Chapter 13

Energy-Dependent Mechanisms of Cholinergic Neurodegeneration

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Additional information is available at the end of the chapter

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1. Introduction

Dementia is a typical symptom of many neurodegenerative diseases. The characteristic feature of this pathology is preferential loss of cholinergic neurons in the brain septum, that are responsible for almost all cognitive functions in humans and animals. Alzheimer disease (AD) is one of the most common neurodegenerative diseases in elderly populations. There are estimations that 30 million people worldwide are suffering from AD. Incidency of AD continues to grow, becoming not only a medical but also a socio-economical problem, especially when number of patients by 2050 will triple in connection with the lengthening of the human life span. The human brain constitutes only 2% of body weight, but consumes about 20% of the total body energy output under resting conditions. In contrast to other tissues, glucose is an almost exclusive energy substrate for the brain. In hypoxia or ketonemia brain may consume certain amounts of lactate and beta-hydroxybutyrate, which, however, cannot fully replace glucose to meet brain demands for energy. That is due to the fact that neurons, constituting 10% of all brain cells, produce and consume about 80% of its energy. In addition they have no capacity to store an inventory of high energy compounds. Therefore, the effective functioning of neurons is dependent on the continuous supply of equivalent amounts of glucose and oxygen. Most of the energy produced in the neurons, (60-70%) is consumed for the maintenance and restoration of the pre-and postsynaptic membrane potentials.

Energy homeostasis of the brain is a very complex process due to the high sensitivity of neurons to metabolic stress, isolation of the brain due to the existence of the blood brain barrier, high energy requirements of the brain, and finally due to limited glycogen stores, as a dynamic source of energy. However the first step in neurodegeneration is mitochondrial dysfunction. This appears during some pathologic conditions such as: hypoxia, hypogly-

cemia, amyloid β accumulation, Zn, Fe, Al excess, free radicals formation and thiamine deficiency. All these pathologic signals strongly inhibited activity of the key enzymes engaged in energy metabolism.

In some cholinergic encephalopathies an impairment of brain energy metabolism occurs, a process known as hypometabolism. Studies of brain PET using [^{18}F] fluorodeoxyglucose reveal impaired glucose uptake and metabolism in different regions of an encephalopathic brain. The extent of these deficits correlates with the degree of cognitive impairment in the AD patients. On the other hand, PET combined with Pittsburgh compound-B application, can specifically determine the amyloid-β accumulation in the patient's brain. Hence, it is possible to diagnose the AD in the early stages. Another characteristic feature of neurodegeneration of AD type, is inhibition of tricarboxylic acid cycle and the respiratory chain enzymes activities. Thus, there is a reduction in the synthesis and utilization of acetyl-CoA resulting from significant decreases in pyruvate dehydrogenase (PDHC) and α-ketoglutarate dehydrogenase (KDHC) complex activities. Marked inhibition of aconitase and isocitrate dehydrogenase (IDH) activities was also reported in brain regions affected by AD pathology. This particular susceptibility of cholinergic neurons to several neurotoxic signals may be caused by the fact that they use acetyl-CoA not only to produce energy but also to synthesize acetylcholine. Thus the changes observed in AD brains concern he loss of several cholinergic markers including choline acetyltransferase (ChAT), acetylcholine esterase (AChE), high affinity choline uptake system (HACU), vesicular acetylcholine transporter (VAChT) and resulting from them reductions in ACh content and its quantal release. As a consequence, an impairment of signal transduction processes caused by a loss of muscarinic (M_{AChR}) and nicotinic (N_{AChR}) receptors and a decrease in the acetylcholine level take effect. The decrease of different cholinergic markers and protein levels were also observed *post mortem* in affected areas of human brain. It gives rise to a suggestion that impairment of cholinergic neurons in AD may precede later stages of the neurodegeneration process. These observations support the idea the key role of cholinergic dysfunction in triggering the process of AD dementia. It is widely proven that neuroinflammation is a prominent feature in AD brains and that inflammatory responses play a significant role in progression of the disease. Prolonged and spread activation of microglia in AD brain correlates with the extent of brain atrophy and cognitive decline. However the role of microlglia in the development of AD is controversial. There are some data about impairment of energy metabolism in astrocytes in AD and other neurodegenerative conditions.

Astrocytes play several important functions in the metabolism of the brain including intercompartmental turnover of amino acid neurotransmitters and energy substrates. Among others, these cells provide neurons with lactate, glutamine and aspartate for energy production as well as with the precursors for neurotransmitter. The end-feet of astrocytes occupy a strategically special location in brain between capillary endothelial cells and neurons. In addition, astrocytes as a member of the tripartite synapse remove efficiently neurotransmitters such as glutamate from the synaptic cleft and have important functions in regulating extracellular ion homeostasis. Due to the extensive contacts with both blood vessels and neurons astrocytes play a key role in the control of cerebral energy and transmitter metabolism. Astrocyte function and astrocyte-neuronal interactions are very important for synaptic

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plasticity. Thus impairment of astrocyte metabolism in various brain pathologies also has its negative influence on neuronal functions.

2. Brain energy metabolism

Particular cellular compartments of the brain differ markedly in their rates of energy generation and consumption. Among them, neurons constituting only 10% of the brain cells, consume up to 80% of its total energy output. Neuronal cells have no capacity to store any meaningful reserves of high energy compounds. Therefore, the effective functioning of neurons is dependent on the permanent supply of equivalent amounts of glucose and oxygen. About 60-70% of the energy produced in the neurons is consumed for the maintenance and restoration of the pre-and postsynaptic membrane potentials after the functional depolarization taking place with frequency from several to tens of Hz. Furthermore, the synthesis of neurotransmitters, particularly acetylcholine (ACh), also consumes fraction of pyruvate derived acetyl-CoA, a key substrate for tricarboxylic acid cycle (TCA). Neurotransmission requires a transmembrane lipid asymmetry and the constant rearrangement of phospholipids. The amount of energy consumed in these processes is constitutes about 25% of the total pool [1]. Therefore, the energy expenditures for maintenance metabolic activity of the brain are very high and can be a factor limiting the number of neurons that can be fully active at any given time [2].

Glucose from brain vascular compartment is transported across the blood brain barrier and astrocytes extensions by transporters GLUT1 of high-density and medium affinity for glucose (Km 5-10 mmol/L). Their expression in endothelium is reduced by chronic hyperglycemia [3]. On the other hand, neurons on their plasma membranes contain high density of transporters GLUT3 of high affinity to glucose (Km 1-2 mmol/L), expression of which may increase during chronic hypoglycemia [3-4]. In turn, astrocytes, take up glucose through the transporter GLUT1. The high rate of glucose uptake by neurons and astrocytes makes its concentration in extracellular spaces of the brain to be one third lower than in the blood plasma. Thus, under physiological conditions, the transport of glucose into neurons is the maximum a rate of about 6.5 μmol/s in the whole brain [3]. It should be noted that GLUT1 transporters are insensitive to hypoglycemia, whereas GLUT3 to hyperglycemia [5]. These properties make the transport of glucose into neurons optimized, which assures a relatively constant supply of this energy substrate, despite large fluctuations in blood glucose concentrations under physiological and pathological conditions.

