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Review Article

Nicotinic acetylcholine receptors: Diversity and physiological importance for neurodegenerative disorders and development of organophosphate antidotes

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Abbreviations:
ACh – acetylcholine
AChE – acetylcholinesterase
AChR – acetylcholine receptor
CHRN – nicotinic acetylcholine receptor gene
COMT – catechol-*O*-methyltransferase
DA – dopamine
GABA – gamma-aminobutyric acid
IP3/DAG – inositol trisphosphate/diacylglycerol
L-DOPA – *L*-3,4-dihydroxyphenylalanin (levodopa)
mAChR – muscarinic acetylcholine receptor
MAO-B – monoamine oxidase B
NA – noradrenaline
nAChR – nicotinic acetylcholine receptor
NMDA – *N*-methyl-*D*-aspartate
OP – organophosphorus compound
VTA – ventral tegmental area

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Abstract

The communication between the nervous and other systems in the organism is carried out by the transmission of nerve impulses. Diverse neurotransmitters are released into the synaptic cleft and bind to the specific receptors at the neighbouring cell to transmit the signal further. One of such receptors are nicotinic acetylcholine receptors (nAChR), integrated membrane proteins (ligand-gated ion channels) activated by the binding of a neurotransmitter acetylcholine. nAChR's main characteristic is their diversity, as they consist of five of the same or mutually different subunits, which contribute to the specific receptors properties and biological activity. During the assembly of a pentameric protein structure, various combinations of subunits are linked together. After the discovery of nAChR's involvement in various diseases, they became an important therapeutic target, for example in the treatment of neurodegenerative diseases (Alzheimer's and Parkinson's) and in the treatment of organophosphorus compound poisoning. This paper presents an overview of current knowledge on nicotinic receptors and an accompanying discussion on diseases, poisonings, potential drugs and treatments is given.

INTRODUCTION

By studying nicotine at the turn of the 20th century, the concept of *receptive substance* was set up, from which the idea of a receptor was developed *(1)*. A crucial event in nicotinic receptor studies was the chemical identification of the first neurotransmitter receptor. Successful identification allowed for further metabolic, pharmacological and biochemical research *(2)*. Nicotinic acetylcholine receptors (nAChRs) are proteins integrated into membranes and belong to the superfamily of ligand-gated ion-channels *(3, 4)*. Since they are formed by a specific combination of five subunits, there are different subtypes of nAChRs that mediate different physiological functions *(5)*. Nicotinic receptors are expressed in the peripheral nervous system (PNS), where they mediate the transmission of impulses between nerve and muscle cells; in the central nervous system (CNS) and in other types of cells (keratinocytes, epithelial cells, macrophages, *etc.*; *(5)*). After understanding that the subtypes of nAChR contributed to the neuropathology of many diseases, there arose an interest in the development of therapeutic nicotine agonists and other drugs for specific subtypes and the use of cloned nAChR subunits as possible therapeutic agents *(1)*. nAChRs have be-

come an important therapeutic targets for the treatment of neurodegenerative diseases, primarily Alzheimer's and Parkinson's *(5)*, but also in the treatment of poisoning with highly toxic organophosphorus compounds *(6)*.

Historical overview

After studying nerve-muscle preparations, John Newport Langley found that muscle tissue possesses something that mixed with nicotine and *curare* (reversible inhibitor of nAChR) receives stimulus and transfers it to other cells *(7)*. In the 1960s, David Nachmansohn and other scientists identified the receptor for acetylcholine (ACh) in the electric eel (*Electrophus electricus*) electric organ by radioactive ligands, which was unusually rich in nicotinic synapses *(8)*. In addition, Chen-Yuan Lee with snake venom, α-bungarotoxin (α-Bgt), specifically inhibited *in vivo* nerve-muscle transmission without interaction with acetylcholinesterase (AChE, EC 3.1.1.7, an enzyme that hydrolyses the ACh neurotransmitter in synapses). It has been shown that nAChR is a hydrophobic protein of high molecular weight and can be physically separated from AChE, and studies then confirmed that AChE and nAChR are different protein entities *(9)*. In 1972, significant influence on the investigation of nicotinic acetylcholine receptors was the isolation of the new generation of nAChR from homogenates of marbled electric ray (*Torpedo marmorata*) *(10)*. In 1973, the purified protein nAChR was observed under a microscope and rings-like structures (with a hydrophilic core linked to compact clusters) were detected. Such a compact cluster was made up of several subunits *(11)*. A group of scientists in 1988 was able to generate 3D crystals of *Torpedo* nAChR for the first time *(12, 13)*. Soon thereafter, the physiology and a variety of nicotinic receptors expression in the vertebrates was determined, including five different subunits that could be assembled in different ratios *(14)*. Also, in the 1990s, nicotinic receptors and nicotine were associated with various diseases, such as schizophrenia *(15)*, Alzheimer's *(16)*, Parkinson's disease *(17)* and Tourette's syndrome *(18)* and research took a turn toward finding drugs and effective therapies for nAChR-related states. In association with various diseases, the effect of organophosphorus compounds (OP) on the cholinergic system was also observed *(6, 19)*. More precisely, the symptoms of OP poisoning are caused by the irreversible inhibition of the enzyme AChE, which leads to the accumulation of ACh and extensive receptor stimulation *(20, 21)*. In addition to the inhibition of AChE, several organophosphorus compounds directly affect the receptors of the cholinergic system and modulate the level of their receptor expression *(22, 23)*. OPs thus also affect nicotine receptors, block the opening of the ion channel and desensitize the receptors *(24–27)*.

