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## Chapter

# Role of Acetylcholine in Chronic Diseases

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## Abstract

The complex and extensive network of brain signals plays a vital role in maintaining physiological mechanisms and homeostasis. Acetylcholine, a chief neurotransmitter of the parasympathetic nervous system, is an important component of the cholinergic system along with cholinergic receptors, acetylcholinesterase, and choline acetyltransferase. It is responsible for mediating cell-to-cell communication and regulates various peripheral and non-neuronal cholinergic signals. Any alteration in the levels of acetylcholine leads to chronic diseases. Chronic diseases, the leading causes of disability, require continuing health care, medical attention, and potential therapeutics. This chapter will cover a brief overview of acetylcholine including its synthesis and degradation, the cholinergic system, and the influence of acetylcholine on different chronic diseases including neurological complications, metabolic disorders, cardiac diseases, and immune disorders. Moreover, the mechanistic approach of acetylcholine in different diseases and the therapies for recovering the levels of acetylcholine will be reviewed in this chapter. Further, this will illustrate the acetylcholine interaction with various cells implicated in the diseases. The insights on agonists and antagonists of acetylcholine and different targets of cholinergic receptors that could help to design better strategies to control these chronic diseases will also be provided.

**Keywords:** acetylcholine, chronic diseases, cholinergic, neurotransmitter, mechanistic approach, parasympathetic nervous system, treatment

## 1. Introduction

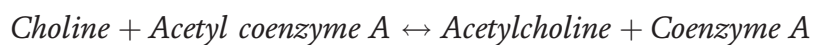
The most common and significant chemical of the nervous system capable of performing numerous roles within the anatomical framework of humans is Acetylcholine (Ach). In particular, it is termed as a chemical messenger liberated via nerve cell for broadcasting signals to other neurons and to other generalized cells, for instance, heart and glandular cells. It is also found at the junctions of neuron and muscles; on the ganglion of visceral motor system and in the numerous spots in Central Nervous System. Acetylcholine, the designated expression has been derived from its structural association as an “ester of acetic acid” and “choline”. Cholinergic tissues are those that use or respond to Ach present within a body while the chemicals that interfere with the influence of Ach on the body are referred to as anticholinergics.

Despite the fact of Ach's presence in body's several regions; a myoneural junction has been marked as a normally linked region. At this region, there is a synaptic association between efferent or motor nerve cell and myofiber. Ach is also been able to act as a chemical transmitter between the neurons of pre-ganglion and post-ganglion in an autonomic nervous system [1].

## **1.1 Biogenesis, retention, and discharge of acetylcholine**

### *1.1.1 Biogenesis*

Acetylcholine is produced from choline and acetyl coenzyme A, its two immediate precursors. The choline acetyltransferase (ChAT) enzyme catalyzes the synthesis reaction in a single step.



ChAT was first detected in 1943 in a cell-free based preparation, and which was cloned and purified from various sources [2]. ChAT purification has enabled the creation of selective antibodies targeted to a particular antigen. The enzyme, acetylcholinesterase (AChE), synthesized by the cells having the site for choline receptors and cholinergic neurons, is in control to degrade acetylcholine. The location of ChAT is mostly present in regions of the brain wherein the production of Ach occurs. ChAT is localized in the nerve endings within cholinergic neurons, but it is also present in axons, in which it is transferred out of its production in the cell body. The sub-cellular fractionation experiments revealed the recovery of ChAT in the synaptosomes and in the synaptosomal complex suggested the cytoplasmic nature of ChAT [3].

Acetyl CoA is generated from pyruvate, which is created from glucose in the mammalian brain. It is unknown how acetyl CoA, which is widely considered to be generated at the inner membrane of the mitochondria, reaches the cytoplasmic ChAT, and this might be a rate-limiting step.

### *1.1.2 Retention*

Following the production of ACh by ChAT at the nerve terminal, ACh is transported to storage receptacles [4]. Vesicular acetylcholine transporter (VACHT) has been cloned and expressed. Because of its sequence, it belongs to the 12-membrane-spanning family of biogenic amine transporters identified in adrenergic nerve terminals [5, 6]. Remarkably, the transporter gene is situated within an intron of the ChAT gene, implying the co-regulation of ChAT and VACHT. A proton-pumping ATPase drives ACh uptake in the vesicle while coupled H<sup>+</sup> and ACh counter transport permit the vesicle to maintain iso-osmoticity and electroneutrality [4].

Vesamicol selectively inhibits Ach transport with an IC<sub>50</sub> of 40 nM, inhibits vesicular ACh uptake [4–6]. Non-competitive mechanism of inhibition was found for vesamicol which means that it works on a location other than the transporter's ACh-binding site. Further, Vesamicol inhibits the induced release of freshly generated ACh while having no effect on the uptake of choline, synthesis of acetylcholine and high-affinity choline uptake, ACh synthesis, or inflow of calcium ions. The notion that the release of ACh is lost as a result of the vesicle's inhibition of absorption clearly implies that the vesicle is the source of ACh release. Moreover, Vesamicol also inhibits the expressed transporter from the cloned cDNA [5, 6].

### 1.1.3 Discharge

It is believed that more than 50% of total choline utilized in the production of acetylcholine, is derived straightforwardly from reprocessing of liberated acetylcholine, which is metabolized/hydrolyzed by cholinesterase to choline. This metabolically generated choline is likely to be swiftly absorbed before it diffuses away from the synaptic cleft. The disintegration of phosphatidyl-choline, which may be accelerated due to local production in Ach, is another source of choline. Choline produced from the two mentioned sources turned accessible to the space outside of the cell which is subsequently taken up by the nerve ending with high affinity. Due to the fact that choline is not able to cross blood brain barrier (BBB) when present in plasma, the metabolic origins of choline found to be more significant in the CNS. Accordingly, in the CNS, elevated choline absorption into the neurons of cholinergic system is not maximal or is saturated, suggesting the choline availability as the rate limiting step in the production of acetylcholine.

## 1.2 Cholinergic system

The cholinergic system consists of organized nerve fibers responsible for projecting nerve impulses, also known as action potentials through acetylcholine. The release of acetylcholine stimulates the nerve impulse during transmission. Memory, selective attention, and emotional processing are among the cognitive activities connected with the cholinergic system.

### 1.2.1 Cholinergic projections

The widely projecting and local circuits constitute the cholinergic system. The extended projections of cholinergic neurons originate in nuclei of basal forebrain and project throughout the brain. Acetylcholine is transmitted to the cerebral cortex via the “nucleus basalis of Meynert” and the “diagonal band of Broca”. There are also cholinergic connections between the nucleus located in septal region and the hippocampus located deep into temporal lobe. The local circuitry of cholinergic system inside corpus striatum (important component of basal ganglia) interacts with neurons of GABA system and nigrostriatal dopamine system engaged in extra-pyramidal movement.