An additional fraction of energy substrates is provided by astrocytes, which by their extensions take up the glucose directly from the circulation and display a high rate of glycolytic cycle. Therefore, they synthesize and release large amounts of lactate, which may be taken up by neurons through their monocarboxylic acids transporters MCT1 and MCT4. Lactate is transported into neurons serving as a source of pyruvate, the direct precursor of acetyl-CoA [6-7]. There are claims, that the lactate under certain physiologic and pathologic conditions may provide up to 25% of the energy in neurons [5,8-10]. In addition, high-fat diets, starvation, as well as diabetic ketoacidosis can activate uptake of BHB, through the beta-hydroxybutyrate dehydrogenase-

acetoacetyl-CoA synthetase-beta-ketothiolase steps. The level of BHB in extracellular compartment is about 3.4 mmol/L. After being taken up into the cells by MCT1/MCT4 it becomes a source of acetyl-CoA independent of pyruvate dehydrogenase complex (PDHC) [4]. Therefore ketogenic diet is used to treat syndromes of congenital deficiency of PDHC, although the effectiveness of this treatment is limited [1,11-13]. Patients improvement is limited to the general conditions including alleviation of seizures. Deep losses of cognitive functions remain uncorrected. Hence, neither lactate nor ketoacids can't completely replace glucose as energy substrate for neurons.In this respect, there is no explanation why under *in vitro* conditions pyruvate/lactate remain better energy/acetyl-CoA sources than the glucose [9,14].

3. Cholinergic neurons and their role in central nervous system

Cholinergic neurons constitute only 1-10% of the total pool of neurons depending on the region of the brain, but are indispensable for its basic function-cognition. With other transmitter systems (glutamatergic, GABAergic etc.) they form structural networks for short-and long-term memory formation as well as multiple associative functions [15]. The cholinergic neurotransmission is linked with cognition, higher feelings, the analysis of visual stimuli, olfactory and auditory processes, sustain attention, recall previously stored memory traces and the regulation of behavior. The cholinergic system regulates cerebral blood flow and controls the level of activity of the cerebral cortex, including the sleep-wake cycle [30] [15,17,30]. It also modulates cognitive functions plasticity processes in the brain [16,18]. Cholinergic motor neurons innervating neuro-muscular junctions are indispensable for contraction of all groups of striated muscles [16,19].

The prevalence of neurodegenerative pathologies increases with age. Many of them, including Alzheimer's disease (AD) or Wernicke or hypoxic encephalopathies, are connected with decay of cholinergic innervation in the regions of brain cortex responsible for diverse cognitive functions. *Post mortem* examinations reveal decrease in their number, atrophy, loss of arborization and the reduction of the level and activity of cholinergic markers such as choline acetyltransferase (ChAT) vesicular acetylcholine transporter (VAChT) or high affinity choline uptake system (HACU). They are linked with the impairment of cholinergic neurotranssmition. They correlate with results of the cognitive status of the patients shortly before their death in a progressive physiological age-associated memory impairment and cognitive function [19-20]. Recent reports indicate that accelerated and excessive cholinergic neuron atrophy and loss of their connections are the main feature of cellular pathology underlying AD [21]. Reductions of the number of septal cholinergic neurons were reported to vary from 10% to 90% [22-23].

4. Selective vulnerability of cholinergic neurons

Cholinergic neurons compared to other types of neurons exhibit significantly higher sensitivity to various pathogenic agents [7,16,24-26]. Different groups of cholinergic neurons in the central

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nervous system are characterized by the different sensitivity to similar, harmful active signals and factors. In AD first of all cholinergic neurons of septum are found to be damaged. This type of neurons have nerve endings in the hippocampus and different regions of cerebral cortex. On the other hand, cholinergic interneurons in the striatum and motor neurons in anterior horns of medulla oblongata remain intact, sometimes to the final stages of the disease. Pathological changes were observed in the cholinergic terminals in medial temporal lobe [27]. Early, selective changes in cholinergic neurons are also observed in the olfactory cortex, amygdala, CA-1 region and subiculum. Recent studies have shown that early amyloid overload in the amygdalar regions was associated with appearance of neurofibrillary tangles inside the neurons. These areas of the brain are known to be responsible for the formation of declarative and long-term memory [28-30]. Abundant deposits of amyloid-β (Aβ) also occur in the frontal, temporal and parietal lobes. In the final stages of AD up to 60-65% losses of cholinergic neurons in different areas of the hippocampus, and the accumulation of neurofibrillary tangles in other neurons have been reported [24]. Abundance of neurofibrillary tangles correlated with gravity of clinical symptoms of dementia. On the contrary, the presence of senile plaques was also found in several older patients, who were free from cognitive deficits [31]. Selective neurodegeneration of specific areas of the hippocampus leads to the functional isolation and contributes to the short term memory impairment, which can be seen particularly in the initial stage of the disease. Variable sensitivity of brain regions rich in cholinergic neurons to neurodegeneration may be due to the influence of other regionally characteristic, diverse neurotransmitter networks, as well as the variable interactions with astrocytic and microglial cells. It can also result from phenotypic diversity of individual groups of cholinergic neurons. The underlying cause of the varying sensitivity of different groups of cholinergic neurons may be the level of their cholinergic neurotransmission, the presence of different classes of glutamatergic receptors as well as the frequency of their basic electrophysiological activity. Studies on different whole brain and cell lines indicate, that particular sensitivity of cholinergic neurons to cytotoxic stimuli may be due to the fact that they are using acetyl-CoA, not only, as the other group of neurons, to produce energy, but also for the synthesis of the neurotransmitter, which is ACh [7,26,32].

5. Alzheimer's disease

Alzheimer disease (AD) is one of the most common neurodegenerative diseases in elderly populations. It is estimated that 30 millions people are suffering from AD around the world. The number of cases of AD continues to grow, it is anticipated that the number of patients by 2050 will triplicate as a result of increasing longevity in modern societies.

AD is characterized by a decrease in the number of neurons and their interconnections, linked with progressive impairments of memory and cognitive functions, disorientation and the appearance of neurodegenerative alterations in affected areas of the brain. Disruption of axonal transport in cholinergic neurons is one of the earliest signs of AD observed both in humans and in experimental studies using transgenic mice [33]. The typical hallmark of AD is preferential loss of cholinergic neurons and their extensions in the olfactory bulbs, hippocampus,

frontal, occipital and parietal lobes [34]. Differential sensitivity of between particular groups of cholinergic neurons may be due to their highly variable phenotypes as well as functional status (septal and motor neurons as an example) [35]. Clinical and animal studies demonstrated that loss of septal cholinergic neurons occurred well before those of other groups of neuronal and glial cells. Particular susceptibility of cholinergic neurons may be caused by the fact that in pathological neurodegenerative conditions, their demand for acetyl units for ACh synthesis overlaps with inhibition of PDHC [7,26,32]. This conclusion remains in accord with studies on human AD brains, that revealed a decrease of PDHC, α-ketoglutarate dehydrogenase (KDHC) and aconitase activities in areas affected by this pathology [7,35-36].

Accumulation of Aβ/senile plaques in extracellular compartment and hyperphosphorylated *tau* protein inside the neurons are characteristic histopathological findings in AD brains [37].

The process of Aβ peptide accumulation and its polymerization under favorable conditions is very slow. It gave rise to the hypothesis that amyloidosis is just an outcome but not the cause of AD degeneration [38-40]. Aβ synthesized mainly as 40 amino acid peptide, with minute fractions of 39, 41 and 43 amino acid peptides, all of none or limited neurotoxicity. The 42 aminoacid Aβ is apparently most toxic peptide in its mono-and oligomeric forms [30,41-42]. Amyloid peptides are formed by proteolytic processing of amyloid precursor protein (APP) in sequential reactions catalysed by β-and γ-secretase, respectively. Amyloid polymers are thought to disrupt the neuronal cells through formation high flow uncontrollable Ca-cation channels in their plasma membranes [41-42]. That triggers intensive red-ox processes being the source of excessive amounts of free radicals. Peroxidation of membrane phospholipids disrupts ions transport across cell membranes, including calcium homeostasis and causes changes in the functioning of the cell membrane receptor proteins. Aggregation and polymerization of Aβ peptide and the accumulation of paired helical filaments in neurons and the synaptic endings impairs axonal transport leading to degeneration and death of neurons.