Acetylcholine receptors and nerve effects

In general, acetylcholine receptors (AChR) are integrated membrane proteins, which are activated by neurotransmitter ACh binding. There are two types of such receptors: ionotropic nicotinic receptors (nAChRs), which exist in two forms (nervous and muscular), and five types of metabotropic muscarinic receptors (mAChR) M1 to M5, which can be classified into two traditional pharmacological groups: M1 and M2 receptors. The M1 group receptors (M1 and M3) interact via the IP3/DAG system (by activating phospholipase C), inactivate K^+ channels, while M2 (M2, M4 and M5) receptors regulate adenylyl cyclase (intracellular cAMP concentration) and activate K^+ channels *(28, 29)*. Like other transmembrane receptors, AChRs are classified according to their pharmacology or affinity and sensitivity to different compounds (Figure 1). Although all acetylcholine receptors, by definition, are sensitive to acetylcholine, they also react to other xenobiotics. For example, mAChRs are sensitive to muscarine *(30, 31)*.

nAChRs are ionic channels for Na^+, K^+ and Ca^{2+} and are particularly sensitive to nicotine. They are found in the central and peripheral nervous system, muscle and many other tissues *(32)*. In the nerve-muscle synapse, they are the primary receptors for communication with the muscles and controlling muscle contraction. In this way, the peripheral nervous system transmits outgoing signals from presynaptic to postsynaptic cells within the sympathetic and parasympathetic nervous system. In the immune system, nAChRs regulate inflammatory processes and participate in signalling different intracellular pathways *(1, 33, 34)*.

Full activation of a postsynaptic nAChR occurs when two molecules of the neurotransmitter ACh are bound to it. The cationic channel of the receptor is opened because of the electrochemical gradients through which Na^+ and

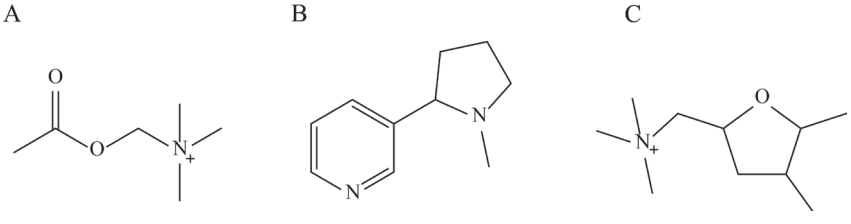

Fig. 1. *Structures: (A) acetylcholine, (B) nicotine and (C) muscarine*

K^+ flow. Every second a nerve vesicle releases neurotransmitters into the synapse. In addition to the acetylcholine receptors, there are many AChEs in the synapses, whose task is to hydrolyse the ACh, thus terminating the signalling *(35)*. The long-term binding of ACh to the receptor results in desensitization and thus inactivation of the ion channel. For example, irreversible AChE inhibitors lead to the accumulation of ACh in a synapse *(36, 37)*, and one molecule of ACh can stimulate a few additional openings of ion channels. This may also have its advantages; like in diseases where the number of nAChR is reduced and causes muscular weakness, AChE-inhibiting drugs enable signal reactivation with the same neurotransmitter molecule, thereby improving signalling and replacing the lack of receptors *(38, 39)*.

Types, localization and receptor structure

It has already been mentioned that nAChRs are ligand-gated ion channels forming pores on a cell plasma membrane mediating fast signal transmission through synapses. They are involved in a wide range of physiological processes and are divided into two groups: nerve and muscle *(40)*. Muscle nAChRs are localized in the nerve-muscle synapses, where the electrical nerve impulse signals contraction to the muscle cells and is responsible for muscle activity. Many different types of nerve nAChR are localized in synapses on postsynaptic nerve cells, as in the CNS, where they are involved in cognitive functions, learning, memory, *etc*. nAChR activation causes cation movement through the ion channel pore with the calcium ions which affect the release of neurotransmitters *(1, 41)*.

Table 1. *nAChR subunits subfamilies based on similarities in protein sequence (42)*

Subfamily	Subtype	Type	Subunits
I		Neuronal	$\alpha9$, $\alpha10$
II		Neuronal	$\alpha7$, $\alpha8$
III	1	Neuronal	$\alpha2$, $\alpha3$, $\alpha4$, $\alpha6$
	2	Neuronal	$\beta2$, $\beta4$
	3	Neuronal	$\beta3$, $\alpha5$
IV		Muscle	$\alpha1$, $\beta1$, δ, γ, ε

Studies revealed the existence of 5 different types of nAChR subunits with small molecular weight differences. Thus, nAChR consists of five subunit types: alpha ($\alpha1$-$\alpha10$), beta ($\beta2$-$\beta5$), gama (γ) delta (δ) and epsilon (ε); found in various combinations. The nAChR subunits have been grouped into 4 subfamilies (I-IV) based on similarities in protein sequence, and subfamily III has been further divided into 3 subtypes (Table 1) *(42)*. The most widely expressed nAChR subtypes are $\alpha4$, $\alpha7$ and $\beta2$ *(43)*. Nerve nAChRs are assembled just from alpha and beta units, while muscle nAChR from all five subunit types *(41, 44)*.

