# NATURAL NEUROTOXINS WITH TARGETING TO CHOLINERGIC NERVOUS SYSTEM (CHOLINOTOXINS)

Jiří PATOČKA
Department of Toxicology, Purkyně Military Medical Academy, Hradec Králové

***Summary***

*Cholinergic nervous system plays an important role in many physiological and behavioral functions in all animals. The activity of central and peripheral nervous system depends on the production and fate of acetylcholine and all compounds influenced its biosynthesis, storage, release, hydrolysis, and interactions with different subtypes of acetylcholine both muscarinic and nicotinic receptors. Many natural neurotoxins also interact with different parts of cholinergic nervous system and these compounds are the aim of this review.*

KEY WORDS: Cholinergic nervous system; Alkaloids; Animal venoms; Neurotoxins.

## 1. Introduction

Natural toxins are chemical agents of biological origin, present in the bodies of some organisms or in the special glands which product venom. Toxins have evolved in microorganisms, fungi, plants and animals over many thousands of years to have specific and unique effects. Venoms from different toxic plants are known and used for different purposes by native civilizations in all continents. Toxins of venomous animals as for instance snakes, scorpions, spiders, bees etc., have fascinated mankind because of their dramatic toxic effect on other animals. These poisons are used by the animals for protection against predators or for capturing prey and very often affect their nervous system (neurotoxins). Many venoms have multiple components or toxins that lead to a complicated

clinical picture. The toxicity of many toxins is very high, very often comparable with chemical warfare agents, and they may be dangerous for humans.

## 2. Neurotoxins

Neurotoxins have common mechanism of action on a target system. There are toxic to the nervous system on different levels. There are known presynaptic toxins which cause abnormalities in depletion of neuromediators, postsynaptic toxins which block different post-synaptic receptors causing paralysis, toxins which block the nerve conduction and at last toxins which cause spontaneous action potentials. Toxins with specific affinity to the structures of both central and peripheral cholinergic nervous system could be sign as cholinotoxins and resumption of them is the theme of this article.

## 3. Cholinotoxins

Cholinotoxins by all means do not represent the greatest group of natural neurotoxins. For all that there are chemically and pharmacologically very interesting compounds which have been used scientifically to elucidate physiological mechanisms in cholineregic nervous system, or as the starting point in the development of new therapeutic agents or even as therapeutically applicable agents. Cholinotoxins, on the other hand, could be misused as chemical warfare agents or as wrecker poisons, because some of them are very toxic for a human being.

All known natural cholinotoxins can be divided into four principal groups: 1. Acetylcholine receptor agonists, 2. acetylcholine receptor antagonists, 3. inhibitors of acetylcholine releasing and neuromuscular junction and 4. inhibitors of acetylcholinesterase.

### 3.1 Acetylcholine Receptor Agonists

At this time many cholinergic agonists are of known but only some of them are of natural origin. Some cholinergic agonists have both muscarinic and nicotinic actions, others are more or less selective to these receptors or to their different subtypes. Well-known natural cholinergic agonists are some toxic plant alkaloids, for example arecoline from *Areca catechu*, pilocarpine from *Pilocarpus jaborandi* and *Pilocarpus microphyllus*, muscarine from *Amanita muscaria*) and nicotine from *Nicotiana tabaccum*. Another antagonist is anatoxin which has been found in blue-green algae *Anabaena ssp*. Up-to-date no acetylcholine receptor agonist has been found as a component of animal venoms.

#### *Arecoline*

Arecoline is the main alkaloid of Betel nuts from *Areca catechu*, which are used by various ethnic groups, including those from India, South-East Asia and parts of Africa, particular manner analogous to chewing tobacco. But seeds of Betel nuts are toxic, resulting in pupillary dilatation, vomiting, diarrhoea and convulsions. Arecoline is connected with oesophageal cancer.

Arecoline is chemically tetrahydropyridine derivative (Fig. 1) and pharmacologically is characterized as a gangliomimetic compound which stimulates the muscarinic as well as nicotinic receptors and acetylcholine is likely to be partially involved in the gangliomimetic effects of arecoline (59).

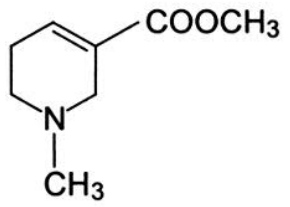


*Fig. 1 Structural formula of arecoline*

#### *Pilocarpine*

Pilocarpine is alkaloid (Fig. 2) from the leaves of Jaborandi bushes (*Pilocarpus jaborandi*) (62). These bushes are mainly found in the rainforests in the North-East of Brazil. For more than 120 years pilocarpine has been already used for the treatment of glaucoma, by reducing the intraocular pressure. Its intraocular administration causes pupillary constriction and spasm of accomodation. Pilocarpine causes bronchoconstriction and enhances tone and motility of the ureters, the urinary bladder, the gallbladder, and the billiary ducts (55). It stimulates exocrine glands.

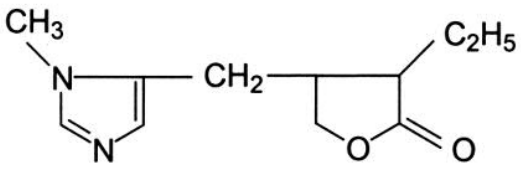


*Fig. 2 Structural formula of pilocarpine*

### *Muscarine*

Muscarine is found in *Amanita muscaria* (Mushrooms), chemically it is a derivative of furane (Fig. 3) and its pharmacological effect is similar to pilocarpine on smooth muscle and exocrine glands. Muscarine can cause a decrease of blood pressure and slowing down a heartbeat. It can cause cortical arousal. Poisoning is manifested by gastro-intestinal irritation, vomiting, abdominal pain and diarrhoea, perspiration, salivation, lacrimation, constriction of pupils and blurring of vision. In severe cases there may by convulsions and coma. Death may be a result of shock, respiratory failure or pulmonary oedema (4).