Biochemical alterations observed in the AD brains are associated with decreased activities of enzymes involved in energy metabolism as well as in those responsible for the biosynthesis, release and breakdown of ACh, such as ChAT, acetylcholine esterase (AChE), HACU or VAChT. The impairment of signal transduction processes caused by decreased densities in muscarinic (M_{AChR}) and nicotinic (N_{AChR}) receptors and inhibition of the ACh synthesis and quantal release were also reported [7,30,42].

AChE is an enzyme present both in the axons and nerve ending of cholinergic neurons and in postsynaptic neurons in the cerebral cortex. Therefore its activity/level is also decreased in parallel with the loss of cholinergic neurons taking place in AD and other encephalopathies, [43-44]. These changes were also accompanied by impaired axonal transport, which is one of the earliest functional alterations in cholinergic neurons of AD brains [43]. The decrease of activities/levels different cholinergic markers were also observed *post mortem* in affected brain areas [45-46]. It gave rise to the suggestion that impairment of cholinergic neurons in AD may precede later stages of neurodegeneration process [30]. These observations support the hypothesis of the pivotal role of cholinergic dysfunction in the pathomechanisms of AD dementia.

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6. Hypometabolism in Alzheimer's disease

Energy homeostasis of the brain is a very complex process. This is due to the high sensitivity of neurons to metabolic stress, existence of the blood brain barrier, high-energy requirements of the brain, and finally due to limited reserves of energy precursor substrates. In AD an impairment of brain energy metabolism occurs, a process known as hypometabolism [1,47-48]. Studies with positron emission tomography (PET) using [^{18}F] fluorodeoxyglucose exhibit impaired glucose metabolism in brain regions of both sides in the temporal, parietal and cingulate cortex. The extent of these changes correlates with cognitive impairment in the affected patients. These changes are one of the well established diagnostic criteria for AD. PET combined with marking Pittsburgh blue (Pittsburgh compound-B) can specifically determine the Aβ deposits in the brain, so it is possible to diagnose the AD in its early stages [49-54]. Disturbances in glucose metabolism are associated with the reduction in the density of glucose transporters GLUT1 and GLUT3 in the neurons. Also activity of phosphofructokinase and glyceraldehyde-3-phosphate are diminished yielding suppression of the glycolytic metabolism, and facilitation of amyloidogenic transformation of APP and apoptosis [55-56].

However, the most important alteration in AD brains seems to be suppression of acetyl-CoA synthesis and TCA as well as the respiratory chain proteins. Reductions of PDHC, KDHC complex activities may be key factor in this pathomechanism due to reduction of acetyl-CoA synthesis and its utilization in TCA cycle, respectively. Studies of cholinergic septal neuronal cell lines have shown, that neurotoxins associated with AD pathomechanisms caused direct/instant inhibition of aconitase, PDHC, KDHC and suppressed synthesis and utilization of acetyl-CoA in mitochondria yielding increased mortality in septal cholinergic SN56 neuronal cells with high expression of the cholinergic phenotype [7,32]. One of the main changes observed an early stage of AD is the impairment of oxidative phosphorylation, which leads to decrease of electron transport in the respiratory chain, mainly in complex IV, which is associated with inhibition/decreased expression of cytochrome oxidase and ATP synthase. In this way, in the AD brains reduced of ATP level occurs. At this stage of the disease morphological changes of mitochondria were also observed. Disturbances in membrane fluidity and structure, reduction of the mitochondrial combs, density of mitochondria were also observed [57-59].

7. Pivotal role of acetyl-CoA

The principal, immediate source of acetyl-CoA in the brain is pyruvate formed from the glycolytic metabolism of glucose. The reaction of the oxidative decarboxylation of pyruvate supplying acetyl-CoA is catalyzed by PDHC, located in the mitochondria. More than 97% of acetyl residues *via* citrate synthase is metabolized to citrate and consumed in TCA cycle to produce the energy needed to restore the membrane potential during depolarization-repolarization cycles of several Hz frequency. Only 3% of the pool of generated acetyl-CoA is used in the synthesis of ACh, which takes place in the cytoplasmic compartment [60-63]. However,

under resting conditions acetyl-CoA molecules practically do not pass through the inner mitochondrial membrane into the cytoplasm. Therefore acetyl moiety for ACh synthesis must be transported to the cytoplasm through the intermediate metabolites, such as citrate, acetyl-L-carnitine, for which the inner mitochondrial membrane has a suitable transport systems [64]. In cytoplasm, acetyl-CoA is resynthesized from these compounds. It has been found, that in brain nerve terminals about 30-50% of acetyl-CoA pool is transported from the mitochondria to synaptoplasm, as citrate [65-67]. In cholinergic neurons and nerve terminals the metabolic flow through this pathway is facilitated by the preferential localization of ATP-citrate lyase (ACL) [67-70].

In various forms of dementia including AD, thiamine deficiency, hypoxia or dialysis evoked encephalopathies in humans and animal models of dementia, loss of cognitive functions correlated with preferential deficits of cholinergic markers. Another striking feature in all of these pathologies was the decrease in energy metabolism in the affected regions of the brain [7,21,35,57-58,71]. The decreases in glucose metabolism and reduced stores of phosphocreatine and ATP have been shown during the life of the patients, by PET investigations [51-52,54]. This is confirmed by *post mortem* studies, which show that the cause of these changes may be decreased activity of PDHC, aconitase and KDHC in pathologically altered regions of the central nervous system [26,71-74]. These changes correlated with both the loss of cholinergic markers and the degree of degreased cognitive scores, before the death of the patient [19,30,75]. Studies on isolated cholinergic murine septal neuronal cell lines displayed strong inverse correlations between rates of cell death and PDHC activities or acetyl-CoA levels in their mitochondrial compartment under various neurodegenerative and neuroprotective conditions [7,40,76-80]. On the other hand, ChAT activity, ACh level and synthesis as well as quantal release correlated directly with levels of acetyl-CoA in cytoplasmic compartment of the cholinergic neurons [7,81].

8. Acute and chronic neurotoxicity

Cognitive deficits, the main clinical symptoms of cholinergic encephalopathies may in some cases combine with motor disability [82]. These changes correlate well with the degree of functional and structural losses of basal forebrain cholinergic neurons projecting axons to hippocampus and different cortical areas, motor neurons innervating different groups of striated muscles [45]. In these cases suppression of energy metabolism, correlates with losses of cholinergic markers in affected areas of brain cortex or spinal cord segments. Dysfunction of brain mitochondria is thought to be both the consequence of pathologic insults as well as a source of signals triggering neurodegeneration. Therefore, alterations in PDHC synthesized acetyl-CoA metabolism in the cholinergic neurons should be considered both as a source of disturbances in their transmitter functions and viability (Fig. 1) [7,32]. Several pathologic disturbances of aging brain cause excessive depolarization and overload of neuronal cells with $Ca^{2+}$and other divalent cations yielding diverse cytotoxic reactions.