Nicotinic receptors are always pentamers (Figure 2), with subunits disposed symmetrically in a circle around the central receptor channel pore. The receptors always contain two or more α-subunits, which are crucial for ACh binding *(45, 46)*. The ACh binding site is formed

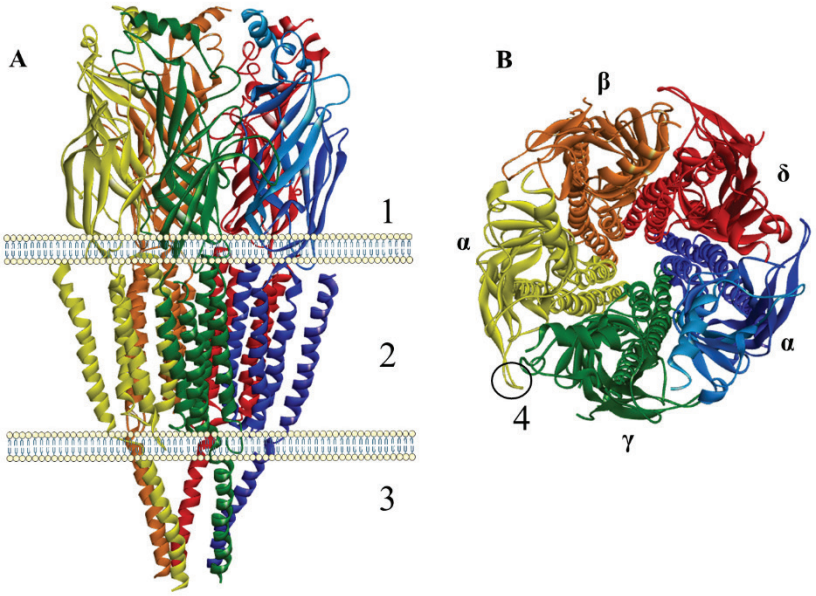

Figure 2. *The basic structure of nicotinic acetylcholine receptors. (A)* Torpedo marmorata *nAChR structure (model from PDB code 2BG9; 49) with extracellular (1), transmembrane (2) and intracellular (3) domains, (B) the receptor is formed from 5 subunits around the central pore with Cys-loop (4) in α subunit.*

when dimers of α-subunits and adjacent subunits associate *(47, 48)*.

Nerve nAChRs are divided into two main groups based on their sensitivity to the snake venom α-bungarotoxin. α-Bungarotoxin-sensitive receptors are homomers (α7 and α9), while α-bungarotoxin-non-susceptible receptors are heteromeric (other subunits types). Most nAChRs are asymmetric heteromers in which neither ACh binding sites nor subunit-subunit interactions are identical *(48, 49)*.

After years of experiments, the nicotinic receptor localization and structure, which contains the extracellular synaptic domain, four transmembrane α-helix and the intracellular cytoplasmic domain, have been proposed (Figure 2). Further analysis revealed the existence of so-called Cys-loops in nAChR structure *(2, 47)*. nAChR is also a glycosylated protein and has N-linked glycosylation sites which vary depending on the subunits. A cys-cys pair is required for agonist binding and its presence denotes the existence of an α-subunit *(41, 47)*. A Cys-loop is characteristic for this type of receptor and in mammals these cysteines are most often separated with 13 conserved amino acids. Other receptors that belong to the same receptor superfamily as nAChR include: 5-hydroxytryptamine (5-HT3), γ-aminobutyric acid type A ($GABA_A$) and $GABA_C$, glycine receptors, glutamate and histidine receptors *(50)*.

The variety of nicotine subunits

The diversity of nAChR subunits is the main feature of the specific properties and functions of mature receptors. Receptor pentamers can be assembled from various combinations of α, β and other structural subunits (*38, 41*). Nowadays, 16 genes encoding for nAChR subunits are known, named *CHRNA1–10* for α subunits, *CHRNB1–4* for β subunits, *CHRNG*, *CHRND* and *CHRNE* for γ, δ and ε, respectively (*1, 52, 53*). Different nAChR gene details are presented in Table 1. Mutations in *CHRN* genes (causing loss or gain of nAChR function, increased or decreased sensitivity to ACh or desensibilisation of the nAChR) can cause phenotypes with different manifestations such as congenital myasthenic syndrome, nocturnal frontal lobe epilepsy and multiple pterygiums (*54–56*). The genes *CHRNA3/CHRNA5/CHRNB4* or *CHRNB3/CHRNA6* are in gene clusters on chromosomes 15q25 or 8p11, respectively; while other *CHRN* genes are separately located on chromosomes 1, 2, 4, 8, 11, 15, 17 or 20 as shown in Table 2.