### 1.2.2 Cholinergic system: the role in cognition

The cholinergic system has been linked to several cognitive functions such as memory, attention, memory, and processing of emotions. Research done on humans and animals indicated that sustained attentional performance has/had been driven by the cholinergic input from the basal forebrain [7]. In general, cholinergic system activation aids better focussed ability to retain the relevant stimuli and filter out the irrelevant ones, but particular projections to the medial prefrontal cortex regulate anxious reactions to contextual cues acetylcholine influences working memory and the attentional processes necessary for error detection by regulating arousal and attention. The cholinergic system is also tightly linked to emotional processing; moreover, the inputs of cholinergic neurons to the frontoparietal cortex modulate the direction of attention toward emotional expression. In view of the fact that the system is governing so many aspects of cognition, diminished cholinergic tone correlated to Alzheimer's disease and leads to poor intellectual execution that includes attentiveness, memory, attention, and executive functioning [8].

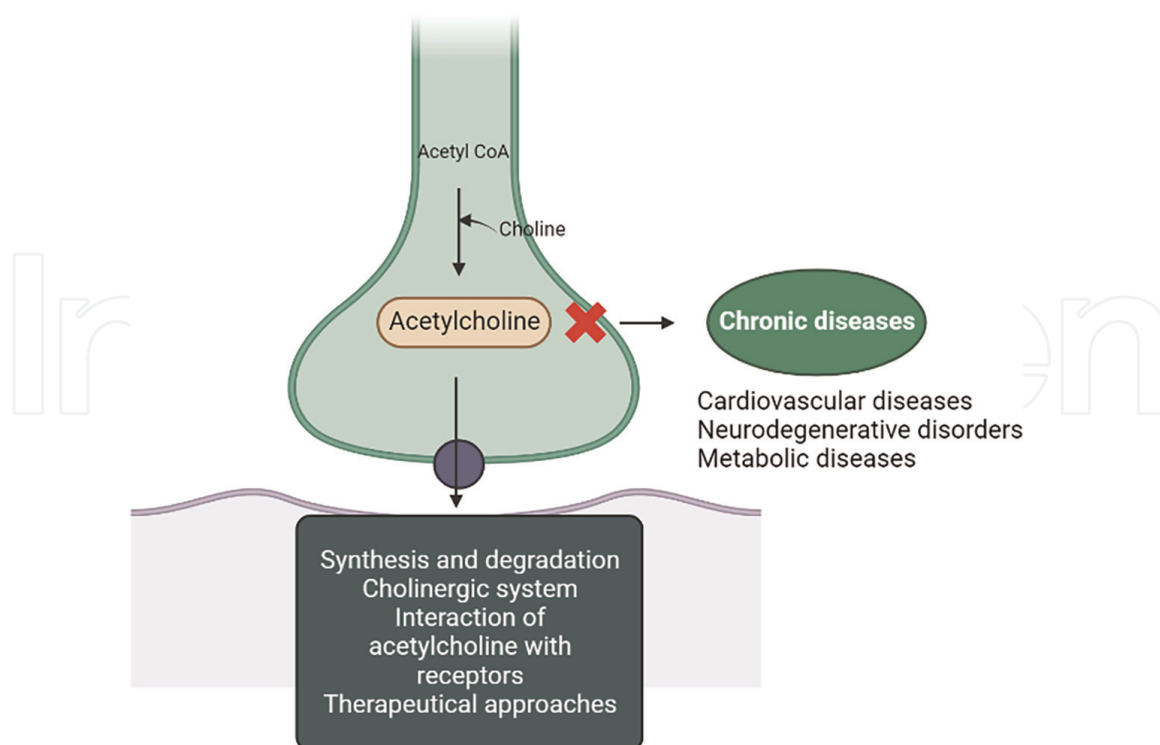
### **1.3 Cholinergic receptors**

Nicotinic acetylcholine receptors are ion channels that are activated by a ligand. They are made up of five polypeptide subunits, including 2  $\alpha$  subunits plus  $\beta$ ,  $\gamma$  and  $\delta$  subunits with its further two subtypes viz. Muscular (N1) and Neuronal (N2). N1 consists of  $\alpha_1$ ,  $\beta_1$ ,  $\gamma$ ,  $\delta$  (2:1:1:1) subunits in the case of embryo while  $\alpha_1$ ,  $\beta_1$ ,  $\delta$ , and  $\epsilon$  (2:1:1:1) in case of adults. N2 possesses 2 $\alpha$ , 3 $\beta$  subunits that can form twelve different combinations of nicotinic receptor subunits. When two acetylcholine molecules bind to the nicotinic acetylcholine receptor, there exists a change in the internal conformation of the pentameric structure, forming a trans-membrane aperture that allows the movement of  $\text{Na}^+$ ,  $\text{K}^+$  (3 sodium ions out of cells while pumping 2 potassium ions into cells) and  $\text{Ca}^{2+}$  ions to pass through. Depending on the intensity of the initiating stimulation, the transport of these ions will result in cell depolarization [9].

mAChR, known as “Muscarinic acetylcholine receptors” belong to the subfamily of G-protein coupled receptor (GPCR) complexes, commonly found in brain, bladder, sweat glands, eye and gland, constitute a single polypeptide chain with 7 distinct sections organized in an alpha-helical structure. Hydrophobic residues in these alpha helices permit the polypeptide for spanning the neural membrane about 7 times. The 5th cytoplasmic facing loop along with the carboxy tail of this polypeptide communicates with G-proteins (made of  $\alpha$ ,  $\beta$ , and  $\gamma$  subunits), also known as secondary messengers. When acetylcholine binds to the muscarinic receptor, there is a change in its configuration, prompting the alpha sub-unit for releasing the naturally attached inactive metabolite (purine nucleotide diphosphate consisting of guanine and pyrophosphate) called guanosine di-phosphate, GDP and swap this for tri-phosphate molecule (guanosine triphosphate, GTP).  $\alpha$  subunit dissociates from the distinct components ( $\beta$  and  $\gamma$ ) upon binding of triphosphate molecule to alpha component. Further, GTP collaborate in accompany to other signaling proteins. An inherent activity of GTPase in  $\alpha$  subunit metabolize GTP again to GDP, thus shutting off the second messenger system over time. Muscarinic receptors have numerous tissue-dependent activities in the physiological system of humans. These activities could be either of two: STIMULATORY or INHIBITORY due to their utilization of secondary messengers to achieve the desired results. M2 and M4, the subtypes of Muscarinic receptor, inhibits adenylate cyclase to function. There is an inhibition of adenylate cyclase by the alpha component of G protein upon binding of Ach to Muscarinic receptor subtypes (M2 or M4) that results in decrement of intra cellular cAMP and as there is an essential role of cAMP in activating/inhibiting the numerous down-stream elements of signaling pathway, it's decreased concentration leads to plethora of pitfalls. The actions of M-type subreceptors (M1, M3 & M5) have been carried out via stimulating phospholipase C (PLC). The active complex of G protein communicate to PLC and activates it that cause hydrolysis of phosphatidyl-inositol to Inositol tri-phosphate (IP3) and diacyl-glycerol (DAG). The increase in intra cellular calcium concentration in the cytoplasm of the cell is due to the interaction of secondary messenger IP3 with its receptors located in SER (Smooth Endoplasmic Reticulum) [10].