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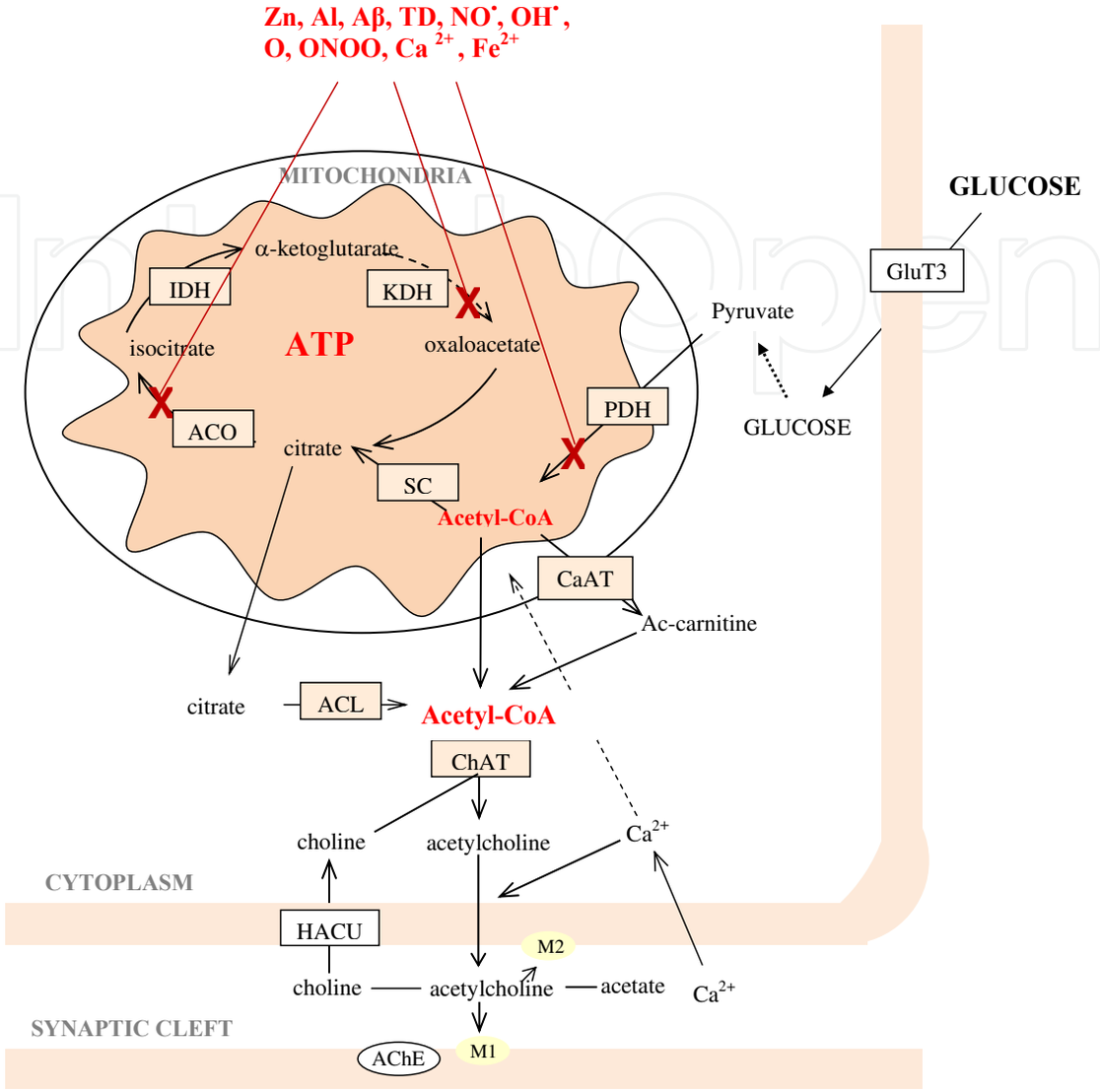

Figure 1. Putative neurotoxic signals affecting pathways acetyl-CoA and energy metabolism in brain cells and their specific interactions with cholinergic neurons.

Glutamateric neurotransmitter system constitutes 50% of all brain neurons and synaptic terminals. Prolonged pathologic depolarization yields an excessive co-release of glutamate and Zn from brain terminals triggering action potentials through NMDA, AMPA receptors and other voltage gated Ca channels located on postsynaptic neurons including cholinergic ones [83-85]. They cause dysfunction of postsynaptic neurons that may lead to apoptosis and necrosis [86-87]. Energy deficits also inhibit uptake of glutamate by adjacent astrocytes, due to the down-regulation of EAA, GLAST and GLT1 transporters and inhibition of their glutamine synthetase [88]. Sustained elevations of glutamate and Zn levels within the synaptic clefts, yield prolonged depolarization of postsynaptic neurons, as well as astroglial and microglial cells [89]. The disruption of Ca^{2+}homeostasis affects enzymes linked with pathways involved in energy, neurotransmitter, and NO metabolism. The Ca^{2+}excess in the mitochondria

compartment may lead to PDHC activity inhibition due to activation of PDH kinase. That may cause acetyl-CoA deficits in subcellular compartments of cholinergic neuronal cells [40,90-91]. During brain hypoxic/ischemic episodes the earliest event is excitotoxic activation caused by prolonged release of glutamate and Zn from glutaminergic nerve terminals. The excess of glutamate/Zn in the synaptic cleft results in, through multiple receptors and transporters, excitotoxic depolarization of postsynaptic neurons and adjacent glial cells as well. These alterations pave the road to subsequent chronic steps of neurodegeneration yielding characteristic histopathologic picture of amyloidosis-β and tauopathy [92-93].

9. Amyloid-β toxicity

It has been found that AD frequently combines with stroke and cerebral vessel thrombosis and other defects of capilary circulation [94]. Transient hypoxic and hypoperfusion conditions, frequent in eldery people brains, may also augment Aβ accumulation by activation of γ and β-secretases. They catalyze amyloidogenic cleavage of APP and increase Aβ accumulation in extra-and intracellular compartments of the brain [95].

There is a common view that different extra-and intracellular deposits of Aβ are the main cause of neuronal injury in the course of AD. Neurotoxic properties of Aβ have been demonstrated in several experimental paradigms. It has been shown, that Aβ added to the cell cultures inhibited the key enzymes of TCA cycle, as well as PDHC [77, 92,96]. It resulted in depletion of acetyl-CoA yielding supression of respiratory chain and ATP levels in affected neuronal cells [76-77,97]. These alterations could be aggravated by Aβ-evoked disruption of endogenous metal homeostasis, including calcium, iron, zinc and copper [98]. Accumulation of these metals as well as xenobiotic. Espesially aluminium, has been found in AD amyloid lesions. Each of these metals may aggravate inhibitory effects of Aβ on oxidative/energy metabolism and cholinergic neurotransmission, yielding increased mortality of cholinergic neurons both in cultures and in brain tissue *in situ* [32]. Aβ fibrilar polymers were reported to form high conductance Ca-channels in cell plasma membranes, with apparent impairment of energy metabolism and activation catabolic pathways [99-100]. Subtoxic levels of Aβ were found to directly inhibit PDHC activity in brain nerve terminals [96]. Accumulation of extracellular Aβ aggravated suppressive effect of NGF mediated by p75 receptors abundantly expressed in septal cholinergic neurons, yielding different suppressive and neurotoxic reactions [32,101]. Aβ also facilitaed inflammatory responses of microglial cells, that promote neurodegenerative processes through excessive production of inflammatory cytokines [102]. However, a recent report reveals that Aβ accumulation in sensitive regions of human cortex correlated neither with loss of cholinergic innervation nor with impairment of respective cognitive functions [103]. That supports earlier notions that Aβ should be considered rather as an outcome than the cause of AD encephalopathy. Nevertheless, that does not rule out the possibility that accumulated Aβ may combine with preceding cytotoxic signals, yielding augmentation of neurodegeneration processes.