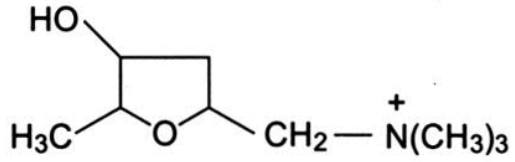


*Fig. 3 Structural formula of muscarine*

### *Nicotine*

Nicotine is an alkaloid obtained from the dry leaves and stems of the *Nicotiana* species. These include *N. tabacum* (Cultivated tobacco), N. attenuata (Wild tobacco), *N. glauca* (Tree tobacco), and *N. trigonophylla* (Desert tobacco).

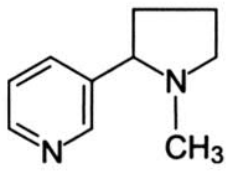


*Fig. 4 Structural formula of nicotine*

Nicotine is 1-methyl-2-(3-pyridyl)-pyrrolidine, tertiary amine consisting of a pyridine and pyrrolidine ring (Fig. 4). It is a colorless, strongly alkaline, volatile liquid which turns brown and acquires a characteristic odor of tobacco on exposure to air. (S)-Nicotine, found in tobacco, is binded stereoselectively to nicotinic cholinergic receptors and the main actions are: light stimulation followed by paralysis of autonomic ganglia and the central nervous system. This drug has a characteristic curare action on skeletal muscle. Nicotine is readily absorbed from the oral and gastrointestinal mucosa and from the respiratory tract. The drug is detoxified mainly by the liver, and the remainder is excreted, mainly in the urine. (R)-Nicotine, found in small quantities in cigarette smoke due to racemization during the pyrolysis process is a weak agonist of cholinergic receptors (7).

Nicotine is known as one of the most lethal poisons. However, despite its common use, there is a very low incidence of reported toxicity. This low incidence of reported poisoning may be due to the strong emetic effect of nicotine as well as the slow absorption when oral tobacco is ingested. At present, virtually all toxicity from nicotine is reported to be from cigarettes (6). More than 90 per cent of toxic exposures to cigarettes are reported in children less than 5 years of age. None nicotine-related death has been reported in recent years.

Nicotine maintains tobacco addiction and has therapeutic utility to aid smoking cassation (7) and possibly to treat other medical diseases (31, 56).

### *Anatoxin*

Anatoxin-a is the term used for the potent alkaloid neurotoxin produced by the freshwater cyanophyte Anabaena flos-aquae. Structurally, anatoxin-a is similar to the tropane alkaloids, especially cocaine (Fig. 5). Anatoxin-a is a potent depolarizing neuromuscular blocking agent possessing both muscarinic and nicotinic activities. According to Thomas et al. (57) anatoxin-a is a potent stereospecific nicotine receptor agonist on neuronal nicotinic acetylcholine receptors. The potencies at chick nicotinic receptors as the agonist was expressed by $ED_{50}$ = 0.58 μM (5). Therefore anatoxin-a is a potent neurotoxin which blocks neuromuscular activity by its pharmacological action as a nicotinic agonist. In various pharmacology studies it was between 7 and 136 times more potent than nicotine and offers significant potential as a basis for new molecular probes for the characterization of neuronal nicotinic acetylcholine receptors.

Under blue-green algae can certain conditions, massively increase in numbers in some water bodies and may form visible surface blooms. Toxic effect on animals ingesting significant quantities of blue-green algal cells, usually from such blooms, have been recognized for over a century and some of the toxins were classified according to their mode of action, e.g. neurotoxins and hepatotoxins.

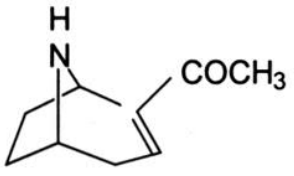


*Fig. 5 Structural formula of anatoxin-a*

### 3.2 Acetylcholine Receptor Antagonists

The best-known acetylcholine receptor agonists are belladona alkaloids atropine and scopolamine, natural anticholinergics of some Solanaceae (*Atropa bella-dona, Hyoscyamus niger, Datura stramonium*) that have been used as both medicinal and hallucinogenic agents for centuries. Ancient Romans and Egyptians used these substances to dilute the pupils of young girls and enhance their beauty, hence the name „belladona". These two alkaloids were also used as poisons during the Roman Empire and in the Middle Ages (11). Another acetylcholine receptor antagonist is represented by snake toxin entitled cobratoxin, principal toxic compounds of Monocled cobra venom.

#### *Atropine*

Atropine, a naturally occurred alkaloid (Fig. 6) of some *Solanceae*, is a competitive antagonist of muscarinic cholinergic receptors (10). It is absorbed from the gastro-intestinal tract, and is excreted in the urine. Atropine undergoes hepatic metabolism and has a plasma half-life of 2-3 hours. Atropine decreases bronchial and salivary secretions, blocks the bradycardia associated with some drugs used in anaesthesia such as halothane, suxamethonium and neostigmine, and also helps to prevent bradycardia from excessive vagal stimulation. Typical toxic effects are dry mouth, dysphagia, thirst and dryness of skin, excitement, confusion, hallucinations, fever, rapid pulse and respiratory rate, pupils are dilated. Death for respiratory failure is uncommon, within 15 hours. Only one mg may causes toxic symptoms in man, but a lethal dose for children is above 10 mg and for adult above 100 mg.

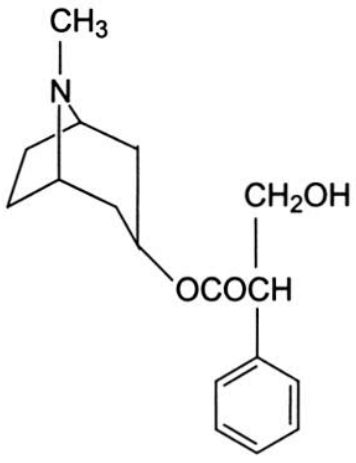


*Fig. 6 Structural formula of atropine*

#### *Scopolamine*

Scopolamine is tropane alkaloide (Fig. 7) isolated from *Datura metel, Scopola carnioloca* and other *Solanaceae*. The compound is rapidly absorbed through mucous membranes and skin. Scopolamine produces inhibition of structures innervated by postganglionic cholinergic nerves and stimulation of the central nervous system followed by depression. Typical approves of intoxication are auditory and visual hallucinations attend by headache, vertigo, manic excitement of short duration leading to depression (27). Large doses produce vertigo, tinnitus with blurring speech, restlessness and excitement, tonic convulsions and respiratory arrest. A lethal dose for man may be as low as 8 to 10 mg.