In some brain areas, diverse specific subtype subunits participate in order to form an nAChR of high affinity for substrates. In the *basal ganglia*, including the *ventral tegmental area* (VTA) and *substantia nigra*, the α6 and β3 nAChR subunits were included in α4β2 nAChR complexes to create a high affinity receptor for ACh (Table 3). Currently, these are the only areas in the brain where α6 and β3 subunits are co-expressed alongside the α4 subunit, which is very important for the appearance and therapy of Parkinson's disease *(57)*. The autonomic nervous system is characterized by an abundant expression of α3 and β4 nAChR, while expression of α4 and β2 subunits dominates the central nervous system. In example, coordinated expression of essential subunits is strongly regulated in the brain during the development of the organism and during various injuries *(58)*.

Table 2. *nAChR genes: subunit receptor name, gene names, chromosomal location, gene length (kb), gene cluster (if applicable), and number of aminoacids (AA) in expressed protein (1, 52)*

Subunit receptor	Gene name	Chromosome	Gene (kb)	Gene cluster	Protein (AA)
α1	*CHRNA1*	2q31.1	16.64		482
α2	*CHRNA2*	8p21.2	18.51		529
α3	*CHRNA3*	15q25.1	28.24	*CHRA3-A5-B4*	622
α4	*CHRNA4*	20q13.33	14.75		627
α5	*CHRNA5*	15q25.1	29.71	*CHRA3-A5-B4*	515
α6	*CHRNA6*	8p11.21	15.93	*CHRNB3-A6*	494
α7	*CHRNA7*	15q13.2	142.25		534
α9	*CHRNA9*	4p14	19.63		479
α10	*CHRNA10*	11p15.4	5.8		450
β1	*CHRNB1*	17p13.1	12.65		501
β2	*CHRNB2*	1q21.3	12.25		502
β3	*CHRNB3*	8p11.21	39.99	*CHRNB3-A6*	458
β4	*CHRNB4*	15q25.1	17.48	*CHRA3-A5-B4*	498
δ	*CHRND*	2q37.1	10.48		517
γ	*CHRNG*	2q37.1	6.6		517
ε	*CHRNE*	17p13.2	5.3		496

Table 3. *The presence of different α and β subunits in certain parts of the brain (*specific subtype; - not determined) (62).*

Localization	Type nAChR	Involved in specific neurotransmitter release
Amygdala	*α4β2*, *α7*	–
Cerebellum	*α4β2*, *α7*, *α3β3**, *α3β4**	Glu
Cortex	*α4β2*, *α4α5β2*, *α7*	Glu, ACh, DA
Hedial habenula	*α4β2*, *α3β2**, *α7*, *α3β3β4*, *α3β4**	–
Hippocampus	*α4β2*, *α4α5β2*, *α3β4*, *α7*	Glu, ACh, DA
Hypothalamus	*α4β2*, *α7*	NA
Interpeduncular nucleus	*α4β2**, *α7*, *α3β3β4*, *α3β4*, *α2β2**	ACh, GABA, NA
Locus coeruleus	*α3β4*, *α6β2β3**	–
Olfactor bulb	*α4β2*, *α7*	Glu, DA
Pineal gland	*α3β4*, *α7*	–
Rapche nuclei	*α4β2*	–
Spinal cord	*α4β2*, *α7*, *α3β2**	–
Striatum	*α4β2*, *α4α5β2*, *α6β2β3*, *α6α4β2β3*	Glu, DA
Substantia nigra, VTA	*α4β2*, *α4α5β2*, *α7*, *α3β4**, *α6β2β3*	–
Thalamus	*α4β2*, *α4α5β2*	DA, GABA

α7 are the most researched subunits of all known nicotinic subunits types. Receptors assembled of *α7* subunits have a high Ca^{2+}:Na^{+} bandwidth ratio *(59)*. As a result, the opening of the *α7* nAChR channel may cause several Ca^{2+}-dependent mechanisms, including the activation of secondary signal pathways *(60)*. Co-expression and assembly, i.e. binding of the *α7* nAChR subunit to other subunits, affects receptor ionic permeability, so nAChR consisting of *α7* and *β2* subunits has pharmacological properties different from those homozygous *α7* nAChR *(61)*.

Pentameric structures, unlike those with pair subunits structures (*e.g.*, tetramers), require multiple mechanisms for overcoming problems related to receptor assembly. Coordination of regulatory mechanisms between transcription and receptors assembly, which respond to external changes, are a common biological feature of nicotinic receptors. The first level of regulation of the region-specific expression of nAChR is transcriptional control of subunit expression.

Posttranslational modifications are crucial for the regulation of mature receptor functions, their expression and localization. There are several checkpoints in the cell to ensure proper assembly. Experiments with the chimeric recombinant subunits showed that the 23-amino acid region (from G23 to D46) contains the residues needed for proper *α7* subunit assembly to the homopentamer receptors and a Cys-loop is needed for proper domain folding *(63)*. Another significant control point for nAChR is the endoplasmic reticulum where degradation of proteins is an important part of regulating receptor concentration. In total, 80% of the synthesized subunits are mproperly assembled and never leave the endoplasmic reticulum, where they are degraded *(64)*. Reduction of the degradation of precursor subunits in the endoplasmic reticulum results in increased concentration of nAChR on the cell membrane *(65)*. Also, continuous nicotine exposure increases the concentration of nAChR on the surface of the cell by reduction of the degradation of the intracellular pool of receptors *(66)*. Another important posttranslational regulation is the modification achieved by N-glycosylation, where more sites in the NH_2-terminal domain of the nAChR subunit are glycosylated. It has been found that muscular nAChR glycosylation is not required for receptor assembly or the formation and functioning of Cys-loops *(67)*. Still, proper glycosylation is necessary for their subsequent incorporation into the plasma membrane *(68, 69)*. In addition, palmitoylation and phosphorylation are also mechanisms that regulate the expression, receptor function and binding efficacy of different nAChR subunits. Palmitoylation is a reversible posttranslational modification where the palmitate covalently binds to Cys residues in the endoplasmic reticulum *(70, 71)*. As opposed to that, in phosphorylation, phosphate binds to specific residues within the cytoplasmic domain *(72)*. Besides, as mentioned nicotinic receptors also possess the ability of increasing upregulation when exposed to high nicotine concentrations; still, some subtypes have a lower level of expression *(73)*.

