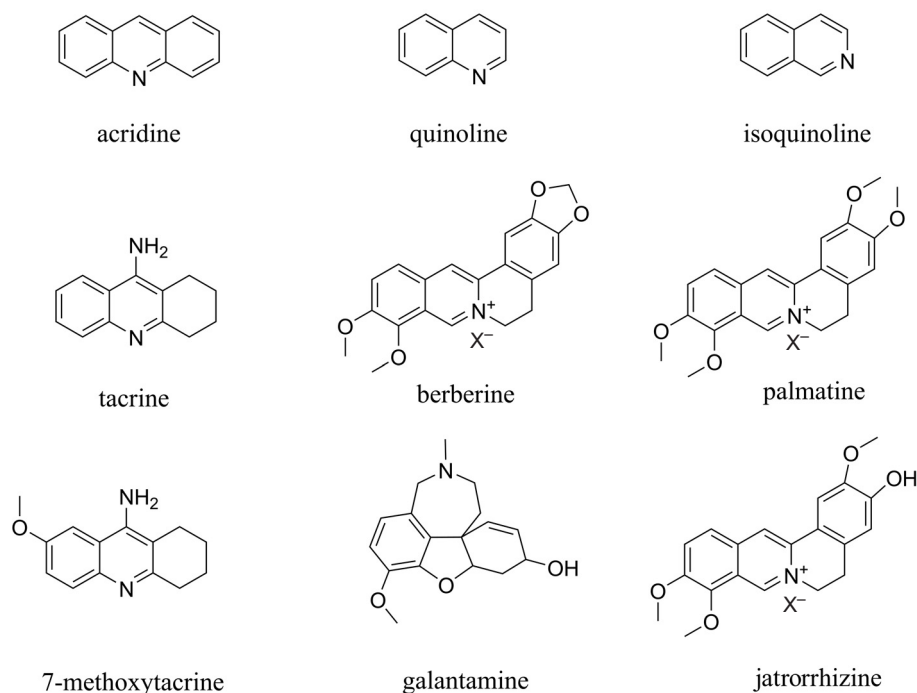


Fig. 5. Selected inhibitors that bind onto the α -anionic site.

the aromatic gorge due to electrostatic interaction¹⁰⁶. But the main decamethonium effect is not on AChE (ref.¹⁰⁷).

Inhibitors of peripheral (β) anionic site

The peripheral anionic site is the main target of many pharmacologically important compounds rather than toxins. Much attention is given to the peripheral anionic site due to the link to Alzheimer's disease. Lack of acetylcholine was considered as a major factor in the cause of Alzheimer's disease (AD). The deposition of amyloid plaque in AD may be accelerated or even triggered by interaction of β -amyloid with the peripheral anionic site. Inhibitors binding at the peripheral anionic site are considered not only symptomatic drugs for Alzheimer's disease, but also probably causative ones¹⁰⁸. It should be emphasized though that the etiology of Alzheimer's disease is not thoroughly understood and the actual function of AChE is still being investigated.

Aflatoxins are natural hepatocarcinogens activated by liver cytochrome P450. They probably interact with

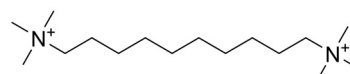


Fig. 6. Decamethonium (anions are not considered).

the peripheral anionic site. Despite strong inhibition of AChE, it seems that BuChE has no sensitivity to aflatoxin as shown for aflatoxin B1 (ref.¹⁰⁹⁻¹¹¹). Inhibition of AChE was confirmed after the onset of cholinergic symptoms following aflatoxin exposure¹¹². The mechanism of aflatoxin interaction with AChE is not well-explained and more supporting experiments are needed. Double-stranded DNA fluorescence dyes are also inhibitors of AChE. Propidium¹¹³ as well as ethidium¹¹⁴ are proven inhibitors, binding at the peripheral anionic site. The structure of aflatoxin B1, ethidium and propidium are depicted below (Fig. 7).