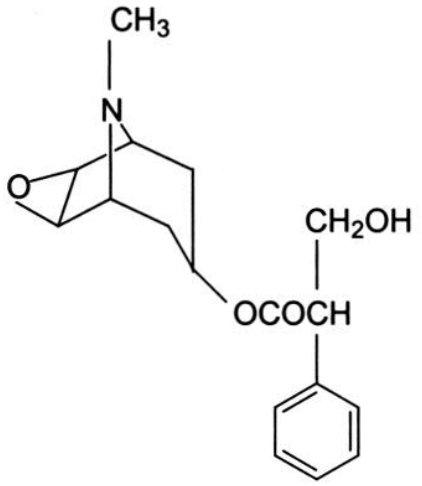


*Fig. 7 Structural formula of scopolamine*

#### *Cobratoxin*

Cobra neurotoxins are a family of toxic protein isolated from the venom of the snake *Naja naja siamensis* (Monocled cobra). Long neurotoxin 1 known as alpha-cobratoxin is composed from 71 amino acids cross-linked by five disulfide chains between cysteins 3-20, 14-41, 26-30, 45-56, and 57-62 (66, 36). Cobratoxins produce peripheral paralysis by blocking neuromuscular transmission. It is binded to the nicotinic acetylcholine receptor. $LD_{50}$ is 0.1 mg/kg by intravenous injection to mice.

### 3.3 Acetylcholine Releasing and Neuromuscular Junction Inhibitors

#### *Erabutoxin*

Erabutoxin is toxic protein from the sea snake *Laticauda semifasciata* which is known under the names Broad-banded sea-snake or Erabu sea-snake. Erabutoxin is composed from 83 amino acids

cross-linked by 4 disulfide chains between cysteins 24-45, 38-62, 64-75, and 76-81 (61, 38). We know erabutoxins A, B, and C, which have little differences in amino acid sequences (54, 25). Little differences also exist between Japanese and Philippines sea snake Laticauda semifasciata (61). Erabutoxin produces peripheral paralysis by blocking neuromuscular transmission on the postsynaptic site and binds to the nicotinic acetylcholine receptors. Its $LD_{50}$ is 0.15 mg/kg by intramuscular injection to mice.

### *Notexin*

Notexin is a presynaptic neurotoxin from the venom of Australian tiger snake *(Notechis scutatus scutatus)* (23). Notexin is a protein which consists of a single chain of 119 amino acids cross-linked by seven disulfide bridges and has a formula molecular weight of 13.58 kDa. Disulfide bridges are located between cysteins 11-71, 27-118, 29-45, 44-99, 51-92, 60-85 and 78-90 (15). Notexin catalyzes the calcium-dependent hydrolysis of the 2-acyl group 3-SN-phosphoglycerides and inhibits the release of acetylcholine at the neuromuscular junction and is directly toxic to skeletal muscle upon local application in vivo (dystrophic effect). The $LD_{50}$ is 25 μg/kg by intravenous injection to mice.

### Textilotoxin

Textilotoxin is a presynaptic neurotoxin from the venom of the Australian common brown snake, *Pseudonaja textilis* (63). It has the highest lethality and is structurally the most complex of any known snake venom neurotoxins (45, 46). It is composed from five non-covalently linked subunits A, B, and C, and two identical covalently linked D subunits. All subunits are necessary for maximum lethality. $LD_{50}$ is 1μg/kg by i.p. injection to mice.

Textilotoxin A chain is composed from 118 amino acids cross-linked by seven disulfide bridges between cysteines in positions 11-71, 27-117, 29-45, 44-98, 51-91, 60-84, and 78-89. Molecular weight of the A chain is 13.849 kDa. Textilotoxin A subunit is a potent presynaptic neurotoxin possessing phospholipase activity and is lethal to mice at 4 mg/kg i.v. It is essential for the neurotoxicity of textilotoxin. Textilotoxin A has catalytic activity and catalyzes the calcium-dependent hydrolysis of the 2-acyl--groups in 3-SN-phosphoglycerides.

Textilotoxin B chain contains 121 amino acids and seven disulfide bridges in positions 11-72, 27-120, 29-49, 44-101, 51-94, 61-87, and 80-92. Its molecular weight is 13.798 kDa.This subunit is not neurotoxic itself, but it is essential for the neurotoxicity of textilotoxin. Subunit B possesses a very low phospholipase activity.

Textilotoxin C chain is composed from 118 amino acids cross-linked by seven disulfide bridges in positions 11-71, 27-117, 29-49, 44-98, 51-91, 60-84, and 78-89, molecular weight of 13.010 kDa. Subunit C possesses a very low phospholipase activity.

Textilotoxin D chain is composed from 133 amino acids cross-linked by six disulfide bridges (19-85, 35-132, 37-53, 52-113, 59-106, and 69-99), two D chains are connected by disulfide bridge between cysteins in position 23 of each D subunit. Molecular weight of D subunit is 14.923 kDa. Also subunit D is not neurotoxic itself, but it is essential for the neurotoxicity of textilotoxin.

Separation of textilotoxin into its subunits is reversible and reformed textilotoxin has the same molecular weight and lethality in mice as the natural toxin (63).

### *Crotoxin*

Crotoxin, the major toxin of the venom of the South American rattlesnake, *Crotalus durissus terrificus*, is made of two subunits: component B, a basic and weakly toxic phospholipase $A_2$, and component A, an acidic non-toxic protein that enhances the lethal potency of component B (2). Crotoxin is a mixture of similar crotalid presynaptic neurotoxins that results from the association of several isoforms of its two subunits (19). Several isoforms of subunit A and two isoforms of subunit B were isolated and compared to purified and characterized subunit isoforms from pooled venom (18).

Component A comprises three peptides that are cross-linked by seven disulfide bridges between cysteins in positions 42-131, 44-60, 59-111, 65-138, 66-104, 73-97, and 911-102. It has 138 amino acids and molecular weight 15.968 kDa. Inhibits neuromuscular transmission by blocking acetylcholine release from the nerve terminals and acts presynaptically.