## **2. Influence of acetylcholine on chronic diseases**

Acetylcholine, being a mediator of cell to cell communication, and responsible for various peripheral and non-neuronal cholinergic signals. Any alteration in the levels of acetylcholine leads to chronic diseases. The same has been depicted in **Figure 1**.



**Figure 1.**  
The graphical illustration of acetylcholine effect on chronic disorders.

## 2.1 Acetylcholine and cardiovascular system

The heart's purpose is to pump blood to body tissues and organs. Myocardial infarction, being an index event, causes anatomical and physiological changes in the heart, called "cardiac remodeling". These alterations have been seen only at molecular and cellular stages in the cardiomyocyte. Based on the extent of the damage, cardiac remodeling may escalate to heart failure and, eventually, death [11]. Despite significant efforts in understanding the components implicated in how heart failure evolves, the mode of action underlying the phenomenon remains unknown.

ACh is secreted in the heart via para-sympathetic division of the ANS and frequently serves in offsetting the effects of fight/flight system. A highly innervated heart's atrial valve activates cholinergic system that reduces heart rate, atrioventricular contractility, and node conduction velocities. Although ventricles displayed cholinergic fibers, however their density within ventricles remain substantially lower than in the atrium [12].

ACh has been universally considered as the primary neurotransmitter of parasympathetic nervous system since the Otto Loewi experimental times till present day investigations. The processes involved in its production and release have now been thoroughly elucidated. The operations of choline acetyltransferase (ChAT), which converts acetyl-CoA and choline into ACh in the cytoplasm, are required for ACh synthesis. The vesicular ACh transporter (VACHT) stores cytoplasmic ACh in synaptic vesicles, hence the level of ACh discharged is directly related to the quantity of VACHT. Increased expression of VACHT leads to increased ACh release, whereas decreased VACHT expression leads to decreased ACh release.  $\text{Ca}^{2+}$  promotes the exocytosis of ACh-filled vesicles by stimulating vesicle fusion with the biological membranes, permitting ACh to be released into the extracellular environment [13].

ACh, being liberated into the extracellular environment, can adhere to nicotinic (nAChR) or muscarinic (mAChR) receptors, activating particular signal transduction series in various types of cells. The muscarinic receptor, a G protein-coupled receptor, is the most important ACh receptor in the heart. The subtypes of Muscarinic receptors (M1–M5) seem to be present all across the systemic circulation and have a range of physiological roles. The most frequent heart's muscarinic receptor subtype is type 2 i.e., AChR-M2). The G-inhibitory (Gi) protein is connected to AChR-M2, whose expression has been thought to counterbalance adrenaline activation via the G-stimulatory (Gs) protein. Cholinesterases, found in the extracellular environment, are present numerously in the heart, break ACh molecules quickly. Incredibly little quantity of ACh persist in the extracellular environment, eliminating the actions of non-receptor-mediated signals. The high-affinity choline transporter transports the choline produced by ACh breakdown again to the cytoplasm of the cell (CHT 1). Considering the fact that ACh-synthesizing cells require choline from the extracellular environment in order to synthesize ACh, CHT1 activity is a step-limiting element in ACh production. The ACh release by heart myocytes reliant on the activity of VACHT. This premise is supported by three pieces of evidence. First, vesicle-like structures were identified to present together in accompany of VACHT in cardiac myocytes. Secondly, knocked out VACHT derived heart myocytes from mice have decreased Ach secretion. Third, ACh was found in the supernatant of in vitro cardiomyocyte preparations, however absent in VACHT mutant animals (cardiac specific) [14].

### *2.1.1 Cardioprotective actions of Ach*

ACh is found to protect the heart against a variety of pathological diseases, including isoproterenol-induced hypertrophy, hypertension, myocardial infarction, chronic chagas cardiomyopathy, and angiotensin II (Ang-II)-induced cardiac dysfunction. Cholinergic signaling was manipulated pharmacologically by using cholinesterase inhibitors such as pyridostigmine (PYR), surgically by modifying vagus nerve activity, or genetically engineered mice models to investigate the influence of variations in cholinergic activity on heart disease development. Cholinergic signaling was modulated pharmacologically with pyridostigmine (PYR), a cholinesterase inhibitor, by modifying the activity of vagus nerve with surgical procedures, or genetically engineered mouse models to examine the effect of alterations in cholinergic activity on the development of heart disease. In infarcted mice, eating improved hemodynamic measures, autonomic balance, and ventricular dysfunction. Likewise, Gavioli and colleagues discovered that PYR therapy decreased heart hypertrophy and ventricular dysfunction in two different animal models of hyperadrenergic stimulation. Even though these research findings consistently demonstrated that PYR therapies provide cardio protection in various mouse injury models, it could be fascinating to determine for certain if PYR's potential benefits have been recognized massively in human patients. In this spirit, the Alzheimer's patients who intake the inhibitors of cholinesterase are at lower risk of mortality. The study by Li et al. described how chronic heart diseased rats survived upon the stimulation of vagus nerve. Although stimulated nerve had no effect on infarct size, it did enhance the functionality of cardiac cell and decrease hypertrophic cardiomyopathy. It is important to note here that rats whose nerve got stimulated survived 70% more compared to those that were not stimulated. Vaseghi et al. demonstrated in another work employing infarcted pigs that stimulated vagus nerve enhanced the "rest and digest" system and decreased

abnormal heart rhythms of the ventricles, most likely by stabilizing the infarct border zones [15].

In conclusion, growing evidence suggests that cardiac cholinergic transmission (both neuronal and non-neuronal) has a biological purpose in damaged conditions, despite the fact that considerations for every ACh generator to recovery remains to be firmly defined yet.