The effects of certain ions on AChE remain unclear. Oxidative state III+ aluminum ions have been investigated.

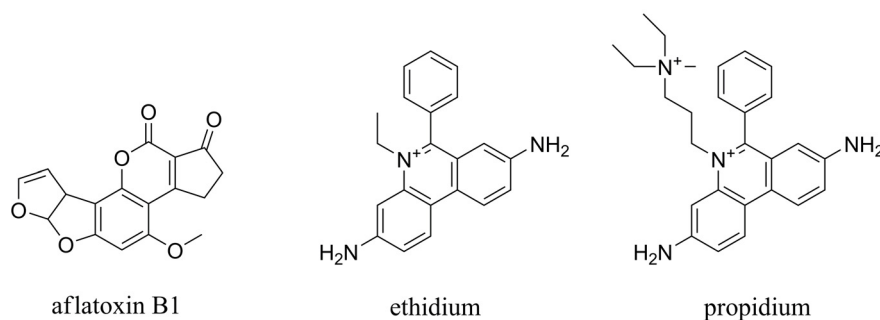


Fig. 7. Selected structures that bind to peripheral anionic site (anions are not considered).

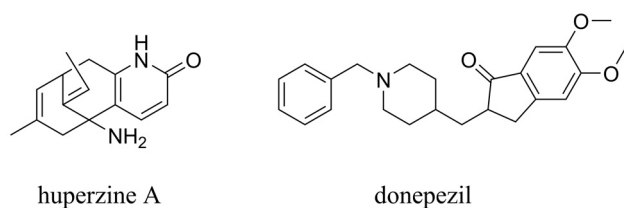


Fig. 8. Alzheimer disease drugs binding into peripheral anionic site.

Aluminum can act as a neurotoxin and it has been investigated as the causative agent of Alzheimer's disease for many years; however, no decisive connection has been found^{115,116}. Contradictory changes in brain AChE activity after aluminum exposure have been found in different brain regions^{117,118}. On the other hand, AChE is not only affected by aluminum. Double-valent ions also have the ability to inhibit AChE activity. Magnesium ions are also an AChE inhibitor¹¹⁹.

The peripheral anionic site is a target of newly synthesized drugs for Alzheimer's disease treatment. Inhibition of the peripheral anionic site in Alzheimer's disease has probably not only symptomatic effects due to enhancement of acetylcholine availability. It can also slow down the deposition of amyloid plaque^{120,121}. Inhibition of the peripheral anionic site can be considered the most promising for Alzheimer's disease treatment. Drugs that bind at the peripheral (e.g. donepezil, huperzine) as well as at the anionic site (e.g. tacrine, galantamine) cause elevated expression of AChE. In contrast, inhibitors binding at the esteratic site do not cause significant expression of AChE (ref.¹²²). The up-regulation of AChE expression is an unwanted effect that lowers treatment efficacy. The commercially available drugs for Alzheimer's disease that inhibit AChE through the peripheral anionic site are e.g. alkaloids huperzine A as well as B and man-made donepezil. Huperzine comes from the firmoss *Huperzia serrata* (Huperziaceae) (ref.^{123,124}). Huperzine A is a more potent AChE inhibitor than huperzine B. Huperzine can be isolated from the *H. serrata* that contains above 0.025 (w/w) of huperzine A and less huperzine B; synthetic production is available as well¹²⁵. Donepezil is another drug suitable for Alzheimer's disease treatment with good penetration through the blood brain barrier and slow excretion. It is marketed under the trade name Aricept¹²⁶. The structure of huperzine and donepezil is depicted in (Fig. 8).

CONCLUSION

Cholinesterases play an important role in the human body. They are a regular target of a large number of toxins including chemical warfare agents. However, the current focus of investigation is development of *Myasthenia gravis* and Alzheimer's disease drugs. Understanding of cholinesterase structure and the biological mechanism of their inhibition is necessary for novel effective drug development.

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