Insight into the function of nAChR

Activation of mammalian nerve nAChR promotes the opening of an unselected cationic channel leading to Na^{+} flow, depolarization of the membrane and activation of Ca^{2+} channels (*49*). Ca^{2+} flow through nAChR or voltage-gated channels is essential for nicotine modulation, synaptic plasticity, nerve viability, differentiation and migra-

tion. In the nervous system expressing specific nAChR subtypes, nicotine mediates activation of several Ca^{2+} independent kinases including PI3K, protein kinase C (PKC), protein kinase A (PKA) and extracellular signal-regulatory kinase (ERK) *(74, 75)*. Also these kinases activate a large number of transcription factors by downstream regulation, such as cAMP-binding protein (CREB) that further activates transcription factor 2 (ATF-2) *(76)* and the ETS-like transcription factor (Elk-1), and transcript signal transducer (STAT3) *(77)*.

nAChR are present at essential regulatory sites and lead to multiple outcomes in sensitivity to exogenous stimuli or participation in neurodegeneration and inflammation. The presence of expressed nAChR in adipose tissue has provided additional research into the effects of nicotine on the body. It has shown that average smokers are skinnier and more likely to develop metabolic syndrome such as type II diabetes.

Also, patients with ulcerative colitis who stopped consuming tobacco developed more serious progression of the disease, *i.e.* nicotine had a protective effect. By contrast, patients with Crohn's disease improve disease severity if they smoke *(78)*.

However, small concentrations of nicotine protect cells from damage induced by β-amyloids, inflammation, alcohol, hypoxia, but mechanism of action is unknown (*40, 79, 80*). High nicotine concentrations increase the number of nAChRs, due to desensitization instead of enhanced receptor function. Nicotine effects on nAChR are complex at molecular level and it is difficult to separate their consequences at system level *(81)*.

Neurodegenerative disorders

The involvement of nicotinic receptors in many diseases, autoimmune responses and epilepsy, stimulated the development of nAChR subtypes of specific ligands as possible drugs *(80)*. Reduced function and/or nAChR expression in the brain is associated with the pathophysiology of for example Alzheimer's disease (AD) or Parkinson's disease (PD), severe, incurable, degenerative brain disorders *(40)*. Since the expression of the receptors cannot be induced easily, drug design for the treatment of neurodegenerative disorders is based on improving existing receptors' stimulation. One of the approaches is the use of nicotinic agonists or AChE inhibitors that are investigated to prevent present neurotransmitter acetylcholine from being degraded rapidly, which in turns enables activation of nAChR and the signal transmission to the next postsynaptic cell *(82)*.

AD progression (Figure 3) leads to dementia, memory loss, thinking impairment, and changes in behaviour and personality. AD and other forms of dementia affect about 47 million people worldwide, in Croatia about 86,000 *(83)*. The histopathology of the disease is well-known and is accompanied by a loss of cholinergic transmission, deposition of extracellular amyloid β-peptides (Aβ) in plaques, and hyperphosphorylation of τ-protein leading to excessive formation of neurofibrillary tangles *(84, 85)*. Also, receptor loss in Alzheimer's disease is much higher than in a normal aging brain *(86, 87)*. Specifically, the degree of loss of $\alpha4\beta2$ nAChR and $\alpha7$-expressed expression are well correlated with the progressive range of cognitive decline in patients with mild to moderate AD *(88)*. Two effects are important for cognitive improvement of AD, improvement of synaptic transfer and reduction of neurodegeneration *(89)*. Interestingly, $\alpha4$ nAChR expression decreases by 80% in AD *(90)*.

The simplest therapy to nAChR loss is the long-term use of nicotine *(91)* and in human clinical trials; nicotine alleviated AD symptoms *(92)*. Additionally, nicotine has shown beneficial effects on learning, memory, attention, and cognitive functions in patients with AD. Now, the focus of the development of AD therapy is on specific nAChR agonists (Table 4), which should have low affinity for muscle nAChR to reduce unwanted muscle stimu-