## **2.2 Acetylcholine and neurodegenerative diseases**

### *2.2.1 Alzheimer's disease*

Alzheimer's disease (AD) is a neurodegenerative, inevitable, progressive disorder that affects memory, thinking behavior and other potential activities, the early symptoms of which include trouble in recalling recent talks, names or events; depression; lack of interest (apathy) and later signs are confusion, hindered communication, disorientation, behavioral changes and poor judgment [16]. Because the cholinergic system is disrupted in this condition, the "cholinergic hypothesis" was proposed. Cholinergic innervation may be disrupted even in the early stages of Alzheimer's disease, according to researchers. Neurons of nucleus basalis are especially vulnerable to this degradation. It is widely accepted about the functionality of cholinergic system that could be increased utilizing the Nicotinic/muscarinic receptor agonists and antagonists, thus these two approaches have been into action to treat Alzheimer disease. The hyperactivity of AChE produces a drop in the levels of Ach, leading to cholinergic system degeneration. The usage of acetylcholinesterase inhibitors may enhance a patient's life; however, these medications are merely indicative, meaning it results in the delaying of symptom onset, thus cannot be considered as definitive treatment. AChE activity assessments is of little relevance in the initial phases of the disease since only a small reduction in its effects has been seen. AChE is found in both the main cleft and the postjunctional fold, however the majority of it is found in the basal lamina. The location of AChE is in close proximity to the surface of a muscle as compared to the pre-synaptic layer present in the main cleft, but it is present along the whole stretch of the postjunctional fold, reaching its highest concentration down past the fold. According to research, the alterations in early AD are presynaptic. This is consistent with prior research, which found that AChE activity declines very little in early illness. Neuronal apoptosis occurs over time in Alzheimer's disease. AChE may potentially have a role in this. The tissues having more concentration of AChE are more vulnerable to apoptosis. In one of the study Tau Glycogen synthase kinase 3 (GSK3) was activated by the transfection of N-AChE-S in cell culture. GSK3 caused the tau hyperphosphorylation and apoptotic induction [17]. The amyloid hypothesis is another effort to explain the etiology of AD. Its supporters claim that the illness is caused by the buildup of protein called beta amyloid (AB) in brain. It is also thought about the neurodegeneration and symptom manifestations by these AB deposits. Amyloid beta is neurotoxic to mature neurons, causing them to die. Amyloid beta is generated by proteolysis of amyloid precursor protein (endosomal/lysosomal/at the plasma membrane surface) (APP). This process is triggered by the presence of alpha secretase (unit of preselin 1). AChE has also been found to participate in beta amyloid buildup. AChE has variable sensitivity to inhibitors in Alzheimer's disease, inhibited by indoleamine and bacitracin [18]. Furthermore, this enzyme can directly communicate with beta amyloid. The amyloid beta-AChE complex is more hazardous to the brain than only the aggregates of beta amyloid [19].



### *2.2.2 Parkinson's disease*

Parkinson's disease (PD) is a kind of neurological disorder. After Alzheimer's disease, it is the second most prevalent neurodegenerative disease. This illness is hypothesized to be caused by Lewy body (LB) and neurite aggregates. They accumulate inside "substantia nigra" (SN) & gradually degenerate the system producing dopamine neurons via neuronal destruction. The manifestations arise after half of the neurons deteriorate. Parkinson's disease also results in the malfunctioning of the cholinergic system, causing weakening of Meynert basalis nucleus, other cognitive disfigurements and hence dementia. Cholinergic deficiencies are more evident in Parkinson's disease than in Alzheimer's disease [20]. AChE activity decreases significantly with Parkinson's disease. This decline is due to the degeneration of cholinergic neurons which has decreased independently of any movement action and illness severity. Dementia patients have greater impairment in AChE activity. The individuals who were not reported with dementia but with a lower concentration of AChE in outer layer of the cerebrum have been found to have low intellectual disability, which coincides with the degradation of neurons producing choline. This association, though, varies. The number of cholinergic terminals was reduced in around one-third of the individuals. An association of various brain's areas in Parkinson's disease results in a wide range of apparent symptoms. The vulnerability of degrading choline-producing neurons of neo-cortex is more in men as compared to women [21]. An early buildup of  $\alpha$ -synuclein in cholinergic neurons in the basal forebrain has been linked to the development of LB and neuronal loss in the SN. AChE activity was also shown to be decreased in individuals with early Parkinson's disease dementia, namely in the cerebellar medial occipital cortex. This is the location with the most cholinergic denervation. Cholinergic denervation adds to depressed symptoms in Parkinson's disease. It becomes more obvious, however, when the patient also develops dementia.  $\beta$ A deposits are also significant in the pathogenesis of Parkinson's disease. As previously stated, AChE may play an essential role in deposition of  $\beta$ A in the brain. It is probable that it will also improve Amyloid beta aggregation in Parkinson's disease. Postural instability and gait difficulties are motor subtypes in Parkinson's disease (PIGD). This kind of Parkinson's disease is distinguished by a limited sensitivity to dopaminergic medications. PIGD is one of the elements that contribute to the development of dementia. This subtype frequently has an accumulation of  $\beta$ A in the brain, which magnifies cognitive deficits in addition to those attributed with PIGD. It has also been demonstrated that  $\beta$ A deposition in Parkinson's disease patients might independently worsen apathy. In these cases, there was a strong association between  $\beta$ A binding and apathy [22].  $\beta$ A might be deposited in both the cerebral cortex and the striatum. Gait disruption in Parkinson's disease is linked to cholinergic deficiencies in the basal forebrain and an increased risk of cognitive loss. Gait speed was correlated in patients with cholinergic and dopaminergic degeneration. Furthermore, cortical AChE activity was lower than normal in certain cases. Impaired postural control and gait abnormalities are related with pedunculopontine nucleus dysfunction. Increased postural sway is related with reduced cholinergic innervation of the thalamus and, as a result, lower AChE activity. In Parkinson's disease, p-tau is also deposited. Deposition of this molecule/protein have been found in the olfactory bulb of up to 80% of Parkinson's disease patients. Its buildup is most likely linked to cognitive deterioration and the progression of dementia in patients with idiopathic Parkinson's disease. AChE increases the formation of p-tau in the brain.

Acetylcholinesterase, furthermore, contribute significantly to the development of ocular disorders. The favorable effect has been observed on retinal development after inhibition [23]. Visual abnormalities occur in Parkinson's disease, with reasons spanning from the retina to higher cortical parts of the brain. Dopamine insufficiency is assumed to be the primary cause of the retinal alterations. Furthermore, it is not ruled out that AChE may have a role in the pathophysiology of ocular abnormalities in Parkinson's disease. Several mutations, including those in the LRRK2 and DJ-1 genes, can alter the course and onset of Parkinson's disease. In hereditary Parkinsonism, mutations in the LRRK2 gene are prevalent. It is passed down in an autosomal dominant manner. In clinical practice, this type of Parkinson's disease is not distinguishable from idiopathic Parkinson's disease. LRRK2 is involved in inflammation. The activity of AChE in carriers of this mutation was compared to that of AChE in individuals with idiopathic Parkinson's disease. AChE activity was shown to be considerably greater in LRRK2 gene mutation carriers. This is consistent with patients who carry this mutation having a shorter illness course and hence fewer severe non-motor symptoms. Enhanced AChE activity has been linked to increased neurotransmission at cholinergic synapses in the thalamus and cerebellar cortex. It is true that oxidative stress plays a crucial role in the etiology of Parkinson's disease. Its primary cause is glial cell activation. AChE receptor is the most likely type responsible for oxidative stress. Stress causes an increase in AChE by increasing the expression of this type. The rise in AChE-R is mostly due to astrocytes. In Parkinson's disease, AChE is implicated in neuronal death via apoptosis [24]. AChE expression increased in PC12 model cells for Parkinson's disease and SNpc in a mouse model. A lack of the enzyme reduced dopaminergic neuron death.

