NATURAL CHOLINOTOXINS AND PROGRESS IN PSYCHPHARMACOLOGY

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Summary

The paper reviews the impact of natural cholinotoxin research on the recent knowledge of brain cholinergic neurotransmission, which represents the biological background for learning and memory processes as well as for various psychiatric disorders. Several natural cholinotoxins helped to analyze cholinergic receptor functions on molecular level; others served as models for the synthesis of agents with perspective use in psychopharmacology.

Naturally occurring cholinotoxins have played an important part in the physiological research of the neurotransmission in the cholinergic system. Already at the beginning of the 20th century, when acetylcholin (ACh) has been identified as cholinergic neurotransmitter, experiments using two plant alkaloids with ACh resembling effects, *muscarine* and *nicotine,* helped to distinguish two main classes of ACh receptors, which differed both in function and localization and were named after mentioned cholinotoxins. It was found that **muscarinic receptors**, which are activated by *muscarine* and blocked by *atropine* or *scopolamine,* are present on cells of smooth muscles and glands, on autonomic ganglion cells and on CNS neurons. **Nicotinic receptors,** which are activated by *nicotine* and blocked by *curare,* are localized at the motor endplate of vertebrate skeletal muscle, on autonomic ganglion cells and CNS neurons. The use of several other natural cholinotoxins with specific binding affinities (*epibatidine, anabasein, bungarotoxin, methyllycaconitine, tubocurarine, mamba toxins*) in combination with recent progress in molecular biology and autoradiographical labelling techniques led to the discovery of various receptor subtypes in both ACh receptor classes and enabled more detailed analysis of cholinergic neurotransmission processes in physiological as well as pathological conditions. Model experiments with *scopolamine* and *atropine* or natural Ach esterase inhibitors *physostigmine* and *galantamine* carried out both in animals and man indicated the significance of the central cholinergic system for the neuro-psycho-behavioural reactivity. This was the reason for increased attention which has been paid in last years to the research of potential relations between disturbed or deviated central cholinergic neurotransmission and various neuropsychiatric diseases (e.g. Alzheimer's disease, Parkinson's disease, schizophrenia, Tourette's syndrome, drug addiction) with the aim to develop rational and effective tools for psychopharmacological therapy.

The majority of studies - both experimental and clinical - concern Alzheimer's disease (AD) and the pivotal role of the central cholinergic system impairment in the cognitive decline and behavioural deterioration of AD patients. The neuropathobiology of AD is characterized by the degeneration of the basal forebrain cholinergic projections to the neocortex, hippocampus and amygdala, β-amyloid plaque formation and hyperphosphorylated tau protein deposition in the brain. Specific deviations were found in brain muscarinic and nicotinic receptors in AD.

Muscarinic ACh receptor (mAChR) belongs to the family of G-protein-coupled receptors. There are five subtypes of mAChRs (M-1 to M-5), which were identified using *mamba toxins* (8). They control a large number of physiological processes such as the function of heart and smooth muscles, glandular secretion, release of neurotransmitters and cognitive functions. The brain imaging methods have shown that in AD, there is a deficit of M2 subtype mAChRs (10, 11) placed mostly on presynaptic cholinergic terminals which undergo to progressive degeneration in AD. On the other hand, postsynaptic M1 mAChRs are relatively preserved. Consequently, stimulation of these M1 mAChRs with selective agonists (1, 5) as e.g. xanomeline (2, 15) was used as a therapeutic strategy for AD to increase central cholinergic neurotransmission. Later investigation indicated that the beneficial effect of these M1 mAChR agonists in AD is wider as they

modulate the processing of amyloid precursor protein and tau phosphorylation and thus prevent or retard β-amyloid plaque formation and neurofibrillary tangle deposition in AD brains (5, 6).

Nicotinic Ach receptors (nAChRs) are ligand gated cation channels, which have a pentameric structure composed of five mebrane spanning subunits. Multiple subunit izoforms were identified (with substantial aid of natural cholinotoxins *epibatidine, anabaseine, bungarotoxin, methyllycaconitine)* named with Greek letters in order of their molecular size -α(1-9), β(1-4), γ, δ. Different combinations of these izoforms in one heterooligomeric complex provide the basis for heterogeneity of structure and function observed in nAChR subtypes. For brain nAChRs, subunits α3, α4, α7 in combination with β2 are characteristic, whereas nAChRs in the muscle are composed mainly of subunits α1 β1 γ δ (12). Neuronal nAChRs are involved in processes of attention and learning and - via presynaptic location on different neurons - in the release of various neurotransmitters (Ach, dopamine, serotonin, GABA, glutamate) (4, 9, 12). Unlike other receptor agonists, repeated administration of nicotine in experimental animals as well as in man results in up-regulation of brain nAChRs (7, 10). Activation of central nAChRs (α7, α4 β2) was reported to have neurotrophic (3) and neuroprotective (6) effects.

Studies on nAChR distribution in the brain of AD patients - both in necroptic brains and *in vivo* using non-invasive imaging techniques such as PET and SPECT - indicated significant reduction of nAChRs in the hippocampus and prefrontal cortex concerning mostly α4 β2 subunits (9, 10, 12). Deficits of neuronal nAChRs were described also in Parkinson's disease (α6) and in schizophrenia (α7). Interesting are the relations between tobacco smoking (nicotine intake) and psychiatric pathologies. Some epidemiological studies carried out in familiary AD cohorts reported that the onset of AD was postponed by several years in those family members who were smokers (13, 16). These findings initiated special therapeutic strategy in AD: sustained transdermal delivery of nicotine using nicotine patches available for smoking cessation treatment (17). Similarly, a relief of Parkinsonic or schizophrenic symptomes was described following cigarette smoking (9). These effects may be explained by nicotine-induced up-regulation of neuronal nAChRs and facilitation of dopamine release. There are, however, important objections against the use of nicotine in either form for neuropsychiatric preventive or therapeutic strategy: a) the action of nicotine is non-selective as it activates all nAChRs (neuronal, muscular, ganglional) and may cause serious undesirable side-effects; b) in tobacco smoking, the risk of unfavourable sequels (cardiovascular damage, pulmonal carcinoma) due to toxic products of burning exceeds the potential benefit of nicotine. On the other hand, selective agonists of nAChR subtypes the activation of which could be useful in relevant psychiatric disorder, might represent promising way of treatment. The development of this type of agents which in addition would penetrate easily into the brain and have low general toxicity, should be the aim of future psychopharmacological research.

One way of AD therapy, which is nowadays widely used, consists in blocking ACh-degrading enzyme - ACh-esterase (AChE) - by AChE inhibitors and thus increases and prolongs the action of Ach in the synaptic cleft. Besides known synthetic AChE inhibitors (tacrine, donepezil, exelon), there are two plant alkaloids - natural cholinotoxins - with this activity: *physostigmine* and *galantamin.* Recently, several clinical studies confirmed the efficacy of *galantamine* in improving the symptoms of AD (14). Its beneficial effects may be due to its dual mechanism of action: it functions not only as ACHE inhibitor, but also as allosteric modulator of nAChRs.

As it is evident from this review, the study of natural cholinotoxins contributed in many respects to the analysis of brain functioning, both in physiological and pathological conditions. In addition, the chemical structures of these natural cholinotoxins have served as an inspiration for the synthesis of new, more adequate tools for the neurophysiological research as well as for the development of novel psychotropic drugs.

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