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10. Zinc neurotoxicity

Zinc is an essential trace element for living organisms, being the component of active centers of about 300 enzymes and proteins including: carboxypeptidase, aspartate carbamoyltransferase, alcohol dehydrogenases, peroxide dismutase, zinc finger structures of transcription factors and several others [104-105]. It down-regulates the activity of NMDA receptors and other transporter proteins. As a crucial structural element in zinc-fingers, Zn is a regulator of transcription and other adaptative reactions of the organism [106-107]. It inhibits the opening of NMDA channels [108], that during sustained depolarization may take up the excess of this metal from-the synaptic cleft into the postsynaptic neurons [108].

Zinc concentration in synaptic vesicles of glutaminergic terminals may reach levels of few hundred mmol/L as it forms complex with L-glutamate to assure isoosmolality of the vesicular fluid. In accordance with this the highest whole tissue concentration of Zn, about 0.15 mmol/L, was found in the grey matter. During pathologic brain depolarization glutamate is released with zinc from glutaminergic terminals to synaptic clefts, where it can reach concentrations as high as 0.3 mmol/L. Under physiological conditions Zn is quickly cleared from the synaptic cleft mainly by astrocytes and postsynaptic neurons.

There are three groups of proteins specifically regulating Zn distribution in brain cells. They include: ZnT1, located in the neuronal plasma membranes; ZnT2 in endoplasmic reticulum and ZnT3 in synaptic vesicles of nerve terminals [109]. These proteins are activated when zinc concentration in the cytoplasm is elevated. Apart from that, the neuron-specific membrane transporters Zip1, 4, 6 participate in zinc turnover [110]. Zip 1 and 4 remove zinc from the cell, whereas Zip 6 accumulates this cation in the intracellular compartment [111]. It is however not known how ZnTs functions combine with various Ca-channel/transporter activities in the regulation of Zn levels and compartmentalization in the neuronal cells.

Several pathologic conditions cause excessive release of zinc from presynaptic glutamatergic vesicles. High amounts of free Zn are taken-up by postsynaptic neurons and adjacent glial cells. There is no evidence whether large amounts of Zn can be released from other locations apart synaptic vesicles. There was increasing Zn^{2+}accumulation in degenerating neurons after excitotoxic stimulation of transgenic mice, lacking ZnT3 transporter that results in no zinc accumulation in vesicles [112]. Our earlier study revealed that high zinc accumulation in cultured neurons caused inhibition of key enzymes of energy metabolism [40,80]. Namely, Zn^{2+}directly inhibited PDHC and KDHC as well as aconitase activities which led to reduction of acetyl-CoA and ATP levels [40,80]. These Zn/glutamate induced energy deficits along with sustained depolarization along may cause Ca and free radical overloads. That triggers excessive synthesis of nitric oxide (NO), by nNOS and iNOS present in adjacent postsynaptic neuronal and glial cells, respectively. As a result excess of highly toxic peroxynitrite radicals accumulate in affected area. NO excess was reported to cause irreversible inhibition of aconitase and isocitrate dehydrogenase and the reversible one PDHC and KDHC [32,77,98]. These effects apparently aggravated cytotoxic effects of Zn, triggering vicious cycle of cholinergic neurodegeneration [76,80-81]. There are evidences that aberrant Zn homeostasis is involved in the pathogenesis of AD [113]. Zn may be directly involved in the process of

amyloidogenesis as APP protein was found to contain Zn binding motif [113] located within the cysteine-rich region of its ectodomain. This points out that Zn may play a role in yet unknown functions of APP.

High dietary intake of Zn significantly increased the Zn and APP levels in transgenic APP/P1 mouse brains. It also enhanced amyloidogenic cleavage of APP protein both under *in vivo* and *in vitro* conditions [114]. In mouse brain Zn inhibited α-sectetase activity, elevating the β and γ-secretase activities promoting accumulation of Aβ(1-40), the main component of Aβ plaques [108,115]. There was accompanied by the impairment of learning capacity in the Morris water maze test [114]. Zinc cytotoxic effects were observed not only in AD but also in several other brain pathologies including: epilepsy, mechanical trauma, ischemic stroke, hypoglycemia, hypoxia, thiamine deficits and other inherited or acquired metabolic blocks [115].

Besides, chronic pathological conditions may down-regulate expression of different classes of ZnT in astrocytes. In the same conditions Zn may be released to perisynaptic compartments [116]. Hence, Zn excitotoxicity would not be caused by overall increase of its concentration in the brain, but by its aberrant redistribution between different extra-and intracellular compartments of the brain [117].

Increased Zn concentrations in extracellular space may induce oligomerization of Aβ, aggravating its cytotoxic effect in AD brains. That is why short-time elevation of Zn concentrations in extracellular fluid (ECF) might trigger the long-term amyloidogenetic process. These signals were found to exert negative influence on cholinergic neurons that are responsible for cognitive functions and short-time memory formation [32]. It seems that high expression of the cholinergic phenotype in neurons (SN56) of septal origin makes them particularly susceptible to Zn-cytotoxic signaling [7,40,80].

There was also reported that xenobiotic metal Al may also accumulate in the brains in age-dependent manner [118-119]. It could inhibit calcium channels and Na/Ca exchanger in mitochondrial membranes what might increase mitochondrial and decrease cytoplasmic calcium levels in nerve terminals and cholinergic neuronal cells [32,74]. All these pathogens either alone or in combination were found to cause the decrease acetyl-CoA synthesis in neuronal mitochondria and reduction of energy production yielding increased cholinergic neuron susceptibility to degeneration [32,80]. In addition, lowering the cytoplasmic level of calcium could reduce direct transport of acetyl-CoA from mitochondria to cytoplasm through permeability transition pores (PTP) [32,74]. Shortages of acetyl-CoA in cytoplasmic compartment cause inhibition of acetylcholine synthesis and release [40].

On the other hand, primary or secondary Zn deficits could also induce neurodegenerative brain injury. Such conditions were found in the elderly people who maintained themselves on Zn-deficient diet [41]. Some life periods such as intensive growth, pregnancy, lactation, intensive physical exercises increase demand for Zn facilitating appearance of its deficits. That is why numerous therapeutical and schedules recommend taking supplements that contain Zn organic complexes: zinc bisglicine, or zinc bisaspartate. They are claimed to be safer in use than nonchelatable inorganic Zn salts. However, there is no convincing data that would

support this claim. Zn deficits in experimental animals were reported to cause to have increased oxidative stress and/or had greater rate of lipid peroxidation [120].