### *2.2.3 Huntington's disease*

Huntington's disease (HD) is another neurological disorder. It is passed down to progeny as "autosomal dominant" manner. The trigger in IT15 gene (found on the short arm of chromosome 4) results in HD. Mutated IT15 gene encodes "Huntingtin" (HTT) protein that builds up inside tissues, causing the deterioration of nerve cell, which eventually leads to death. The mutation increases the amount of "CAG repeats", that causes the prolonged chain of glutamine (Q) giving polychain Q of greater than 36 toward its anionic terminal. The number of repetitions is inversely proportionate to the age at which the sickness manifests itself. After around 15–20 years, the condition causes cachexia and death. Chorea-like motions, cognitive impairment, and mood disturbances are among the symptoms [25]. It has been discovered that in HD, there is a decline in the production of genes and proteins in this system, rather than a loss of cholinergic neurons. It was demonstrated that AChE activity was lowered in mouse models of HD (R6/1), and as a result, they showed cognitive abnormalities in the middle stage of the illness. Activation of microglial cells has been observed in the carriers of mutation that can be diagnosed 15 years prior to the beginning of HD. Furthermore, activation of these cells coincides with striatal neuron dysfunction. By secreting proinflammatory cytokines, microglia may activate A1 astrocytes [26]. Furthermore, it has been demonstrated that thalamostriatal degeneration may lead to dystonia in HD. It has also been proposed that the cholinergic system is implicated in dystonia. Cholinergic transmission has been established repeatedly in HD. However, it has been proposed that therapy with AChE inhibitors is not recommended in HD [27].

#### *2.2.4 Multiple sclerosis*

Multiple sclerosis is an usual de-myelinating disorder of the central nervous system (CNS). Multiple, diffuse autoimmune inflammatory alterations contribute to myelin and oligodendrocyte destruction in SM. T-lymphocytes (mostly CD8+) dominate the inflammatory cell infiltrates. Neurotransmission has been disrupted. Axons are preserved at an initial phases of illness, but they have been irreparably destroyed with increasing time. Inflammatory alterations are dynamic and occur mostly in the substantia alba, also called as white matter. Remyelination occurs at all phases of SM, although mostly during the quiescent period [28]. SM causes a rise in proinflammatory cytokines. AChE activity was shown to be higher for those that possessed this condition compared to the reference healthy population. The effect of AChE has been linked to low levels of ACh and persistent inflammatory diseases. According to investigations, periphery acetyl-cholinesterase action has been a supplementary metric for assessing the “non-neuronal cholinergic system’s” functionality in inflammation regulation. The patients suffering from relapsing-remitting SM type (RR-SM), lower ACh levels were associated with higher levels of pro-inflammatory cytokines such as IL-17 and IL1 in both CSF and blood. There is an inverse relation reported between the Ach and AChE in RR-SM patients. More the activity of AChE, less the level of Ach. Furthermore, AChE transcript expression increased. When the individuals with RR-SM were differentiated by the reference group, the enzyme level rose by more than 60%. The enhanced serum AChE activity was caused by its G4 form. Every element that is required for the production of Acetylcholine was unchanged in SM patients, demonstrating that cholinesterases are to blame in generating low levels of Ach. Moreover, greater effect of AChE was linked to elevated concentrations of variety of interleukins (IL-12, 18 and 23) and Tumor necrosis factor. The inflammation of myelin sheath promotes cholinergic dysfunction, which contributes to SM. Because ACh levels alter cytokine levels, uncontrolled metabolism of acetylcholine could be an another pathogenic cause of SM [29]. An imbalance of cholinergic activity has also been detected in the hippocampus of SM patients. This is consistent with some persons experiencing a range of cognitive deficits as a result of this medical issue. In the hippocampus of the patients investigated, ACh levels were lower, ChAT activity was lower, but AChE activity remained same. These diseases may be caused by hyper action of AChE in proportion to the chemical messenger (Ach). Another research found that AChE activity remained constant upon comparing to the reference population. Individuals suffering from SM, on the other hand, showed considerable intellectual disability already. The hike in glial AChE levels has been hypothesized for the cognitive decline that compensated for neuronal AChE’s drop. That might explicate the negative association between AChE activity and the neuropsychological examination results, that could suggest a increased response of glial cells in individuals with larger cognitive impairments, according to these researchers. Furthermore, cholinergic equilibrium within AChE & ChAT has been well recognized during the remission period [30]. There is a rise in ChAT and a reduction in AChE at this stage of SM. This balance is reversed in the acute phase of the illness, with AChE hike and reduced ChAT.

#### *2.2.5 Amyotrophic lateral sclerosis*

Amyotrophic lateral sclerosis (ALS) is a neurological disorder. The degeneration of nerves occurs in the brain stem, pathways of the cortico-spinal route, and in the horn

cells of the anterior region, which results in manifestations that are either motor or non-motor. However, the illness' etiology is still unclear. ALS is more prominent in individuals of ages between 50 and 75 years. The risk of ALS reduces beyond the age of 75 years. Neuronal loss was accompanied by the responses of inflammation entailing the growth of microglial and astrocytic cells in both ALS patients and animals. The defining hallmark of ALS is accumulation of the malfunctioning protein, TDP-43. This protein is generally found in the nucleus of the cell. TDP-43 abnormal folding results in the deposition of aggregates in the cytoplasm, resulting in the motor deficits and disrupted transcriptional process. The condition begins locally and later spreads [31, 32]. This enzyme was created as a consequence of overactive motor neurons. An inflammatory response is prevalent as ALS progresses which leads to an increase in pro-inflammatory cytokines including Interleukins (IL-1, 6), Tumor necrosis factor-alpha, and Interferon alpha [33]. Further, in ALS, there is a presence of microglial cells and reactive astrocytes. Excessive oxidative stress is also present in ALS [34]. R-AChE plays an important function in oxidative stress; however, its source might be reactive astrocytes. This molecular type of AChE may have a role in the development and pathophysiology of ALS.

## **2.3 Acetylcholine and metabolic diseases**

### *2.3.1 Diabetes*

Type 1 diabetes (T1D) is an autoimmune condition characterized by autoreactive T lymphocytes destroying insulin-producing  $\beta$ -cells in the pancreatic islets of Langerhans. The illness begins with the loss of  $\beta$ -cells in individuals with a genetic predisposition and under particular environmental conditions, followed by the migration and activation of inflammatory cells (T and B cells, myeloid, and natural killer cells) to the islets, resulting in insulinitis [35]. The vagus nerve innervates the pancreas via parasympathetic nerve terminals that produce the neurotransmitter acetylcholine (ACh). ACh, in turn, can bind to the nAChRs and mAChRs expressed on pancreatic cells, controlling pancreatic metabolic activities such as glucose homeostasis. Increased vagal activity stimulates insulin production by activating mAChRs on pancreatic cells. Although  $\beta$ -cells appear to express a variety of muscarinic receptor subtypes, M3 mAChR is the most numerous and the one that mediates insulin release. M3 mAChR of pancreatic cells deficient mice had poor glucose tolerance and considerably lower insulin secretion. Mice overexpressing pancreatic M3 mAChR, on the other hand, had increased glucose tolerance and insulin production. There is additional evidence that pancreatic  $\beta$ -cells functionally express distinct nAChR subunits although the role of these receptors in  $\beta$ -cell function is still debated. While some studies found that nAChR agonists had no effect on hyperglycemia or  $\beta$ -cell function, others found that administering particular 7nAChR agonists lowered hyperglycemia in diabetic animal models [36]. The vagus nerve also connects the central nervous system and the immune system via the cholinergic anti-inflammatory pathway, where ACh inhibits the production of pro-inflammatory cytokines (TNF, IL-6, HMGB1), reducing the inflammatory response in sepsis and inflammatory disorders. The 7nAChR, in particular, has been linked to the suppression of pro-inflammatory cytokine production by macrophages, as well as other immunological processes such as T cell death and the suppressive activity of T regulatory cells. In addition, the presence of a cholinergic system in non-neuronal cells, including immunocompetent cells, has been well established. These cells include the enzymes choline acetyltransferase

(Chat) and acetylcholinesterase (AChE), as well as the choline transporters required for ACh synthesis. Furthermore, immune cells express both muscarinic and nicotinic ACh receptors, suggesting that the cholinergic system is involved in immune response control. The 7nAChR is expressed on neutrophils, macrophages, B and T cells, and dendritic cells, as well as enterocytes, endothelium, and microglial cells, and has been linked to the pathophysiology of autoimmune disorders. Activating the cholinergic nerve system with particular acetylcholinesterase inhibitors (AChEI) prevents the occurrence of hyperglycemia and experimental diabetes [36].