### *Bungarotoxin*

Bungarotoxins represent a group of neurotoxic proteins from the venom of the banded of Formosan krait *Bungarus multicinctus*. Alpha-bungarotoxin blocks nicotinic acetylcholine receptors and has been used to isolate and study them. Beta- and gamma bungarotoxins act presynaptically causing acetylcholine release and depletion (53).

Beta-bungarotoxin is known in four forms: A1 chain, A2 chain, A3 chain, and A4 chain.The A1 chain is composed from 120 amino acids cross--linked by seven disulfide bridges in positions 27-119, 29-45, 44-100, 51-93, 61-86, and 79-91 of molecular weight 13.489 kDa. Beta-bungarotoxin A1 chain is dimer of dissimilar chains linked by one disulfide bond between cysteins in position 15 of both subchains A and B (35). This neurotoxin inhibits neuromuscular transmission by blocking

acetylcholine release from the nerve termini and acts presynaptically. The A1 chain was found in beta-1 and beta-2 bungarotoxins. $LD_{50}$ is 0.019 mg/kg by intraperitoneal injection in beta-1 bungarotoxin and 0.028 mg/kg in beta-2 (1).

Beta-bungarotoxin A2 chain is composed from 145 amino acids, six disulfide bridges (52-144,54-70, 69-118, 86-111, and 104-116), molecular weight 16.296 kDa and its two subchains A and B are connected by disulfide bridge between cysteins in positions 40 (35). The A2 chain was found in beta-3 and beta-4 bungaroroxins. The chains have phospholipase $A_2$ activity and the B chains show homology with the basic protease inhibitor. Neurotoxin inhibits neuromuscular transmission and blocking acetylcholine release at nerve terminals. $LD_{50}$ is 0.006 mg/kg by intraperitoneal injection in beta-3 bungarotoxin and 0.073 mg/kg in beta-4.

Beta-bungarotoxin A3 chain has similarly as A1 chain 120 amino acid residues and six disulfide bridges (27-119, 29-45, 44-100, 51-93, 61-86, and 79-91) and two subchains A and B are connected in positions 15 of both chains by disulfide bridge. Its molecular weight is 13.439 kDa. The A3 chain was found in beta-5 bungarotoxin. It inhibits neuromuscular transmission and acetylcholine release similarly as both A1 and A2 chains. Its $LD_{50}$ is 0.13 mg/kg by intraperitoneal application to mice (35).

Beta-bungarotoxin A4 chain is composed from 147 amino acids, molecular weight of 16.177 kDa, with six disulfide bridges (54-146, 56-72, 71-127, 78-120, 88-113, and 106-118) and one disulfide bridge in position 42 of both subchains A and B which connectes it with heterodimer. The A4 chain has phospholipase $A_2$ activity and the B chains show homology with the basic protease inhibitors (17).

### *Botulotoxin*

Botulinum toxin is toxic protein from *Clostridium botulinum*. There are seven antigenically distinct forms of botulinum neurotoxins: Types A, B, C1, D, E, F, and G. All belong to peptidase family M27 (zinc proteases) and they are also known as a tetanus/botulinum neurotoxin subfamily.

Botulotoxins are disulfide-linked heterodimers of a light chain (L) and heavy chain (H). The light chain has the pharmacological activity, while the N- and C-terminal of the heavy chain mediate channel formation and toxin binding, respectively. For example, G type botulotoxin has 1296 amino acids (molecular weight = 14.013 kDa). Amino acids from 1 to 441 create light chain and from 442 to 1296 create heavy chain (12).

Botulinum toxin acts by inhibiting neurotransmitter release. It is binded to peripheral neuronal synapses, internalized and moved by retrograde transport up to the axon into the spinal cord where it can move between postsynaptic and presynaptic neurons. It inhibits neurotransmitter release by acting as a zinc endopeptidase.

## 3.4 Acetylcholinesterase inhibitors

### *Physostigmine*

Physostigmine is the first inhibitor of cholinesterases known to a man. Physostigmine, also called eserine, is an alkaloid obtained from the leguminous plant Calabar bean - a dry, ripe seed of perennial plant in the tropical West Africa *Physostigma venenosum* Balf. The Calabar bean once was used by native tribes as an „ordeal poison" in trials for witchcraft (30). One year later, it was obtained in a crystalline form be Vee and Le Ven (64). Physostigmine as N-methylcarbamate (Fig. 8) is a strong pseudoirreversible inhibitor of cholinesterases (51).

The toxicity of physostigmine varies with the route of administration and species. $LD_{50}$ values of physostigmine in various species of animals (mouse, rat, guinea-pig, rabbit) and various routes of administration varied between 1.0 (mice. i.p.) to 11.2 mg/kg (rabbit, p.o.). Physostigmine has been widely employed for various therapeutic purposes. Recently, it is promising drug for the treatment of dementias, especially of the Alzheimer's disease (42). Physostigmine is also considered to be a potent prophylactic antidote for organophosphate poisoning (37).

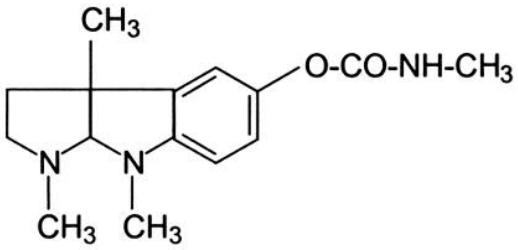


*Fig. 8 Structural formula of physostigmine (eserine)*

### *Galanthamine*

Galanthamine is an alkaloid that has been isolated from the bulbs of the caucasia snowdrop *Galanthus woronowii* (48) and the common snowdrop *G. nivalis* (9). In some regions of Bulgaria, Anatolia in Turkey and the Caucasus mountain range, the *Galanthus* species have been used for hundreds of years to treat painful neurological disturbances such as facial neuralgia. Chemically, galanthamine is tetracyclic tertiary amine derived

from benzofurobenzazocine (Fig. 9) and its compound was as identified as a cholinergic agonist and a reversible inhibitor of acetylcholinesterase (8, 29). The affinity constant $K_i$ for electric eel acetylcholinesterase was 0.12 μM (20). The compound can penetrate the blood-brain barrier, enabling it to activity in the central nervous system. Detailed kinetic studies of the interactions of galanthamine with acetylcholinesterase have been performed by Russian workers (58). The pharmacology of galanthamine is very similar to other acetylcholinesterase inhibitors (24). Galanthamine is very prospective drug for a treatment of Alzheimer's disease (49).