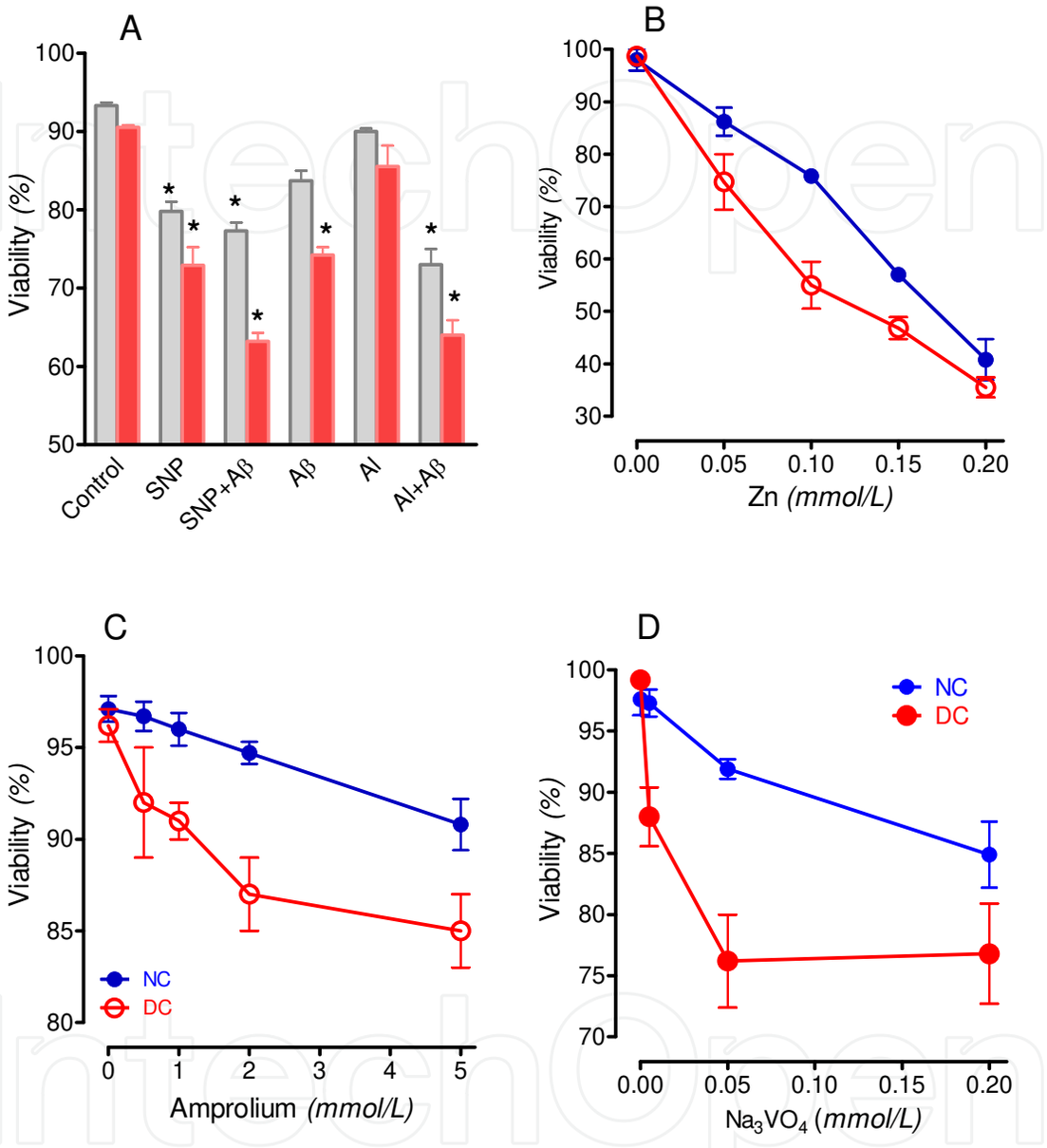

Figure 2. Differential neurotoxicities in nondifferentiated and differentiated cholinergic SN56 neuroblastoma cells. Recalculated from: [32,40,81,158-159].

11. NO excess

Glutamate-Zn evoked increases of $[Ca^{2+}]$/[calmodulin-Ca] in cytoplasmic compartments of postsynaptic neurons and adjacent glial cells activated nNOS and iNOS, respectively. It seems however, that only increased expression of Ca-independent iNOS in the microglial/astroglial

cells may contribute significantly to neurodegeneration. It has been demonstrated, that only iNOS-dependent activation may elevate the NO levels in the brain up to low micromolar, pathologically relevant, concentrations [121]. In fact, bacterial lipopolysaccharides could induce several-fold increase of NO synthesis by microglia [121]. On the other hand, fraction of NO produced by nNOS/eNOS may reach levels two orders of magnitude lower, and is likely to play a physiologic roles of "volume transmitter" and guanyl cyclase activator [89]. Peroxynitrite radicals were found to react with wide range of intracellular biomolecules linked with energy and glycolytic metabolism and several regulatory and transport or neurotransmitter pathways, as well as with antioxidant systems. Excess of endogenous NO exerts rapid but reversible inhibition of cytochrome c oxidase and less potent one for other proteins of respiratory chain and ATP-synthetase, as well [122]. However, NO may also inhibit earlier steps of energy metabolism including: PDHC, aconitase, isocitrate NADP-dehydrogenase, as well as KDHC [40,76,77]. Other enzymes of TCA cycle: succinate dehydrogenase, fumarase, and malate dehydrogenase were not affected by these conditions. That could cause deficits of acetyl-CoA and ATP in NO/ONOO-exposed neuronal [32,76]. Cholinergic neurons with residual expression of the cholinergic phenotype appeared to be more resistant to NO neurotoxicity than those with high expression of the cholinergic phenotype, apparently due to negligible demand for acetyl-CoA to support ACh synthesis in the former.

Lipoic acid or acetyl-L-carnitine were found to exert positive effects on viability in NO or Zn-exposed cholinergic SN56 cells through preservation of acetyl-CoA availability in their mitochondrial and cytoplasmic compartments [32,77]. However, delay in cytoprotectant application markedly diminished their efficacy, apparently due to instant, irreversible inactivation of aconitase by Zn and $NO/ONOO^-$ [40,123]. ChAT appeared to be resistant to direct, acute exposition to NO-excess. However, its expression was adaptatively down-regulated by chronic cytotoxic conditions decreasing acetyl-CoA provision into cytoplasmic compartment [124].

12. Thiamine deficiency

Thiamine pyrophosphate (TPP) is a cofactor for E1 subunits of PDHC and KDHC, that are key rate limiting steps regulating acetyl-CoA synthesis and its metabolic flux through TCA cycle, respectively [61,71,118,125-126]. Activities of these enzymes in the brain mitochondria are several times higher than in nonneuronal tissues, due to high demand for energy in this tissue. Therefore, thiamine pyrophosphate deficits (TD) evoked by chronic alcoholism, starvation or thiamine depleting diets caused dramatic clinical symptoms of motor, cognitive and metabolic disturbances in the form of Wernicke–Korsakoff encephalopathy, muscular dystonia, edema and lactic acidosis, with frequently fatal outcomes [125,127-128]. On the other hand, early supplementation of TPP deficient subjects with thiamine, reversed symptoms of these pathologies [129]. The majority of TD-evoked neurologic and cognitive disturbances may be explained by the impairment of cholinergic neurotransmission. In TD brains there are two major mechanisms that are responsible for dysfuctioning and loss of cholinergic neurons: the primary limitation of acetyl-CoA provision and excytotoxic Zn overload. The first one is caused