### *2.3.2 Diabetes heart disease*

Diabetes heart disease (DHD) is the leading cause of mortality in diabetics, accounting for more than 80% of fatalities. This high fatality rate is notable in light of major advances in modern health-care systems and diabetes therapy. Insulin sensitivity and metabolic disturbances in type 2 diabetes mellitus (T2DM) restrict glucose homeostasis in the heart by downregulating the expression of glucose transporter-4 (GLUT-4). Continued exposure to metabolic changes worsens vascular permeability, leading to coronary artery disease (CAD) and coronary microvascular disease (CMVD). CAD and CMVD reduce coronary artery blood circulation and myocardium perfusion, increasing heart muscle stress and promoting the onset of DHD [37]. Prior literature has shown that cardiomyocytes have a robust inherent cholinergic machinery called the non-neuronal cholinergic system (NNCS). The NNCS in cardiomyocytes is made up of many components that work together to keep acetylcholine (ACh) homeostasis and allow ACh to serve as an autocrine/paracrine mediator. These components are choline acetyltransferase (ChAT) to synthesize ACh; choline transporter1 (CHT1) for the reuptake of choline into the cardiomyocytes for ACh synthesis; vesicular ACh transporter (VAChT) to store and release ACh; acetylcholinesterase (AChE) to degrade ACh in the extracellular space as well as type-2 muscarinic ACh receptor (M2AChR) for ACh binding and signal transduction. ACh secreted by cardiomyocytes functions as an autocrine/paracrine mediator. Its interaction with M2AChR activates the pro-survival phosphatidylinositol-3-kinase (PI3K)/protein kinase B (Akt)/hypoxia-inducible factor 1-alpha (HIF1) signaling cascade. It has been observed that activating this pathway increases the activation of downstream effectors such as (1) glucose transporter-4 (GLUT-4) to promote glucose absorption and energy preservation and (2) vascular endothelial growth factor (VEGF) to promote angiogenesis. However, the role of cardiac NNCS in DHD pathogenesis is uncertain. This is especially important since diabetes is linked to impaired glucose homeostasis, cell survival, and angiogenesis [38].

### *2.3.3 Adipose tissue dysfunction (obesity-related diseases)*

The nicotinic acetylcholine receptor 3 subtype (3-nAChR) is essential for controlling inflammatory responses. Inflammation causes adipose tissue malfunction, which raises a likelihood of cardiac and metabolic illness.

Obesity is now recognized as a key contributor to a variety of chronic illnesses, incorporating inflammatory & blood-vascular & disorders [39]. Increase in the body's most efficient energy storage tissue called White Adipose Tissue (WAT) leads to obesity because of the fact that WAT produces adipokines. These adipokines are responsible for several pathological processes like inflammation and insulin resistance. Furthermore, obesity causes aberrant productions of inflammatory cytokines and

other adipokines from WAT, which can be interpreted as adipose tissue malfunction. Obesity-induced adipose tissue dysfunction always results in persistent low-grade inflammation, which frequently leads to poor organ connections and metabolic abnormalities in various tissues. Indeed, adipose tissue inflammation has been linked to cardiovascular disease and insulin resistance. Diverse adipokines generated by adipose tissue can also alter liver, skeletal muscle, and cardiac functions. Nicotinic acetylcholine receptors (nAChRs) are integral membrane proteins that are members of the ligand-gated ion channel superfamily that mediates and/or modulates cellular signaling. nAChRs are generated in mammals by the assembly of particular combinations of five transmembrane subunits chosen from a pool of 16 homologous polypeptides ( $\alpha$ 1-7,  $\alpha$ 9-10,  $\beta$ 1-4,  $\delta$ ,  $\epsilon$ ,  $\gamma$ ). Various physiological activities may be mediated by nAChRs assembled with different subunits. It has been proposed that nAChRs, particularly 7-nAChR, play a key regulatory role in the cholinergic anti-inflammatory pathway. The role of nAChRs in regulating adipose tissue functions has also been investigated. TNF- $\alpha$  (tumor necrosis factor) production from adipocytes was lowered by activating nAChRs, indicating that nAChRs may alleviate adipose inflammation. Bai et al. hypothesized that in high-fat diet-fed ApoE/mice with 3-nAChR blocked and in IL-6-stimulated adipocytes with  $\alpha$ 3-nAChR gene silenced, the productions of leptin, resistin, triglyceride, cholesterol, and low-density lipoprotein were significantly increased, but the generations of adiponectin and high-density lipoprotein were significantly decreased [40]. Meanwhile, inflammatory cytokine production was significantly increased. Furthermore, JAK2/STAT3 activation was engaged in the  $\alpha$ 3-nAChR-dependent signaling pathways in the control of adipose tissue dysfunction.

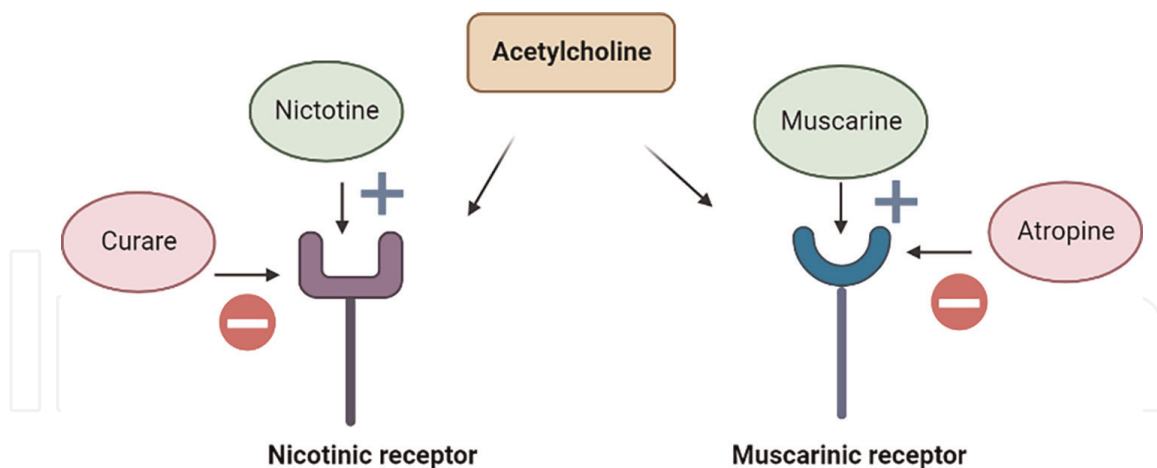
### 3. Therapeutics

#### 3.1 Cholinergic agonists and antagonists

Cholinergic agonists including parasympathomimetics, especially muscarinic agonists and cholinergic antagonists encompassing parasympatholytics or muscarinic antagonists are the two categories of medications influencing the parasympathetic nervous system.

