SUMMARY

BIOCHEMICAL BASIS OF ALZHEIMER'S DISEASE

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The aim of the presented work was to contribute to the elucidation of the etiology of Alzheimer's disease and to create experimental models to help with this endeavor. The approach and the tasks we set to achieve this goal were determined according to the results achieved, which gave us guidance for further research. A huge role in determining the upcoming tasks was the information on the progress of research in this area received from publications in specialized publications. Also crucial for choice and preparation of the next study was the development of scientific techniques and our access to them.

The main results are listed below as conclusions:

1. A comparison of Lowry and Bradford protein determination methods showed that the Bradford method did not give reliable results in determining the protein content of tissue fractions containing membrane-bound proteins. This method is also not reliable in the study of fractions stored at -20° C.
2. The neurotransmitter glutamate affects the metabolism of amyloid precursor protein (APP). Stimulation of the metabotropic glutamate receptor leads to an increase in APP secretion, while stimulation of the AMPA receptor decreases secretion. By counteracting receptors, glutamate may modulate APP processing and a change in balance may be important in the development of Alzheimer's disease (AD).
3. The APP secreted by brain sections has no feedback with its secretion. Following secretion, APP undergoes additional $Ca2^{+}$-dependent processing, which takes place physiologically in brain tissue, most likely by membrane-associated factors. This physiological proteolysis of secreted APP may be required to produce degradation products with different biological activity.
4. Vascular endothelial growth factor is involved in APP metabolism by influencing APP processing and modulating the activity of α- and β-secretases, and thus β-amyloidogenesis, and is therefore a potential participant in the etiology of AD.
5. Interleukin-1β inhibits the signaling transmitted by muscarinic acetylcholine receptors, which may be involved in the pathogenic mechanisms leading to cholinergic

deficiencies observed in AD. This is most likely due to impaired activation of the transcription factors NFκB and AP-1.

6. Induced *in vivo* cholinergic hypoactivity in the cerebral cortex by lesion of cholinergic neurons in the basal nuclei of the forebrain of wild-type mice with immunotoxin 192IgG-saporin leads to a decrease in APP secretion in the cortex and a decrease in ChAT activity, which is reversible by NGF grafting. These first *in vivo* results indicate that APP processing in cortical neurons receiving cholinergic innervation is under cholinergic regulation.
7. *In vivo* immunolesion of cholinergic neurons in the basal nuclei of the forebrain of transgenic mice (Tg2576) with a presence of amyloid pathology causes an increase in amyloid pathology, as well as the manifestation of other important pathological signs of AD. Reduced cholinergic innervation and AChE activity, neurodegeneration and atrophy, and loss of synapses were observed in the hippocampus.
8. Synaptosomes (synaptosomal fraction) are a suitable model for studying the processing of APP, and in particular the neurotransmitter control of its processing due to the direct access of stimulatory agents and the release of APP directly into the incubation environment, which eliminates the risk of further processing in the complex brain tissue.
9. The results of the study of changes in APP expression during ontogenesis showed:

   a) at the protein level - tracking the content of APP in growth cones and synaptosomes during development gives grounds to assume that APP is involved in the germination and pathfinding of neuritis in the early stages of development, as well as in synaptogenesis and maintenance of normal synaptic functions;

   b) at the mRNA level - during ontogenesis APP695 is closely related to the processes of differentiation of nerve cells not only in the central but also in the peripheral nervous system, while the forms APP751 and APP770 are much lower expressed in the brain than in peripheral tissues and organs;

   c) the comparison of the expression of APP in the brain and in peripheral tissues gives grounds to conclude that: the expression and processing of APP in the brain has specific characteristics for this organ; APP secretion is a brain/nerve tissue specific process; APP is expressed mainly in the brain, where its concentration is many times higher than in other studied organs.
10. The amyloid β-peptide affects the electrical activity of neuronal cells:

a) the biologically active fragment of the amyloid β-peptide (Aβ25-35) decreases rapidly, concentration-dependently and reversibly the electrical activity of neural networks. From the obtained results it can be concluded that the reduction caused by Aβ, to stop, of the electrical activity leads to disruption of communication between neurons and subsequently to degeneration of synapses and is a key element in the pathology of AD;.

b) the mechanism of action of Aβ is not through oxidative stress, which acts through cytotoxicity and causes a general, nonspecific effect on the electrical activity of neural networks;

c) Aβ most likely acts as a receptor agonist for the inhibitory neurotransmitters GABA-A and glycine. This is confirmed by the great similarity with the effect of diazepam on the electrical activity of neural networks;

d) the effects of the biologically active fragment Aβ25-35, as well as of the biologically represented forms Aβ1-40 and Aβ1-42, differ in their effect on the electrical activity of neural networks, and this may be due to different mechanisms of action or depending on the effect of their structure;

e) Aβ1-42 affects the electrical activity of neural networks in monomeric form, but not in aggregate state.

11. In adult synaptosomes, compared to young ones, a huge number of new long intervening non-coding RNAs are transcribed. This reveals a new level of transcriptional regulation that is affected by aging. Circular RNAs also accumulate in adult synaptosomes. Their expression in synaptosomes differs from that in brain homogenate. It can be assumed that their potential role is to enhance the interaction with proteins in response to aging processes.
12. Lead treatment reduces the secretion of APP in the cerebral hemispheres and the cerebellum of mice, which can lead to an increase in the intracellular concentration of iron ions and hence to neurotoxicity.