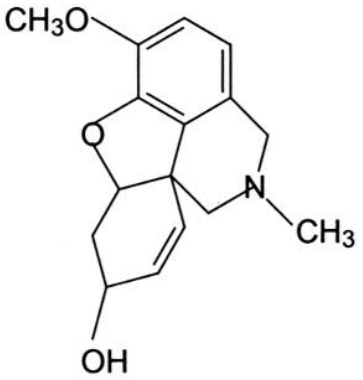


*Fig. 9 Structural formula of galanthamine (galantamine)*

### *Huperzine A*

Huperzine A is alkaloid which was prepared from the moss *Huperzia serrata*, the traditional Chinese herbal medicine called Qian Ceng Ta. It is an unsaturated sesquiterpenic compound with pyridone moiety and primary amino group (Fig. 10) of molecular weight 242 Da. Chemically, 9-amino-13--ethylidene-11-methyl-4-azatricyclo[7.3.1.0(3.8)]trideca-3(8),6,11-trien-5-one (Fig. 10). The compound is optically active and in the moss, only its (-)-enantiomer (21) is present.

Huperzine A is a potent reversible inhibitor of acetylcholinesterase, $K_i$ = 20-40 nM for mammalian acetylcholinesterases. An analysis of the affinities of structural analogues of huperzine A correlated with their interactions with the protein. It shows the importance of individual hydrophobic interactions between huperzine A and aromatic residues at the active-site gorge of acetylcholinesterase (50).

Huperzine A has a similar action as drugs currently approved to treat Alzheimer's disease, i.e. inhibits brain acetylcholinesterase and blocks the breakdown of acetylcholine, a chemical messenger in the brain that is essential to memory function (67). Huperzine A is a candidate drug against Alzheimer's disease as well as a candidate drug against organophosphate nerve agent toxicity for its advantageous pharmacokinetic properties and low toxicity (22).

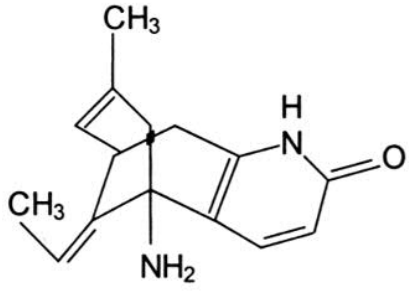


*Fig. 10 Structural formula of huperzine A*

### *Anatoxin-a(s)*

Anatoxin-a(s) is a neurotoxic alkaloid associated with the blue-green alga *Anabaena flos-aquae* clone NRC 525-17 (40) and is different from anatoxin-a, a known depolarizing agent produced by the same alga, clone NRC 44-1 (52). Anatoxin-a(s) is chemically organophosphate agent derived from imidazoline (Fig.11) and inhibits cholinesterases by phosphorylation their active site (39, 43), similarly as organophosphate nerve agents. In vitro inhibition of electric eel acetylcholinesterase and horse serum butyrylcholinesterase by anatoxin-a(s) was time- and concentration-dependent. The inhibition of both cholinesterases follows first order kinetics, indicative of irreversible inhibition (39). Anatoxin-a(s) given intraperitoneally to rats at doses from 0.1 to 1.0 mg/kg caused signs of severe cholinergic overstimulation as salivation, lacrimation, urinary incontinence, defecation, convulsion, fasciculation, and respiratory arrest (41). The potent toxicity of anatoxin-a(s), $LD_{50}$ = 20-40 μg/kg in mice, is just attributed to exceptional anticholinesterase activity. Anticholinesterase-producing cultures of *Anabaena ssp.* were isolated from some Danish lakes during 1993-1995 when many birds died from cyanobacterial toxicosis (26).

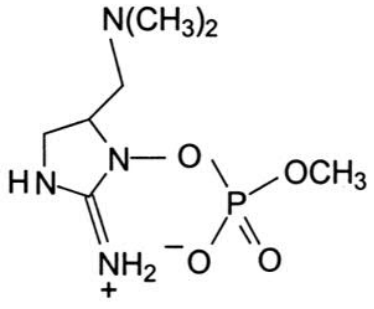


*Fig. 11 Structural formula of anatoxin-a(s)*

### *Fasciculin*

Two toxins that are potent inhibitors of acetylcholinesterase have been isolated from the venom of the snake green mamba, *Dendroaspis angusticeps* (16). The toxins have been called fasciculins after injection into mice (i.p. 0.5-3 mg/kg body weight) they cause severe, generalized and long-lasting fasciculations (5-7 h) (44). Fasciculins have 61 amino acid residues and four disulfides between cysteins 3-22, 17-39, 41-52, and 53-59. The sequences of the two toxins differ probably only in one position by a replacement of Tyr with Asn in position 47 (65). 1 g of venom contained about 40 mg of fasciculins, 2/3 of which was fasciculin 2. A similar inhibitor was isolated from *Dendroaspis polylepis* (black mamba) venom. The sequence of fasciculin 2 is in Fig. 12. Most of the positive charges are concentrated in a small section of the central part of the molecule, and most of the negative charge are in the C-terminal region. Fasciculins appear to have a dipole character.