#### 3.2 Acetylcholinesterase inhibitors

They are also referred to as anti-cholinesterases that prevent the cholinesterase to disintegrate Ach, hence boosting the amount and intensity of synaptic activity. AChE inhibitors are classified into two types based on their method of action: irreversible and reversible (**Figure 2**). Reversible inhibitors, whether competitive or noncompetitive, have largely medical benefits, whereas irreversible AChE activity modulators have harmful consequences. Reversible AChE inhibitors are crucial in the pharmacological modulation of enzyme activity. These inhibitors include compounds with various functional groups (carbamate, quaternary, or tertiary ammonium group) and have been used in the diagnosis and/or treatment of diseases such as myasthenia gravis, Alzheimer's disease, post-operative ileus, bladder distention, glaucoma, and as an antidote to anticholinergic overdose. Irreversible anticholinesterases comprise organo-phosphates which produce a phosphorylated enzyme that does not regenerate appreciably when hydrolyzed. They have little therapeutic efficacy but are extremely toxicologically significant. Isoflurophate, the most known and investigated chemical



**Figure 2.**  
Agonists and antagonists of acetylcholine.

Inhibitor	Bioactive component/chemical structure	Mechanism of inhibition (reversible/irreversible)
Rivastigmine	Carbamate	Reversible action
Galantamine	Alkaloid	Reversible action
Huperzine A	Alkaloid	Reversible action
7-methoxytacrine	Pyridine derivative	Reversible action
Tacrine	Pyridine derivative	Reversible action
Physostigmine	Carbamate	Reversible action
Pyridostigmine	Carbamate	Reversible action
Aldicarb	Carbamate	Reversible action
Butylate	Carbamate	Reversible action
Diisopropyl Fluorophosphate	Organophosphorous compound	Irreversible action
Trichlorfon	Organophosphorous compound	Irreversible action
Tabun	Organophosphorous compound	Irreversible action
Echothiophate	Organophosphorous compound	Irreversible action
Diazinon	Organophosphorous compound	Irreversible action

**Table 1.**  
Commonly used reversible and irreversible AChE inhibitors.

in this family; malathion, an extensively employed insecticide; echothiophate, among the first substances in this family possessing a therapeutic efficacy; and tabun, the deadly and poisonous nerve gases, are four examples. Some of the commonly used reversible and irreversible inhibitors of Acetylcholinesterase are listed in **Table 1**.

#### 4. Conclusion

Acetylcholine, being a part of cholinergic system, is a neurochemical and capable of performing plethora of functions in brain and human body. In addition to manage

the sympathetic, para-sympathetic and autonomic functions, its role in chronic diseases has been well understood and reviewed comprehensively. Furthermore, the cholinergic system, its projections and the cognitive role has been discussed along with a brief overview of cholinergic receptors. Also, the influence of acetylcholine on different types of chronic diseases including cardiovascular diseases, neurodegenerative disorders and metabolic diseases has been illustrated in detail. In last, the therapeutic section has been presented, covering the insights on agonist and antagonists of Acetylcholine for understanding better treatment options.

### **Conflict of interest**

The authors declare no conflict of interest.


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## References

- [1] Waxenbaum JA, Reddy V, Varacallo M. Anatomy, autonomic nervous system. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021
- [2] Wu D, Hersh LB. Choline acetyltransferase: Celebrating its fiftieth year. *Journal of Neurochemistry*. 1994; **62**:1653-1663
- [3] Taylor P, Brown JH. In: Brady S, Siegel G, Albers RW, Price DL, editors. *Basic Neurochemistry: Molecular, Cellular and Medical Aspects*. United States: Elsevier; 2005
- [4] Parsons SM, Bahr BA, Gracz M, et al. Acetylcholine transport: Fundamental properties and effects of pharmacologic agents. *Annals of the New York Academy of Sciences*. 1987; **493**:220-233
- [5] Erickson JD, Weihe E, Schafer MKM, et al. The VAcHT/ChAT “cholinergic gene locus”: New aspects of genetic and vesicular regulation of cholinergic function. *Progress in Brain Research*. 1996; **109**:69-82
- [6] Varoqui H, Mennier F-M, Mennier FA, et al. Expression of the vesicular acetylcholine transporter in mammalian cells. *Progress in Brain Research*. 1996; **109**:83-95
- [7] Sarter M, Givens B, Bruno JP. The cognitive neuroscience of sustained attention: Where top-down meets bottom-up. *Brain Research. Brain Research Reviews*. 2001; **35**(2):146-160. DOI: 10.1016/s0165-0173(01)00044-3
- [8] Bentley P, Vuilleumier P, Thiel C, Driver J, Dolan R. Cholinergic enhancement modulates neural correlates of selective attention and emotional processing. *NeuroImage*. 2003; **20**:58-70
- [9] Zoli M, Pucci S, Vilella A, Gotti C. Neuronal and extraneuronal nicotinic acetylcholine receptors. *Current Neuropharmacology*. 2018; **16**(4): 338-349
- [10] Haga T. Molecular properties of muscarinic acetylcholine receptors. *Proceedings of the Japan Academy. Series B, Physical and Biological Sciences*. 2013; **89**(6):226-256
- [11] Burchfield JS, Xie M, Hill JA. Pathological ventricular remodeling: Mechanisms: Part 1 of 2. *Circulation*. 2013; **128**:388-400. DOI: 10.1161/CIRCULATIONAHA.113.001878
- [12] Sourkes TL. Acetylcholine—From Vagusstoff to cerebral neurotransmitter. *Journal of the History of the Neurosciences*. 2009; **18**:47-58. DOI: 10.1080/09647040701632305
- [13] Van der Kloot W, Molgó J. Quantal acetylcholine release at the vertebrate neuromuscular junction. *Physiological Reviews*. 1994; **74**:899-991. DOI: 10.1152/physrev.1994.74.4.899
- [14] Roy A, Fields WC, Rocha-Resende C, Resende RR, Guatimosim S, Prado VF, et al. Cardiomyocyte-secreted acetylcholine is required for maintenance of homeostasis in the heart. *The FASEB Journal*. 2013; **27**:5072-5082. DOI: 10.1096/fj.13-238279
- [15] Rocha-Resende C, da Silva AM, Prado MA, Guatimosim S. Protective and anti-inflammatory effects of acetylcholine in the heart. *American Journal of Physiology-Cell Physiology*. 2021; **320**(2):C155-C161
- [16] Bondi MW, Edmonds EC, Salmon DP. Alzheimer's disease: Past,

present, and future. *Journal of the International Neuropsychological Society*. 2017;23(9–10):818-831.  
DOI: 10.1017/S135561771700100X

