NATURAL CHOLINOTOXINS

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Summary

Natural toxins represent interesting and recently very studied group of substances with great practical meaning. Especially toxins with targeting to cholinergic nervous system, which plays an important role in many physiological and behavioral functions in animals, represent meaningful group of neurotoxins with wide spectrum of clinical use. These cholinotoxins are the aim of this paper.

Natural toxins, biotoxins, are chemical agents of biological origin, present in the bodies of many organisms. Venoms of toxic plants and animals are known and used for different purposes by native civilizations in all continents. Biotoxins have evolved in organisms over many thousands of years to have very often unique and interesting chemical structure and no less interesting and specific pharmacological effects. Toxins with specific affinity to the structure of nervous system are known as neurotoxins and some of them with the affinity to cholinergic nervous system could be sign as cholinotoxins and resumption of them is the theme of this article. Cholinotoxins are pharmacologically interesting compounds which have been used scientifically to elucidate physiological mechanisms in cholinergic nervous system or as the starting point in the development of new therapeutic agents or even as therapeutically applicable agents. All known natural cholinotoxins can be divided into four principal groups: Acetylcholine receptor agonists, acetylcholine receptor antagonists, inhibitors of acetylcholine releasing and compounds with effect on neuromuscular junction, and inhibitors of cholinesterases (16).

Acetylcholine receptor agonists of natural origin are some toxic plant alkaloids. **Arecoline**, main alkaloid of Betel nut from *Areca catechu*, which is used by various ethnic groups, including those from India, South-East Asia and Africa, particular manner analogous to chewing tobacco. This tetrahydropyridine derivative is pharmacologically characterized as a gangliomimetic compound which stimulates the both muscarinic and nicotinic acetylcholine receptors (6). **Pilocarpine** from the leaves of Jaborandi bushes (*Pilocarpus jaborandii*), growing in the rainforests of Brazil, is more than 120 years used for the treatment of glaucoma, due to reducing the intraoccular pressure. This alkaloid causes bronchoconstriction and enhances tone and motility of the ureters, the urinary bladder, the gallbladder, the billiary ducts, and stimulates exocrine glands (25). **Muscarine,** furane derivative from mushroom *Amanita muscaria*, is pharmacologically similar to pilocarpine on smooth muscle and exocrine glands. Muscarine can cause a decrease of blood pressure and slowing down the heartbeat, gastro-intestinal irritation, vomiting, abdominal pain and diarrhoea, perspiration, salivation, lacrimation constriction of pupils and blurring of vision (9). **Nicotine** is alkaloid of leaves and stems of the *Nicotiana* species. Its chemical structure is derived from pyridine and pyrrolidine. Nicotine binds to nicotinic acetylcholine receptors and the drug has a characteristic curare action on skeletal muscle (3). **Anatoxin-a** and **homoanatoxin-a** are strong cholinotoxins produced by the freshwater cyanophyte *Anabaena flos-aquae*. Structurally, both compounds are similar to tropane alkaloids; both are strong neurotoxins and potent depolarizing neuromuscular blocking agents, more potent than nicotine (26). **Epibatidine** is an animal alkaloid from Equadorian poison-dart frog *Epipedobates tricolor*, very strong selective agonist of some types of nicotinic acetylcholine receptors (18), likewise **anabaseine**, one of toxic compounds from the venom of North American ant *Aphaenogaster rudis* (14). Numerous selective agonist of nicotinic acetylcholine receptors are occur in venom of cone shell moluscs. There are short peptides, so-called **conotoxins** (11).

Acetylcholine receptor antagonists of natural origin are known only as belladona alkaloids atropine and scopolamine from some *Solanaceae*. **Atropine** is a competitive agonist of muscarinic acetylcholine receptors, decreases bronchial and salivary secretions, blocks the bradycardia associated with some drugs used in anesthesia and helps to prevent bradycardia from excessive vagal stimulation. **Scopolamine** produces inhibition of struc-

tures innervated by postganglionic cholinergic nerves and stimulation of the central nervous system followed by depression. Other reversible and competitive antagonist with high affinity for neuronal nicotinic acetylcholine receptors is **methyllycaconitine**, C_{19}-diterpenoid alkaloid from plants of the *Delphinium* genus, *Ranunculaceae* (8). **Cobratoxin**, let us say cobratoxins, are a family of toxic peptides isolated from the venom of the snake *Naja naja siamensis* (Monocled cobra). Alpha-cobratoxin is composed from 71 amino acids cross-linked by five disulfide chains. Cobratoxins produce peripheral paralysis by blocking neuromuscular transmission.