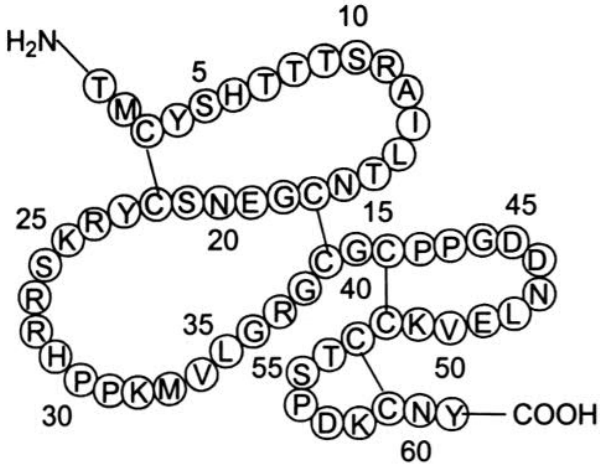


*Fig. 12 Amino acid sequence of fasciculin 2*

*In fasciculin 1 the asparagine in position 47 is changed by the tyrosine.*
*One-letter symbols for the amino acids are used:*
*A = Ala, C = Cys, D = Asp, E = Glu, F = Phe, G = Gly, H = His, I = Ile, K = Lys, L = Leu, M = Met, N = Asp, P = Pro, Q = Gln, R = Arg, S = Ser, T = Thr, V = Val, W = Trp, and Y = Tyr.*

Fasciculin 2 inhibits acetylcholinesterase from human erythrocytes ($K_i$ = 1.1 x $10^{-10}$ M), rat muscle ($K_i$ = 1.2 x $10^{-10}$ M), and electric eel ($K_i$ = 3.0 x $10^{-10}$ M). Fasciculin is binded to the peripheral anionic site, since it can displace propidium, a probe for that site, from acetylcholinesterase (33). The two arginine residues, Arg 24 and Arg 37, probably participate in the binding to acetylcholinesterase and fasciculin covers a large area of the enzyme (13). Fasciculin 2 also inhibits butyrylcholinesterase, but with a much lower affinity ($K_i$ = 3.0 x $10^{-6}$).

When fasciculin 2 was injected into the right striatum of albino rats in total amount of 1.5 μg, the inhibition of acetylcholinesterase activity was 86 and 60 per cent 24 h and 7 days after injection, respectively (14).

Fasciculins are not the only toxic peptides in mamba venoms and there are not the most toxic substances. Characteristic trait of mamba venoms is their synergism. For instance, the $LD_{50}$ of *D. angusticeps* venom is reported to be between 1 to 3 mg/kg mouse (28), but the most lethal component isolated from the venom has $LD_{50}$ value of 23 mg/kg (32). Mamba toxins of subgroup I are anticholinesterases and may therefore act in synergism with facilitatory toxins of subgroup II, since the increase of acetylcholine amount in both subgroups is available?

## 5. Conclusions

Many natural compounds, for example plant alkaloids as well as different peptides which are toxic principle animal venoms, influenced cholinergic nervous system at several levels. These compounds very often appear as very strong neurotoxins (cholinotoxins).

Today it is commonly accepted that central cholinergic nervous system plays an important role in many physiological and behavioral functions in animals and humans. The cholinergic system has been implicated in a wide variety of behaviors, aggression, exploration, social play, odor, aversion, depression, sleep, memory, etc. It is thus readily apparent that the cholinergic system is involved in many different behavioral functions and that its role in neural activities is complex and widespread. Many natural toxins affect via intervention to cholinergic nervous system. In the last decade a new area of neuropsychopharmacological investigation covers the role of cholinergic system in learning and memory which reached the cholinergic hypothesis of geriatric memory dysfunction. Some neurological disorders connected with cognitive decline are associated with decreased central cholinergic transmission.

The number of compounds which influenced cholinergic transmission of various types and various pharmacological effects is very high. As we show, many of them are very toxic and dangerous, but many of them are also very important therapeutics for many diseases.

#### References

1. ABE, T. - ALEMA, S. - MILEDI, R.: Phospholipase activity in beta-bungarotoxin action. Eur. J. Biochem., 1977, vol. 80, p. 1-12.
2. AIRD, SD., et al.: Rattlesnake presynaptic neurotoxins: primary structure and evolutionary origin of the acidic