[17] Zhang XJ, Greenberg DS. Acetylcholinesterase involvement in apoptosis. *Frontiers in Molecular Neuroscience*. 2012;5:40. DOI: 10.3389/fnmol.2012.00040

[18] Ciro A, Park J, Burkhard G, Yan N, Geula C. Biochemical differentiation of cholinesterases from normal and Alzheimers disease cortex. *Current Alzheimer Research*. 2012;9:138-143.  
DOI: 10.2174/156720512799015127

[19] Jean L, Brimijoin S, Vaux DJ. In vivo localization of human acetylcholinesterase-derived species in a  $\beta$ -sheet conformation at the core of senile plaques in Alzheimer's disease. *The Journal of Biological Chemistry*. 2019;294:6253-6272. DOI: 10.1074/jbc.RA118.006230

[20] Guo P, Wang RD, Lian TH, Ding DY, Zhang YN, Zhang WJ, et al. Olfactory dysfunction and its association with neuropathologic proteins in cerebrospinal fluid from patients with Parkinson disease. *Frontiers in Aging Neuroscience*. 2020;12:594324.  
DOI: 10.3389/fnagi.2020.594324

[21] Bohnen NI, Roytman S, Kanel P, Müller ML, Scott PJ, Frey KA, et al. Progression of regional cortical cholinergic denervation in Parkinson's disease. *Brain Communications*. 2022;4(6):fcac320

[22] Zhou Z, Müller MLTM, Kanel P, Chua J, Kotagal V, Kaufer DI, et al. Apathy rating scores and  $\beta$ -amyloidopathy in patients with Parkinson disease at risk for cognitive decline. *Neurology*. 2020;94:e376-e383.

DOI: 10.1212/WNL.00000000000008683

[23] Yi YM, Cai L, Shao Y, Xu M, Yi JL. The protective role of tacrine and donepezil in the retina of acetylcholinesterase knockout mice. *International Journal of Ophthalmology*. 2015;8:884-890. DOI: 10.3980/j.issn.2222-3959.2015.05.06

[24] Zhang X, Lu L, Liu S, Ye W, Wu J, Zhang X. Acetylcholinesterase deficiency decreases apoptosis in dopaminergic neurons in the neurotoxin model of Parkinson's disease. *The International Journal of Biochemistry & Cell Biology*. 2013;45:265-272.  
DOI: 10.1016/j.biocel.2012.11.015

[25] Ahmad Aziz N, Van Der Burg JMM, Tabrizi SJ, Bernhard LG. Overlap between age-at-onset and disease-progression determinants in Huntington disease. *Neurology*. 2018;90:1127.  
DOI: 10.1212/WNL.00000000000005690

[26] Liddel SA, Gattenplan KA, Clarke LE, Bennett FC, Bohlen CJ, Schirmer L, et al. Neurotoxic reactive astrocytes are induced by activated microglia. *Nature*. 2017;541:481-487.  
DOI: 10.1038/nature21029

[27] Giral A, Saavedra A, Alberch J, Pérez-Navarro E. Cognitive dysfunction in Huntington's disease: Humans, mouse models and molecular mechanisms. *Journal of Huntington's Disease*. 2012;1:155-173. DOI: 10.3233/JHD-120023

[28] Dobson R, Giovannoni G. Multiple sclerosis—A review. *European Journal of Neurology*. 2019;26:27-40. DOI: 10.1111/ene.13819

[29] Gatta V, Mengod G, Reale M, Tata AM. Possible correlation between cholinergic system alterations and

neuro/inflammation in multiple sclerosis. *Biomedicine*. 2020;**8**:153. DOI: 10.3390/biomedicines8060153

[30] Di Pinto G, Di Bari M, Martin-Alvarez R, Sperduti S, Serrano-Acedo S, Gatta V, et al. Comparative study of the expression of cholinergic system components in the CNS of experimental autoimmune encephalomyelitis mice: Acute vs remitting phase. *The European Journal of Neuroscience*. 2018;**48**: 2165-2181. DOI: 10.1111/ejn.14125

[31] van Es MA, Hardiman O, Chio A, Al-Chalabi A, Pasterkamp RJ, Veldink JH, et al. Amyotrophic lateral sclerosis. *Lancet*. 2017;**390**:2084-2098. DOI: 10.1016/S0140-6736(17)31287-4

[32] Hardiman O, Al-Chalabi A, Chio A, Corr EM, Logroscino G, Robberecht W, et al. Amyotrophic lateral sclerosis. *Nature Reviews. Disease Primers*. 2017;**3**: 1-19. DOI: 10.1038/nrdp.2017.71

[33] Chen X, Hu Y, Cao Z, Liu Q, Cheng Y. Cerebrospinal fluid inflammatory cytokine aberrations in Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis: A systematic review and meta-analysis. *Frontiers in Immunology*. 2018;**9**:2122. DOI: 10.3389/fimmu.2018.02122

[34] Gerber YN, Sabourin JC, Rabano M, Vivanco MDM, Perrin FE. Early functional deficit and microglial disturbances in a mouse model of amyotrophic lateral sclerosis. *PLoS One*. 2012;**7**:e36000. DOI: 10.1371/journal.pone.0036000

[35] Meglio AD, Molina BE, Oram RA. Type 1 diabetes. *Lancet. The Lancet*. 2018;**391**(10138):2449-2462

[36] Fernández-Cabezudo MJ, George JA, Bashir G, Mohamed YA, Al-Mansori A, Qureshi MM, et al. Involvement of

acetylcholine receptors in cholinergic pathway-mediated protection against autoimmune diabetes. *Frontiers in Immunology*. 2019;**10**:1038

[37] Iismaa TP, Kerr EA, Wilson JR, Carpenter L, Sims N, Biden TJ. Quantitative and functional characterization of muscarinic receptor subtypes in insulin-secreting cell lines and rat pancreatic islets. *Diabetes*. 2000;**49**:392-398. DOI: 10.2337/diabetes.49.3.392

[38] Saw EL, Pearson JT, Schwenke DO, Munasinghe PE, Tsuchimochi H, Rawal S, et al. Activation of the cardiac non-neuronal cholinergic system prevents the development of diabetes-associated cardiovascular complications. *Cardiovascular Diabetology*. 2021;**20**(1): 1-6

[39] Egom EE. LDL-cholesterol and atherosclerotic cardiovascular disease: Innocent bystander or essential ingredient. *Journal of the American College of Cardiology*. 2018;**71**(6): 705-706

[40] Bai X, Ju H, Gao L, Xiong Y, Dai R, Huang X, et al. Regulation of nicotinic acetylcholine receptor  $\alpha 3$  subtype in adipose tissue dysfunction. *Prostaglandins & Other Lipid Mediators*. 2019;**142**:53-58. DOI: 10.1016/j.prostaglandins.2019.04.001