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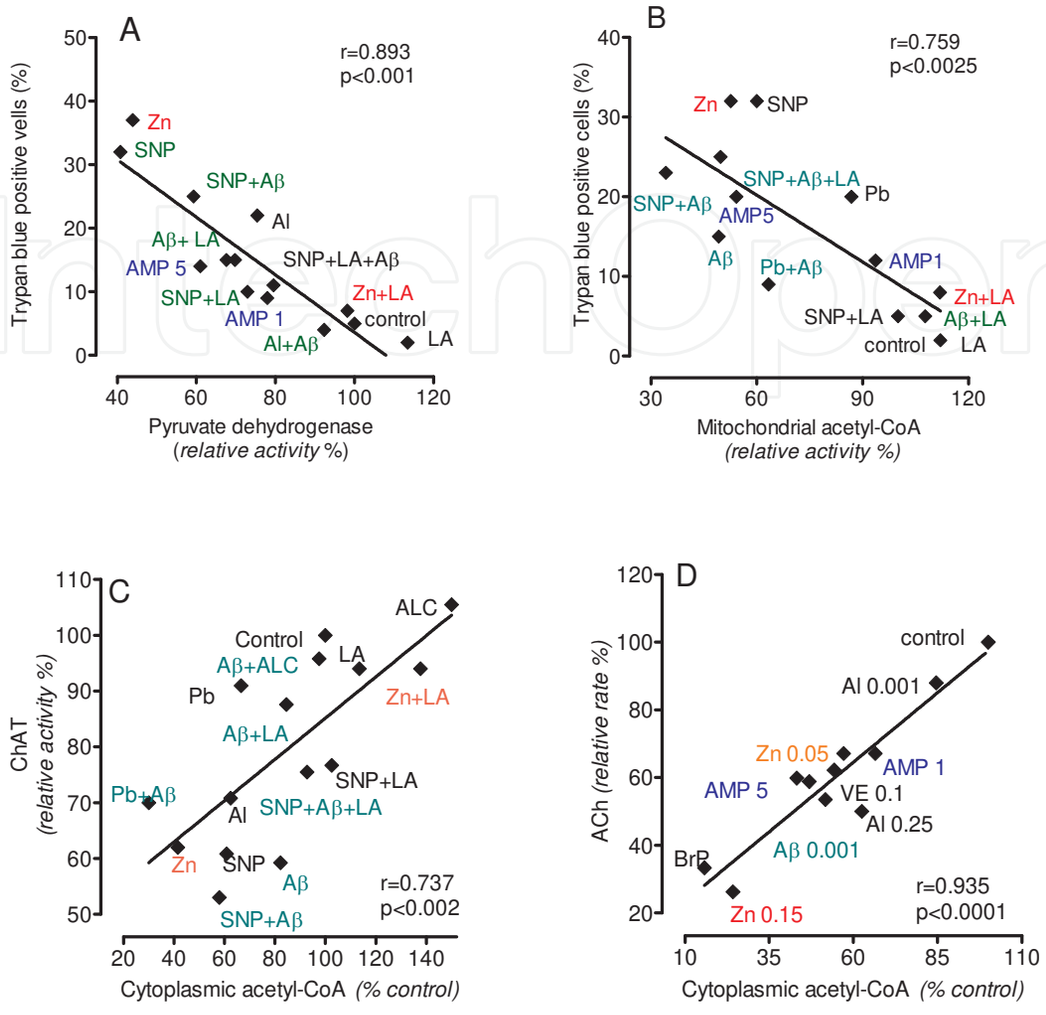

Figure 3. Existence of significant correlations between: intramitochondrial acetyl-CoA metabolism and cholinergic neuronal cell injury (AB) and cytoplasmic acetyl-CoA levels and transmitter functions (CD) of cholinergic neuronal cells of septal origin. Data collected from: [32,40,81,124].

by the impaired synthesis of acetyl-CoA by PDHC, what strightly leads to the excytotoxic release of glutamate-Zn from energy depleted glutamatergic neurons [108]. In whole brain and cellular models of TD, the reduction of mitochondrial levels of acetyl-CoA correlated with losses of cholinergic markers and viability of the neurons [81,119,130-131].

The decreases of cytoplasmic acetyl-CoA in amprolium-induced TD SN56 cells and brain nerve terminals, from pyrythiamine treated rats, resulted from limited synthesis of this metabolite in the mitochondrial compartment by TD-deficient PDHC. In consequence, lower rates of ACh synthesis and its quantal release in TD cholinergic neurons positively correlated with decreased concentration of acetyl-CoA in their cytoplasmic compartment [81,130]. These findings fit to a general rule that the rate of ACh synthesis/release depends on the availability of acetyl-CoA in cytoplasmic/synaptoplasmic compartment of cholinergic neurons, irrespective of the type of neurotoxic signal [7,32]. However, unlike for AD or other neurotoxic conditions, acute

TD altered ChAT activity neither in pyrithiamine-rat brain synaptosomes nor in amprolium-SN56 cells [132]. These data prove that, at least in early stages of TD, the structure of cholinergic neurons remained well preserved and that inhibition of ACh quantal release is exclusively due to the inhibition of acetyl-CoA provision to the site of its synthesis.

13. Glia and neurotoxicity

Astrocytes play several important functions in the metabolism of the brain including inter-compartmental turnover of aminoacid neurotransmitters and energy substrates. They supply neurons with lactate, glutamine and aspartate for energy production neurotransmitter synthesis [133]. The end-feet of astrocytes occupy a strategic sites between capillary endothelial cells and neurons. In addition, astrocytes as a member of the tripartitie synapse remove efficiently neurotransmitters such as glutamate from the synaptic cleft and have important functions in maintenance of ion homeostasis in the extracellular compartments of the brain [134]. Due to the extensive contact with both blood vessels and neurons, astrocytes play the key role in the control of cerebral energy and transmitter metabolism. Astrocyte viability and astrocyte-neuronal interactions take part in processes of synaptic plasticity. Thus impairment in astrocyte metabolism in various brain pathologies also has its negative influence on neuronal functions.

There are some data about impairment of energy metabolism in astrocytes in AD and other neurodegenerative diseases [135]. However, most of them have been collected using isolated astroglial cells or whole brain models without taking into account subcellular distribution of energy metabolism. Therefore, like in the neuronal cells [7] putative aberrations of acetyl-CoA metabolism in the cytoplasmic and mitochondrial compartments of astrocytes, should be investigated in different models of AD and other cholinergic encephalopathies. The main role of astrocytes is to protect and support neurons. Astrocytes are capable to produce net lactate, L-glutamine and accumulate glycogen. They consume about 15-20% of the glucose in the brain [136,137]. Thanks to this they can deliver lactate to neurons, through monocarboxylate transporters MCT1, MCT 2. Lactate, after conversion to pyruvate may serve as an alternative to glucose source of acetyl-CoA under hypoglycaemic or hypoxic conditions. During physiologic activation of glutamatergic endings Na^+dependent transport of glutamate into astrocytes by GLT1 and GLAST transporters was found to be enhanced. Subsequently glutamate was converted there to L-glutamine [136]. There are no Zip transporters on the surface of astrocyte's cellular membrane. Therefore uptake of zinc from synaptic cleft occurs through high density divalent metal transporters: DMT1. Except of Zn ions astrocytes may take up also iron and copper [138]. Apart from that, astrocytes contain high levels of metalothioneins (MTs). In consequence they can take up Zn from synaptic cleft and bind it forming complexes with MTs [139]. That is why impairment of astrocytes under cytotoxic conditions may limit their neuroprotective functions and indirectly facilitate neurodegenerative processes.

There are several therapeutic and preventive approaches to AD and other cholinergic encephalopathies of advanced age. However, now days only cholinomimetics and GABA-

antagonists are approved for treatment of AD and related dementive disorders. They, however neither prevent nor slow down the progress of cognitive loses [102]. Other, therapeutic approaches such as choline supplementation, provision of acetyl-CoA precursors, or free radical scavengers, neurotrophin supply, antiinflamatory drugs application, inhibition of Aβ synthesis or reduction of its overload appeared to be ineffective.