subunit. Biochemistry, 1985, vol. 24, p. 7054-7058.
3. AIRD, SD., et al.: The amino acid sequence of the acidic subunit B-chain of crotoxin. Biochim. Biophys. Acta, 1990, vol. 1040, p. 217-224.
4. AKAIKE, A.: Ionic mechanisms involved in muscarinic regulation of neuronal and paraneuronal activity. Jpn. J. Pharmacol., 1992, vol. 58, p. 83-93.
5. AMAR, M., et al.: Agonist pharmacology of the neuronal alpha 7 nicotinic receptor expressed in Xenopus oocytes. FEBS Lett., 1993, vol. 327, p. 284-288.
6. ARMITAGE, AK.: Turner DM. Absorption and metabolism of nicotine from cigarettes. Br. Med. J., 1975, vol. 4, p. 313-316.
7. BENOWITZ, NL.: Pharmacology of nicotine: Addiction and therapeutics. Ann. Rev. Pharmacol. Toxicol., 1996, vol. 36, p. 597-613.
8. BOISSIER, JR. - COMBES, G. - PAGNY, J.: La galanthamine, puissant cholinegique naturel. Ann. Pharm. Fr., 1960, vol. 48, p. 888-900.
9. BOIT, HG.: Über die Alkaloide der Zwiebeln von Galanthus nivalis (III. Mitteil. Über Amaryllidaceae Alkaloide). Chem. Ber., 1954. Jg. 87, S. 724-725.
10. BRIMBLECOMBE, RW. - GREEN, DM.: The peripheral and central action of some anticholinergic substances. Int. J. Neuropharm., 1968, vol. 7, p. 15-21.
11. BROWN, JH.: Atropine, scopolamine and related anti-muscarinic drugs. In Goodman Gilman A., et al. (Eds.), Goodman and Gilman's the Pharmacological Basis of Therapeutics. New York, Pergamon Press, 1990, p. 150-167.
12. CAMPBELL, K. - COLLINS, MD. - EAST, AK.: Nucleotide sequence of the gene coding for Clostridium botulinum (Clostridium argentinense) type G neurotoxin: genealogical comparison with other clostridial neurotoxins. Biochim. Biophys. Acta, 1993, vol. 1216, p. 487-491.
13. CERVENANSKY, C. - ENGSTRÖM, A. - KARLSSON, E.: Role of arginine residues for the activity of fasciculin. Eur. J. Biochem., 1995, vol. 229, p. 270-275.
14. BOLIOLI, B., et al.: Neurochemical and behavioral correlates of unilateral striatal acetylcholinesterase inhibition by fasciculin in rats. Brain Res., 1989, vol. 504, p. 1-6.
15. CHWETZOFF, S., et al.: On the purification of notexin. Isolation of a single amino acid variant from the venom of Notechis scutatus scutatus. FEBS Lett., 1990, vol. 261, p. 226-230.
16. DAJAS, F., et al.: Rat striatal acetylcholinesterase inhibition by fasciculin (a polypeptide from green mamba snake venom). Neurosci. Lett., 1987, vol. 77, p. 87-91.
17. DANSE, JM. - GARNIER, JM. - KEMPF, J.: cDNA deduced amino acid sequence of a new phospholipase from Bungarus multicinctus. Nucleic Acid Res., 1990, vol. 18, p. 4610-4611.
18. FAURE, G., et al.: *Bon* the origin of the diversity of crotoxin isoforms in the venom of Crotalus durissus terrificus. Eur. J. Biochem., 1994, vol. 223, p. 161-164.
19. FAURE, G., et al.: Multiplicity of acidic subunit isoforms of crotoxin, the phospholipase $A_2$ neurotoxin from Crotalus durissus terrificus venom, results from posttranslational modifications. Biochemistry, 1991, vol. 30, p. 8074-8083.
20. FRIESS, SL., et al.: Some toxicologic properties of the alkaloids galanthamine and securinine. Toxicol. Appl. Pharmacol., 1961, vol. 3, p. 347-357.
21. GEIB, SJ. - TÜCKMANTEL, W. - KOZIKOWSKI, AP.: Huperzine A - a potent acetylcholinesterase inhibitor of use in the treatment of Alzheimer's disease. Acta Crystallogr. C, 1991, vol. 47, p. 824-827.
22. GRUNWALD, J., et al.: Huperzine A as a pretreatment candidate drug against nerve agent toxicity. Life Sci., 1994, vol. 54, p. 991-997.
23. HALPERT, J. - FOHLMAN, J. - EAKER, D.: Amino acid sequence of a postsynaptic neurotoxin from the venom of the Australian tiger snake Notechis scutatus scutatus. Biochimie, 1979, vol. 61, p. 719-723.
24. HARVEY, AL.: The pharmacology of galanthamine and its analogues. Pharmac. Ther., 1995, vol. 68, p. 113-128.
25. HATANAKA, H., et al.: Tertiary structure of erabutoxin B in aqueous solution as elucidated by two-dimensional nuclear magnetic resonance. J. Mol. Biol., 1994, vol. 240, p. 155-166.
26. HENRIKSEN, P., et al.: Detection of an anatoxin-a(s)-like anticholinesterase in natural blooms and cultures of cyanobacteria/blue-green algae from Danish lakes and in the stomach contents of poisoned birds. Toxicon, 1997, vol. 35, p. 901-913.
27. INCH, TD. - BRIMBLECOMBE, RW.: Antiacetylcholine drugs: Chemistry, stereochemistry an pharmacology. Int. Rev. Neurobiol., 1974, vol. 16.
28. IRWIN, RL., et al.: Toxicity of elapid venoms and and observation in relation to geographical location. Toxicon, 1970, vol. 8, p. 51-54.
29. IRWIN, RL. - SMITH, HJ.: Cholinesterase inhibition by galanthamine and lycoramine. Biochem. Pharmacol., 1960, vol. 3, p. 147-148.
30. JOBST, J. - HESSE, O.: Über die Bohne von Calabar. An. Chem. Pharm., 1864, vol. 129, p. 115-121.
31. JONES, GMM., et al.: Effects of acute subcutaneous nicotine on attention, information processing and short-term memory in Alzheimer's disease. Psychopharmacology, 1992, vol. 108, p. 485-494.
32. JOUBERT, FJ. - TALJAARD, N.: Snake venoms. The complete primary structures of two reduced and S-carboxymethylated angusticeps-type toxins from *Dendroaspis angusticeps* (green mamba) venom. Biochim. Biophys. Acta, 1980, vol. 623, p. 449-456.
33. KARLSSON, E. - MBUGUA, PM. - RODRIGUEZ-ITHURRALDE, D.: Fasciculins, anticholinesterase toxins from the venom of the green mamba *Dendroaspis angusticeps*. J. Physiol. (Paris), 1984, vol. 79, p. 232-240.
34. KONDO, K. - TODA, H. - NARITA, K.: Characterization of phospholipase A activity of beta1-bungarotoxin from Bungarus multicinctus venom. II. Identification of the histidine residue of beta1-bungarotoxin modified by p-bromophenylacyl bromide. J. Biochem., 1978, vol. 84, p. 1301-1308.
35. KONDO, K., et al.: Amino acid sequences of three beta--bungarotoxins (beta3-, beta4-, and beta5-bungarotoxins) from Bungarus multicinctus venom. Amino acid substitutions in the A chain. J. Biochem. (Tokyo), 1982, vol. 91, no. 5, p. 1519-1530.
36. LE GOAS, R., et al.: Alpha-cobratoxin: proton NMR assignments and solution structure. Biochemistry 1992, vol. 31, p. 4867-4875.
37. LIM, DK., et al.: Prevention of soman toxicity after the continuous administration of physostigmine. Pharmacol. Biochem. Behav., 1989, vol. 31, p. 633-639.
38. MAEDA, N. - TAMIYA, N.: Correction of partial amino acid sequence of erabutoxins. Biochem. J., 1977, vol. 167, p. 289-291.
39. MAHMOOD, NA. - CARMICHAEL, WW.: Anatoxin-a(s), an anticholinesterase from the cyanobacterium Anabaena flos--aquae NRC-525-17. Toxicon, 1987, vol. 25, p. 1221-1217.
40. MAHMOOD, NA. - CARMICHAEL, WW.: The pharmacology of anatoxin-a(s), a neurotoxin produced by the freshwater cyanobacterium Anabaena flos-aquae NRC 525-17. Toxicon, 1986, vol. 24, p. 425-434.
41. MAHMOOD, NA. - CARMICHAEL, WW. - PFAHLER, D.: Anticholinesterase poisoning in dogs from a cyanobacterial (blue-green algae) bloom dominated by Anabaena flos--aquae. Ann. J. Vet. Res., 1988, vol. 49, p. 500-503.
42. MARTA, M., et al.: New analogs of physostigmine: alternative drugs for Alzheimer's disease? Life Sci., 1988, vol. 43, p. 1921-1928.
43. MATSUNAGA, S. - MOORE, RE. - NIEMCZURA, WP.: Anatoxin-a(s), a potent anticholinesterase from Anabaena flos-aquae. J. Am. Chem. Soc., 1989, vol. 111, p. 8021-8023.
44. OSMAN, OH. - ISMAIL, M. - EL-ASMAR, MF.: Pharmacological studies of snake (*Dendroaspis angusticeps*) venom. Toxicon, 1973, vol. 11, p. 185-192.
45. PEARSON, JA., et al.: Studies on the subunit structure of textilotoxin, a potent presynaptic neurotoxin from the venom

