Abstract

The topic of this doctoral thesis focuses on the analysis of metabolic changes occurring in nerve cells during the aging process, using a mouse animal model and cell lines. Particular attention is given to the impact of inhibiting energy metabolism, especially glycogen metabolism, on the functionality of nerve tissue in the context of age.

Aging is commonly recognized as a complex process involving progressive functional degeneration at both the cellular and organ levels. In the case of the brain, these processes are associated with structural and anatomical changes that lead to decreased synaptic plasticity and impaired cognitive functions. In recent years, it has increasingly been emphasized that the age-related decline in cognitive abilities may be a result of disturbances in energy metabolism. This hypothesis is closely related to the concept of the Astrocyte-Neuron Lactate Shuttle (ANLS) [1].

The ANLS mechanism suggests that glucose is primarily metabolized in astrocytes via glycolysis to lactate, which is then transported to neurons and used as the main energy substrate in oxidative metabolism. Recent studies indicate that lactate delivered to neurons during increased synaptic activity predominantly originates from glycogen stored in astrocytes. It has been proven that inhibition of glycogen breakdown (glycogenolysis) leads to significant cognitive dysfunctions in young animals [2], [3].

Previous studies have mainly focused on young organisms, overlooking the significant impact of aging on brain energy metabolism. Research conducted on aged animal models shows that inhibiting glycogenolysis has a positive effect on cognitive functions, dendritic spine morphology, and the proteomic profile [4], [5], [6]. However, little is known about the metabolic homeostasis changes that positively affect synaptic plasticity and the overall condition of nerve tissue in older animals.

This doctoral thesis presents a set of three scientific publications, in which comprehensive metabolomic tools and the glycogen phosphorylase inhibitor BAY U6751 were used to analyze metabolic changes in nerve tissue related to the physiological process of aging.

The first publication presents a methodological work that introduces an innovative and validated LC-MS analytical method, in accordance with the European Medicines Agency guidelines. The applicability of this method was demonstrated by the quantitative determination of the glycogen phosphorylase inhibitor BAY U6751 in mouse tissues. The published results showed the accumulation of the analyte in the examined brain tissues (hippocampus, cerebral

cortex), cerebellum, heart, liver, and muscles. Furthermore, the results confirm that BAY inhibitor crosses the blood-brain barrier and can successfully be used to study the relationship between glycogenolysis inhibition and synaptic plasticity.

The next research paper focuses on a global analysis of changes occurring in the hippocampus, cerebral cortex, and cerebellum in response to aging processes and glycogenolysis inhibition. The animal model-based study showed that the hippocampus (a structure responsible for memory consolidation) is the most metabolically sensitive to age-related changes. A similar trend was observed when analyzing the metabolome in response to glycogen breakdown inhibition. The smallest changes were observed in the cerebral cortex. The paper also includes an analysis of metabolic pathways that encompassed the identified metabolites. The observed metabolic changes were primarily related to alterations in energy, signaling, and lipid metabolism. The published results demonstrate positive changes in the metabolomic profile of aged animals with inhibited glycogenolysis, comparable to those observed in young animals. This also suggests that glycogen metabolism modulation could represent a promising therapeutic strategy for treating age-related disorders.

The last publication, presented as a preprint, shows changes in the metabolomic profile in response to chemical stimulation of LTP and glycogen breakdown inhibition, dependent on glucose concentration. The study was conducted on 14-day-old primary hippocampal neuronal cultures. A unique feature of this work is the departure from the standard protocol of cultivating nerve cells in high glucose concentration (~25 mM), which is ten times higher than the physiological glucose concentration in the brain. The research demonstrated that synaptic activity can be successfully analyzed under cultivation conditions that reflect physiological glucose levels. Furthermore, it was observed that LTP stimulation in low glucose concentrations leads to the accumulation of intracellular TCA cycle intermediates and neurotransmitters. In contrast, the opposite trend was noted in high glucose concentrations. Additionally, it was observed that the action of the glycogen phosphorylase inhibitor BAY led to a reduction in c-Fos expression (a plasticity marker) in high glucose concentrations, while this effect was not observed in low glucose conditions.

The results presented in this doctoral dissertation, in the form of a collection of scientific publications, show that the most significant changes in metabolic homeostasis with aging are visible in the hippocampal structure. Moreover, the use of BAY inhibitor revealed that glycogenolysis inhibition in aged animals leads to positive changes in energy, signaling, and lipidomic pathways. Furthermore, *in vitro* experiments allowed the analysis of changes in the

nerve cell metabolome during synaptic transmission in an environment mimicking physiological conditions.

Bibliografia

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