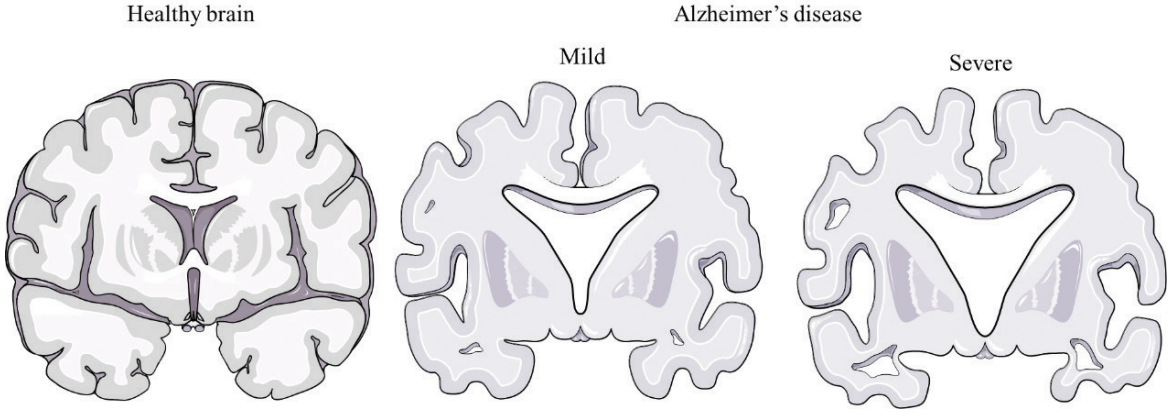

Figure 3. *A sketch of the difference in the brain structure of the healthy brain and in patients with Alzheimer's disease (mild or severe) accompanied by severe cortical shrinkage (according to (99) and (100)).*

lation. Two types of receptors, $\alpha 7$ and $\alpha 4\beta 2$, have become a target for AD treatment, since these receptors are expressed in the brain regions for memory and information processing. A crucial step in AD treatment could be the introduction of positive allosteric modulators (PAMs) to ensure multiple modulation of receptors to suppress disease *(93)*, as well as a combination of drugs based on synergistic mechanisms. For example, a combination of galantamine that would inhibit AChE and modulate nAChRs, curcumine that would inhibit β-secretase and τ-phosphorilation and rivastigmine to reversibly inhibit AChE; or a combination of tacrine, curcumine and huperzine A for the same reason *(94)*. Five medications are currently used to treat the cognitive problems of AD: four are acetylcholinesterase inhibitors (tacrine (withdrawn due to toxicity *(95)*), rivastigmine, galantamine and donepezil) and the other (memantine) is an NMDA receptor antagonist. The way to increase the function of nAChR in the brain without nicotine is to sensitize receptors using allosteric potentiating ligands (APLs) that include drugs such as physostigmine and galantamine, one of the approved drugs for the treatment of AD *(89)*. Second, noncompetitive agonists (NCAs) have agonistic activity and bind to a site close but still further than the ACh binding site on α-subunits of nAChR *(96)*. Although NCA encourages the opening of nAChR channels in many nerve and non-nerve cells, the likelihood of opening channels with such compounds is very low. Namely, galantamine acts as a nicotine NCA but not as a nicotine APL *(97)*. Subunit $\alpha 7$ is the only nAChR subunit in mammals with positively charged residues within the α118-140 segment that is assumed to be an APL binding site *(98)*.

Along with AD, Parkinson's disease (PD) is one of the most common neurodegenerative diseases in the world. It appears in all ethnic groups, in both sexes, although men are affected twice as much than women. The occurrence of illness increases with age and affects 1% of the population over 60. Symptoms usually begin to appear for the first time between the age of 50 and 65, but the disease may occur early in life, childhood or adolescence, albeit rarely. There are around 7-10 million people in the world suffering from Parkinson's disease, while in Croatia the number of registered people with the disease is 20,000 (*122*). Disease is caused by the degeneration of dopaminergic nerves of *substantia nigra* with symptoms of muscle stiffness, bradykinesia and blocking movement, loss of reflexes and tremors. Movement disorders are caused by a lack of dopamine-producing nerve cells, since dopamine is important in movement control. Current treatment for PD includes therapy with dopamine precursor L-DOPA (Figure 4), but neither does it stop the progression of the disease nor does its effectiveness last for a long time (*123*). nAChR-specific nicotine-based agonists are another direction in the development of therapy. One of the synthesized agonists, SIB-1765F, showed high affinity for $\alpha 4\beta 2$, and was as equally effective as nicotine in stimulating dopamine release. This agonist in combination with varenicline can attenuate brain overstimulation (*124*).

Table 4. *Various known nicotinic agonists, antagonists and allosteric modulators*