of the Australian common brown snake (Pseudonaja textilis). 2. The amino acid sequence and toxicity studies of subunit D. Biochim. Biophys. Acta, 1991, vol. 1077, p. 147-150.
46. PEARSON, JA., et al.: Studies on the subunit structure of textilotoxin, a potent presynaptic neurotoxin from the venom of the Australian common brown snake (Pseudonaja textilis). 3. The complete amino-acid sequences of all the subunits. Biochim. Biophys. Acta, 1993, vol. 1161, p. 223-229.
47. PICKWORTH, WB. - BUNKER, EB. - HENNINGFIELD, JE.: Transdermal nicotine: reduction of smoking with minimal abuse liability. Psychopharmacology, 1994, vol. 115, p. 9-14.
48. PROSKURNINA, NF. - YAKOVLEVA, J.: Alaloids of Galanthus woronowii. J. Gen. Chem. USSR, 1952, vol. 22, p. 1899-1902.
49. RAINER, M.: Galanthamine in Alzheimer's disease. A new alternative to Tacrine? CNS Drugs, 1997, vol. 7, p. 89-97.
50. RAVES, ML., et al.: Structure of acetylcholinesterase complexed with the nootropic alkaloid, (-)-huperzine A. Nat. Struct. Biol., 1997, vol. 1, p. 57-63.
51. SOMANI, SM. - DUBE, SN.: In vivo dose response relationship between physostigmine and cholinesterase activity in RBC and tissues of rats. Life Sci., 1989, vol. 44, p. 1907-1915.
52. STEVENS, DK. - KRIEGER, RI.: Effect of route of exposure and repeated doses on the acute toxicity in mice of the cyanobacterial nicotinic alkaloid anatoxin-a. Toxicon, 1991, vol. 29, p. 134-138.
53. SUTCLIFFE, MJ. - DOBSON, CM. - OSWALD, RE.: Solution structure on neuronal bungarotoxin detrmined by two--dimensional NMR spectroscopy: Calculation of tertiary structure using systematic homologous model building, dynamical simulated annealing and restrained molecular dynamics. Biochemistry, 1992, vol. 31, p. 2962-2970.
54. TAMIYA, T., et al.: Cloning and sequence analysis of the cDNA encoding a snake neurotoxin precursor. Biochimie, 1985, vol. 67, p. 185-189.
55. TAYLOR, SE. - AL-HASHIMI, I.: Pilocarpine, an old drug, a new formulation. Tex. Dent. J., 1996, vol. 113, p. 9-13.
56. THOMAS, GAO. - RHODES, J. - GANESH, SA.: Transdermal nicotine for active ulcerative colitis. N. Engl. J. Med., 1994, vol. 330, p. 811-815.
57. THOMAS, P., et al.: (+)-Anatoxin-a is a potent agonist at neuronal nicotinic acetylcholine receptors. J. Neurochem., 1993, vol. 60, p. 2308-2311.
58. TONKOPII, VD. - PROZOROVSKII, VB. - SUSLOVA, IM.: Interaction of reversible inhibitors with catalytic centers and allosteric sites of cholinesterases. Bull. Exp. Biol. Med., 1976, vol. 82, p. 1180-1183.
59. IPATHI, ON.: Arecoline induced nicotinic and muscarinic stimulation of the superior cervical ganglion of cat. Biomed. Biochim. Acta, 1983, vol. 42, p. 275-282.
60. TSERNOGLOU, D. - PETSKO, GA.: Three-dimensional structure of neurotoxin A from venom of the Philippines sea snake. Proc. Natl. Acad. Sci. USA, 1977, vol. 74, p. 971-974.
61. TSERNOGLOU, D. - PETSKO, GA. - TU, AT.: Protein sequencing by computer graphics. Biochim Biophys Acta 1977, vol. 491, p. 605-608.
62. TURSKI, L., et al.: Review: cholinergic mechanisms and epileptogenesis. The seizures induced by pilocarpine: a novel experimental model of intractable epilepsy. Synapse, 1989, vol. 3, p. 154-171.
63. TYLER, MI., et al.: Studies on the subunit structure of textilotoxin, a potent neurotoxin from the venom of the Australian common brown snake (Pseudonaja textilis). Biochim. Biophys. Acta, 1987, vol. 915, p. 210-216.
64. VEE, M. - LE VEN, M.: De l'alcaloide de la feve de calabar et experiences physiologiques avec ce meme alcaloide. J. Pharm. Chimie, 1865, vol. 1, p. 70-72.
65. VILJOEN, CC. - BOTES, DP.: Snake venom toxins. The purification and amino acid sequence of toxin $F_{VII}$ from *Dendroaspis angusticeps* venom. J. Biol. Chem., 1973, vol. 248, p. 4915-4919.
66. WALKINSHAW, MD. - SAENGER, W. - MAELICKE, A.: Three--dimensional structure of the „long" neurotoxin from cobra venom. Proc. Natl. Acad. Sci. USA, 1980, vol. 77, p. 2400-2404.
67. ZHI, QX. - YI, FH. - XI, CT.: Huperzine A - a novel promising acetylcholinesterase inhibitor. Neuroreport, 1996, vol. 8, p. 97-101.

*Correspondence: Doc. RNDr. Jiří Patočka, DrSc.*
*Vojenská lékařská akademie J. E. Purkyně*
*Třebešská 1575*
*500 01 Hradec Králové*

Received: 19. 10. 1998