Neuroinflammation is one of principal pathomechanisms of AD which significantly contributes to the progress of the disease [102]. Prolonged and widely spread activation of microglia in AD brain correlates with the extent of brain atrophy and cognitive decline. However, the role of microlglia in the development of AD is a subject of discrepant reports. On one hand, microglial fagocytosis of Aβ is belived to be a protective mechanism againts neurodegeneration [140]. Both atrocytes and microglia release both pro-and anti-inflammatory cytokines and prostaglandins, as well as oxygen, nitrosyl radicals. Cytokines through TLR-4 receptors were found to stimulate variety of intracellular signaling pathways that have been implicated in neuronal damage in AD. Therefore, people taking chronically nonsteroid anti-inflammatory drugs displayed lower prevalence of this pathology [141]. Microglial activation by many endogenous and signaling compounds such as L-glutamate, ATP, 7-ketocholesterol, cAMP were reported to cause inhibition of several enzymes of their energy metabolism [32,141]. Both Zn and Aβ oligomers are capable of microglia activation. This results in release of soluble neurotoxic compounds that compromise integrity of neurons and synapses [142]. Also Zn in rather low concentrations (30-50 micromol/L) activates microglia through mechanism dependent on activation of transcription factor NF-kappaB [143]. Simultaneously active compounds derived from activated microglia augment Zn release from glutamatergic neuronal endings what may accelerate neurodegenerative processes [144].

The activation of both astrocytes and microglial cells is associated with the induction of major proinflammatory pathways [145]. Gene expression profile analysis confirmed the prominent upregulation of genes associated with the immune/inflammatory pathways, including several chemokines and pro-inflammatory cytokines [146]. Activation the IL-1β pathway has been revealed both, in glial as well as in neuronal cells in brains of chronically epileptic rats [147]. Both the complement pathway and the plasminogen system are also activated within the hippocampus affected by multiple-sclerosis [148-149]. Both IL-1β, complement components and plasminogen activators were found to increase the permeability of the blood brain barrier (BBB) [150,151]. Toll-like receptor (TLR) signaling pathways in brains affected by various pathologies such as epilepsy, ischemia or AD, may contribute to neuronal injury [152]. Moreover microRNAs (miRNA) also play a role in the regulation of the innate and adaptive immune responses. In particular, miR-146a, which can be induced by different pro-inflammatory stimuli such as IL-1β and TNF-α, has been shown to critically modulate innate immunity through regulation of TLR signaling and cytokine responses [153]. Interestingly, this miRNA is upregulated in TLR as well as in experimental models of epilepsy. These observations suggest miRNA as potential targets to modulate inflammatory pathways.

Moreover activation of microglia is the well known source of nitric oxide and other reactive oxygen species (ROS) [154]. There are data showing that NO produced by activated microglia inhibits the activity KDHC [155].

Numberous data proved that prolonged activation of microglia leads to excessive secretion of NO, ROS and proinflamatory cytokines [156]. Lypopolysacharide (LPS) derived from bacteria exerts the capacity to activate microglial cells. In such conditions the cells secrete augmented levels of Il-1β, Il-6, TNF-α. TNF-α in nonactivated microglia is produced in insignificant concentration whereas in LPS-activated cells the level of its release is several times expanded [157]. Microglia may be also stimulated by Aβ what in consequence conducts to excessive release of TNF-α, that becomes the neurotoxic factor. However some data reports that low Il-1β concentrations may have positive effect on highly differentiated cholinergic neurons by increasing the ChAT expression and activity in cholinergic neurons treated by neurotoxic concentrations of Aβ [124]. In consequence the level of ACh was also elevated. Moreover these data also proved that added Il-1β reversed the inhibitory effect of cytotoxic factors on acetyl-CoA level in cytoplasmic compartment. These changes in cholinergic phenotype correlated well with cell viability and morphology. From the other hand Il-1β-activation was completely inhibited by IL-6 or TNF-α.

The other data proves that in the cocultures of neuronal cells with microglial cells the last ones protect neurons from death caused by some cytotoxic factors such as elevated Zn or NO levels (Gul-Hinc et al. unpublished). The cytoprotective effect may be caused by the restoration by microglia the proper level of Il-6 in cholinergic neurons and restoration of the high activity of PDHC and acetyl-CoA level. From the other hand LPS-induced excessive release of TNF-α by microglia exerts the cytotoxic effect that is independent on acetyl-CoA level.

14. Conclusions

There is some data concerning the mechanism of cholinergic encephalopathies in particular Alzheimer disease. They are mainly focused on disturbances in Aβ metabolism and only little of them reflect changes in energy metabolism particularly after various cytotoxic factors. However there is the existence of significant correlation between components of pyruvate-acetyl-CoA-acetycholine pathway. Cytotoxic insults that are responsible for AD such as: Aβ, Zn, Al, $NOO^{\cdot}$, TD directly or indirectly inhibits the activity of PDHC and KDHC what leads to acetyl-CoA synthesis. Consequently, there is inhibition of activity of three carboxylic acid cycle what causes the development of neurodegenerative changes in brain. Characterictic feature of some neurodegenerative diseases in preferential loss of cholinergic neurons what correlates with the degree of energy metabolism inhibition. Some data proved that survival of cholinergic neurons is limited by the level of acetyl-CoA in mitochondrial compartment. Moreover it is independent in the reason. The particular susceptibility of cholinergic neurons to various cytotoxic insults is triggered by relative shortage of this metabolite in mitochondria and used for acetylcholine synthesis. That is why it might be said that PDHC activity strait determine acetyl-CoA level in mitochondria what limits its utilization for energy production and acetylcholine synthesis under cytotoxic insults.

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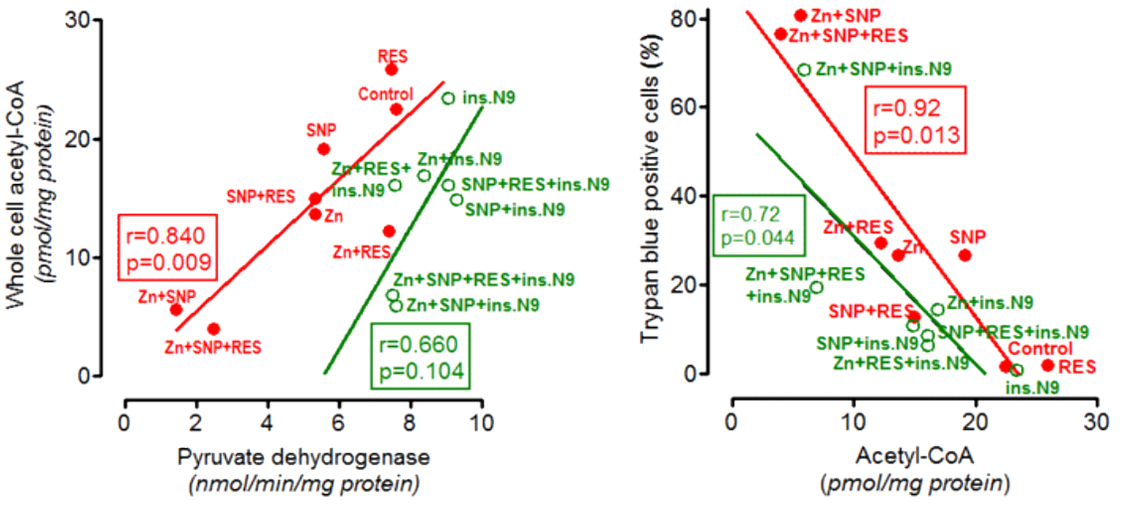

Figure 4. Parcrine effects of microglial N9 cells rescuing cholinergic SN56 neurons under excititocic conditions. A. Microglial effect preserving PDHC activity. B. Microglial effect decreasing mortality without affecting acetyl-CoA levels in cholinergic neurons. *(Gul-Hinc, unpublished).*

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