Name	Action	Specific target	Refs.
18-Methoxycoronaridine (18-MC)	antagonist	$\alpha 3\beta 4$	(*101*)
A-582941	agonist	$\alpha 7$	(*102*)
ABBF	agonist	$\alpha 7$	(*103*)
ABT-089[a]	agonist	$\alpha 4\beta 2$	(*104*)
ABT126[a]	agonist	$\alpha 7$	(*104*)
ABT-418[a]	agonist	$\alpha 4\beta 2$, $\alpha 3\beta 4$	(*104*)
AR-R 17779	agonist	$\alpha 7$	(*105*)
Atropine[b]	antagonist	–	(*106*)
AVL-3288[a]	allosteric modulator	$\alpha 7$	(*107*)
Benthiactzine[b]	antagonist	$\alpha 7$, $\alpha 4\beta 4$, $\alpha 4\beta 2$	(*108*)
CCMI	allosteric modulator	$\alpha 7$	(*104*)
Dihydro-β-erythroidine (DhβE)	antagonist	$\alpha 7$, $\alpha 3\beta 4$, $\alpha 4\beta 2$	(*109*)
Dizocilpine (MK-801)	antagonist	$\alpha 7$	(*110*)
DMPP	agonist	–	(*111*)
d-Tubocurarine (dTC)	antagonist	$\alpha 4\beta 2$, $\alpha 7$	(*112*)
Encenicline[a] (EVP-6124)	agonist	$\alpha 7$	(*113*)
GTS-21	agonist	$\alpha 7$	(*104*)
Ibogaine	antagonist	$\alpha 3\beta 4$	(*114*)
Ispronicline	agonist	$\alpha 4\beta 2$	(*104*)
MB266[b]	antagonist	–	(*106*)
Memantine	antagonist	$\alpha 7$	(*115*)
Metyllycaconitine (MLA)	antagonist	$\alpha 7$	(*116*)
NS-1738	allosteric modulator	$\alpha 7$	(*111*)
PNU-120596	allosteric modulator	$\alpha 7$	(*111*)
PNU-282987	agonist	$\alpha 7$	(*117*)
QNB[b]	antagonist	–	(*106*)
Sazetidine	agonist	$\alpha 4\beta 2$	(*110*)
SEN123333	agonist	$\alpha 7$	(*118*)
SSR-180711	agonist	$\alpha 7$	(*119*)
Tropisetron	agonist	$\alpha 7$	(*120*)
Vinblastine	antagonist	$\alpha 3\beta 4$	(*114*)
α-bungarotoxin	antagonist	$\alpha 7$	(*121*)

[a]in clinical trail phases (*104, 107, 113),* [b]for organophosphorus poisoning treatment (*106, 108*)

Other drugs that cause symptomatic relief and act by raising dopamine levels (L-DOPA, MAO-BI, COMT inhibitors), stimulating dopamine receptors (DA agonists), inhibiting cholinergic effects (anticholinergics) or inhibit-

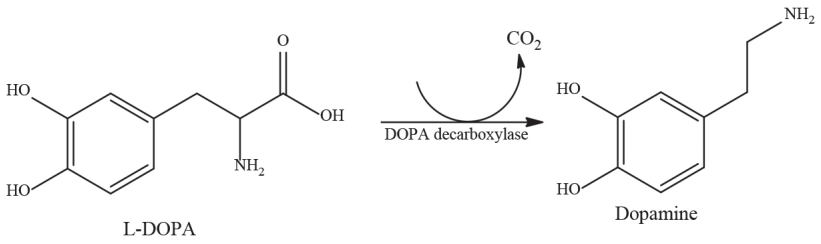

Figure 4. *L-DOPA precursor structure and decarboxylation in dopamine in the organism (123).*

ing glutaminergic NMDA receptors (amantadines). Thus, in such a system, L-DOPA replaces dopamine; COMT inhibitors maintain L-DOPA, which will metabolize and synthesize dopamine within the nerves, which can then be released into the synapse, MAO-B inhibitors serve to preserve existing dopamine and DA agonists mimic dopamine and bind to dopamine binding sites, as it is necessary to increase the dopamine concentration in PD patients. Interestingly, the $\alpha 6$ subtype was discovered in the *substantia nigra* and has become attractive as a pharmacological target for Parkinson's disease therapy *(125).* Epidemiological studies have shown that heavy smokers are less likely to have PD but also a whole spectrum of physiological changes are present in the function and expression of nAChR and desensitization *(126).* However, improvement of dopamine release was observed on rodents exposed to nicotine and degeneration of DA nerves was prevented *(57, 127).* The assumption is that nicotine mediates nerve protection because it prevents cell death, reduces symptoms, binds to nAChR and promotes expression of dopamine receptors in *substantia nigra (128).* The exact mechanism is not known yet, although nicotine effects are well explored.

Along with physiological changes, some studies suggest that DA and cholinergic systems operate in a dynamic balance, whose disturbance causes a variety of neurological disorders such as Parkinson's disease. DA-deficiency triggers a reduction in the efficacy of AChR, which result in increase in ACh release *(129).*

On the other side, an increased kinurenic acid (KYNA), the metabolite of tryptophan that is primarily produced in the brain and releases by astrocytes, follows neurodegenerative disorders and decreased activity/expression of nAChR. Acting as an endogenous activity regulator of $\alpha 7$ nAChR, KYNA can modulate synaptic transmission, synaptic plasticity, nerve endurance, and nervous connectivity in various brain regions *(130).*

Organophosphorus compounds poisonings and nAChRs

Organophosphorus compounds (OP) are a group of highly toxic xenobiotics used as pesticides, lubricants and oils for engines in the industry and as warfare nerve agents. The mechanism of their toxicity is the irreversible inhibition of the AChE, which participates in the regulation of nerve impulse transfer by hydrolising ACh (Figure 5). By chemical structure, OP compounds are esters, anhydrides or halides obtained by complete substitution of phosphorous, phosphonic and phosphinic acids. The base consists of a five-valent phosphorus atom coordinated by covalently bound oxygen or sulfur, and two different substituents (alkyl, aryl, alkoxy, alkylthio, aryloxy, mono- or dialkylamino) and the leaving group (fluorine, cyanide, *etc.*) *(131).*

