Study on the mechanisms of action of yolkin peptide complex as a potential nutraceutical in the prevention and treatment of the disorderds associated with the aging processes

According to the latest research, the share of the aging population (age over 65) in Polish society is 18.8%. This situation creates the need to mitigate the negative consequences of the natural and irreversible process affecting the population, which is aging. Reducing the impact of diseases and disabilities while maintaining high cognitive, mental and physical functions ease the day-to-day activities of elderly people. The brain is an organ responsible for the preservation of mental and physical wellbeing but it decreases in size with aging. Brain shrinkage is related not only to the death of nerve cells, but also to their degeneration, reduction in the number of branches and dendritic spines. Because the aging population is a major challenge in modern societies, natural ways to support the human body are attracting more and more attention. One of the pro-health activities is the use of nutraceuticals, which are natural biologically active substances, obtained, among others, from plants, seafood, beekeeping products, colostrum or eggs. Nutraceuticals can have a beneficial effect on the body by modifying physiological or metabolic processes, and their use has a positive effect on delaying the body's aging processes. One of the very good sources of nutraceuticals are bird eggs, which contain many valuable substances necessary for the growth and protection of the developing embryo. A decade ago, it was discovered that IgY isolated from hen egg yolk is accompanied by an immunologically active peptide fraction with a molecular weight of 1-35 kDa, which was called yolkin. Analysis of the amino acid sequence of the *N*-terminal fragment of yolkin showed homology to the *C*-terminal fragment of vitellogenin II. Since the discovery of the yolkin peptide complex in hen egg yolk many of its properties, mainly immunoregulatory, have been studied, potentially having a beneficial effect on the body.

Literature reports indicate that proteins with immunoregulatory properties may also play an important role in the protection and regulation of functions of the central nervous system. Studies with mice with an experimental model of Alzheimer's disease showed that oral administration of yolkin in mice performed better on behavioral tests designed for assessment of their learning abilities and memory formation. Subsequent studies examining the effect of yolkin on the cognitive functions of aging rats have shown that yolkin increases their locomotor activity and significantly improves their spatial and episodic memory. The studies conducted so far have shown the beneficial effect of yolkin on the improvement of memory and cognitive functions in animals. Hence, an important goal of subsequent research was to explain the molecular mechanism of the action of yolkin on the nervous system. Many studies indicate that the neuroprotective and pro-cognitive properties of substances supporting the nervous system are associated with the activation of pathways that increase neurotrophins' expression and secretion. One of the important neurotrophic factors that plays an important role in the development and functioning of the central nervous system, including the processes of learning and memory formation, is the brain-derived neurotrophic factor (BDNF).

The main objective of this study was to investigate the molecular mechanism of impact of the yolkin peptide preparation in the context of BDNF protein secretion and activation of signaling pathways leading to the activation of the CREB factor, which is the main regulator of *Bdnf* expression. Another aim was to investigate the antioxidant activity of yolkin, its ability to regulate the activity of the antioxidant system and its effect on Aβ1-42 aggregation. The study used PC12-Tet-On rat adrenal phaeochromocytoma cells, which have a neuroectodermal origin. PC12 cells are also reported to differentiate phenotypically into sympathetic neuron-like cells in response to nerve growth factor (NGF). In order to confirm the specific properties of yolkin

obtained with PC12 neuron-like cells, the cell line H19-7, which is immortalized rat hippocampal precursor cells, was also used.

The results presented in this dissertation enabled a thorough characterization of the yolkin peptide complex, which was isolated and characterized for the first time a decade ago. It has been shown that the yolkin peptide complex stimulates PC12 neuron-like cells and immortalized H19-7 hippocampal cells to activate signaling pathways dependent on the activity of PI3K/AKT and cAMP-PKA, which are involved in pro-survival and protective processes, through the activation of the CREB factor and increased *Bdnf* expression. What is more, despite the fact that yolkin does not have its own antioxidant activity, it is able to activate the antioxidant defense system and to protect cells against the harmful effects of free oxygen radicals and the toxic impact of Aβ1-42 protein aggregates. The proven protective and pro-cognitive effect of yolkin creates an opportunity for the substance to be a potential candidate for a nutraceutical that may be successfully used in the prevention and treatment of disorders related to the aging processes.