Acetylcholine releasing and neuromuscular junction inhibitors of natural origin are largely toxic peptides from venom of different snakes, only best-known toxin of this type, botulotoxin, is product of anaerobic microorganism. **Botulinum toxin (botulotoxin)** is toxic protein from the *Clostridium botulinum*. There are seven antigenically distinct forms of botulinum neurotoxins: Types A, B, C1, D, E, F, and G. Botulotoxins are disulfide-linked heterodimers of a light chain (L) and heavy chain (H). The L chain has the pharmacological activity, while the H chain mediate channel formation and toxin binding, respectively. Botulotoxin acts by inhibiting neurotransmitter release. It is bind to peripheral neuronal synapses, internalized and moved by retrograde transport up to the axon into the spinal cord where it can move between presynaptic and postsynaptic neurons (7). **Bungarotoxins** represent a group of neurotoxic proteins from the venom of the banded of Formosan krait *Bungarus multicinctus*. Only beta- and gamma-bungarotoxins act presynaptically causing acetylcholine release and depletion, while alpha-bungarotoxin blocks nicotinic acetylcholine receptors (24). **Crotoxin** is the major toxin of the venom of the South American rattlesnake, *Crotalus durissus terrificus*, protein composed from two subunit: component B, a basic and weakly toxic phospholipase A_2, and component A, an acidic non-toxic protein enhancing the lethal potency of component B (2). **Notexin** is presynaptic neurotoxin from the venom of Australian tiger snake *Notechis scutatus scutatus*. It is a protein, which consists of a single chain of 119 amino acids cross-linked by seven disulfide bridges (4). **Textilotoxin** is presynaptic neurotoxin from the venom of the Australian common brown snake, *Pseudonaja textilis*. It has the highest lethality and is structurally the most complex of any known snake venom neurotoxin. 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 (27). **Erabutoxin** is toxic protein from the sea snake *Laticauda semifasciata* and is composed from 83 amino acids cross-linked by four disulfide chains (5).

Acetylcholinesterase inhibitors of natural origin represent a numerous group of chemically very different compounds. Some inhibitors of this very important neuronal enzyme are used as cognitive enhancers in the treatment of Alzheimer disease. **Physostigmine** (eserine) is the best-known substance of this type. It is an alkaloid from the leguminous plant *Physostigma venenosum* known as Calabar bean, remedy used in West Africa as an "ordeal poison" in trials for witchcraft. Physostigmine as N-methylcarbamate is a strong pseudoirreversible inhibitor of acetylcholinesterase (AChE) and has been widely employed for various therapeutic purposes, including Alzheimer disease treatment (22). This alkaloid has been also a model for preparation great many of modern therapeutics. **Galantamine** is an alkaloid from snowdrop (*Galanthus woronowii*, *G. nivalis*) and reversible inhibitor of AChE (23), as well as alkaloid **huperzine A** from the Chinese moss *Huperzia serrata*, the traditional Chinese herbal medicine called *Qian Ceng Ta* (16). **Anatoxin-a(s)** is a neurotoxic alkaloid and as yet the only one organophosphate compound found in nature, product of fresh water blue-green alga *Anabaena flos-aquae* (12). It is ireversible inhibitor of AChE, likewise **onchidal**, lipophilic acetate ester from molluscs *Onchidella binneyi*, *O. nigricans* or *O. patelloides* (1). Other natural inhibitors of AChE are **pseudoazoanthoxanthin** and **parazoanthoxanthin A** strongly fluorescent pigments occurring in zoanthids (20) and **evodiamine** and **dehydroevodiamine** from the plant *Evodia rutaecarpa* (14). Other sort of antiacetylcholinesterase compounds are for example **territrems**, tremorgenic mycotoxins isolated from *Aspergillus terreus* (10), **arisugacins** from some *Penicillium* (13) or interesting group of toxic **3-alkylpyridinium polymers** from the marine sponge *Raniera sarai* (21).

Hitherto quite unique is the discovery of protein with antiacetylcholinesterase activity. It is a family of peptides with 61 amino acid residues and four disulfide bridges, isolated from the venom of mamba

snail (*Dendroaspis angusticeps*) entitled **fasciculins**. They are very strong inhibitors of AChE and poisoning in mammals proceed under an image of "nerve gas" intoxication (19).

It is commonly accepted that central cholinergic 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, aversion, depression, social play, sleep, memory, etc. Neuropsychopharmacological investigations in the last decade illumine the role of cholinergic system in cognitive functions of brain and bring evidence that some neurological disorders connected with cognitive decline are associated with decreased central cholinergic transmission. Natural cholinotoxins whose effects via intervention on cholinergic nervous system represent meaningful group of drugs, which represent hopeful family of new therapeutics.

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