Moshnin and Clermont synthesized the first OP compound, tetraethylpyrophosphate (TEPP). After some years, the potential of TEPP was recognized and it began being used as a pesticide. However, OP compounds showed to not be selective to species. During the Second World War, the toxic effects of OP compounds encouraged research and their use as chemical nerve agents. Soon, two series of nerve agents were developed: the G-series (tabun, sarin, soman and cyclosarin), and V-series (like VX, from *Venom, Victory or Viscous*) *(37, 132).* In the 1990s, the production, stockpiling and use of chemical weapons was prohibited by Organisation for the Prohibition of Chemical Weapons (OPCW), and existing stocks were ordered to be destroyed. Since then, the use of nerve agents for research purposes has been strictly regulated. Still, the use of nerve agents has been recorded several times in recent years by terroristic groups attacking civilians *(133).*

As mentioned before in the text, when OP inhibits AChE in the nervous system, the neurotransmitter acetylcholine (ACh) accumulates in the synaptic cleft and over-stimulates cholinergic receptors, affecting the appearance of some specific symptoms. The effects of excessive activation of receptors are muscular weakness, cramps and tremor, hypertension and tachycardia, with additional effects such as headaches, memory disorders, wakefulness, confusion, loss of reflexes, anxiety, insomnia and respiratory depression and paralysis *(134, 135).* The main cause of the death of poisoned patients is the failure of the respiratory system. It is caused by paralysis of respiratory muscle, bronchoconstriction in combination with increased secretion and depression of the respiratory centre *(136).* Moreover, death can occur within minutes of exposure to nerve agents. Since OPs pass through the blood-brain barrier, they also cause a number of other undesir-

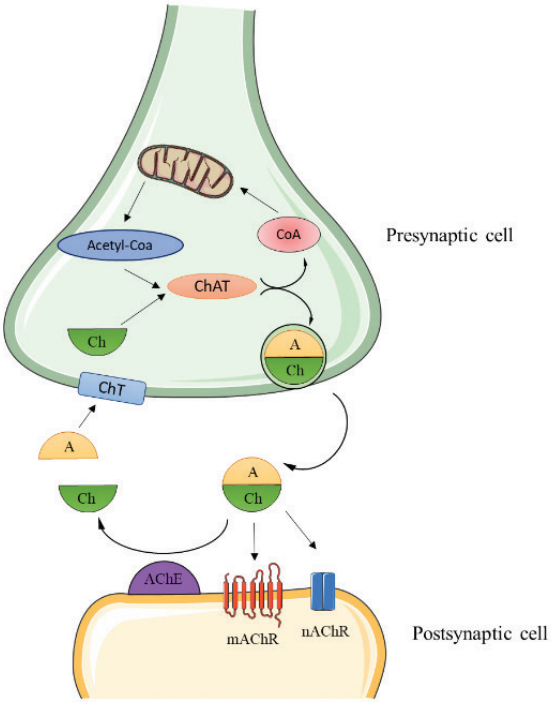

Figure 5. *Synthesis of ACh in the synapse and association with receptors (according to (136))*

able effects due to the action on receptors involved in the transmission of the impulse. Therapy for acute exposure to organophosphorus compounds is a combination of several different antidotes. Atropin is used as an antimuscarinic, ACh antagonist, which reduces the effects of ACh on muscarinic receptors. Compounds known as oximes are used as reactivators of inhibited AChE, which after reactivation reduces the concentration of accumulated acetylcholine in synaptic cleft. The third part of the therapy is administration of benzodiazepine to reduce convulsions and thus prevent brain damage (*137*). However, none of these drugs is effective enough and the effects of accumulated ACh overstimulating nAChRs persist.

There is ongoing research for selective nAChR inhibitors that could serve in therapy of OP poisonings. Scientists have tried different already available nicotinic antagonist drugs and ligands like δ-tubocurarine *(138).* Furthermore, ketamine and similar drugs also have specific anticholinergic inhibitory activity on α7-nicotinic receptors and potentiated testing of a new group of compounds as drug candidates for clinical trials *(139).* In addition, procyclidine (an anticholinergic drug principally used for the treatment of drug-induced parkinsonism) has been shown to be an antimuscarin, antinicotinic and anti-NMDA receptor drug *(140).* Although many drugs are available to test from, the research in this field of nicotine antagonists application is still ongoing and none of the candidates has been introduced into medical practice of treating OP poisoning yet.

Perspectives for future

nAChR isolation paved the way for the design of new pharmacologically active compounds. As they co-operate with other receptors during different physiological processes, it is important to consider them in a wider picture as well. More specifically, the relationship, linkage and communication between different nAChR subtypes and other receptors should be investigated in the future research. In addition, we need to consider that understanding the role that nAChR plays in regulating immune responses inside and outside the central nervous system under normal physiological conditions. Furthermore, the development of ligands that selectively enhance the activity of a particular nAChR subtype is crucial for the future design of drugs for the treatment of, for example, neurodegenerative diseases and OP poisonings. At the same time, the application of such drugs should be as simple, effective and fast as possible to improve existing therapies. In this search, novel *in silico*, *in vitro* and *in vivo* models available for studies of potential agonists and antagonist of nicotinic receptors, could provide an essential platform.

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