“The impact of differences in locomotor activity and stress reactivity in rats on the development of cognitive and depressive-like disorders and the effectiveness of therapy using insulin-like growth factor-1 in a streptozotocin model of Alzheimer's disease”
mgr Joanna Dunacka

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that occurs in two forms: familial (fAD) and sporadic (sAD). The fAD form is caused by mutations in genes that promote the accumulation of β-amyloid (βA) in the brain and is diagnosed in only 5% of AD patients. The sAD form affects as many as 95% of all people with AD, and causes of this form of the disease are varied and include: β-amyloid (βA) cascade, neuroinflammation, oxidative stress, dysfunction of insulin and cholinergic pathways. In the course of AD numerous behavioral changes are observed, i.e. cognitive, anxiety and depressive disorders, which are the result of accumulation of two pathological forms of proteins in the central nervous system (CNS): senile plaques (composed of βA) and neurofibrillary tangles (composed of hyperphosphorylated tau protein). The animal model of sAD is application of streptozotocin (STZ) into the lateral ventricles of the brain (ICV). In this model of sAD a decrease in glucose metabolism is observed, which contributes to the development of insulin resistance, a decrease in the number of neurons and disruption of the neurogenesis process, disturbances in the functioning of cholinergic pathways, the presence of βA deposits, spatial memory deficits and depressive/anxiety-like disorders. One of the substances that restores the physiological function of insulin pathways is insulin-like growth factor-1 (IGF-1). IGF-1 by regulating glucose metabolism affects: neuroprotection, neurogenesis, neuroregeneration, glial support, neuroplasticity and acts as an anxiolytic agent.

The results presented in the doctoral dissertation are based on three scientific articles (Dunacka et al., 2024; 2025a; 2025b) and unpublished data. My experiments focused on investigating whether the influence of individual differences in stress reactivity in Wistar Han rats has an impact on the development of disorders associated with sAD (cognitive/neuropsychiatric disorders, neuroinflammation/accumulation of βA and peripheral inflammation) and on the effectiveness of therapy using IGF-1 administered into the lateral ventricles of the brain.

The experiments were conducted using male Wistar Han rats (total N=72, HR rats N=35, LR rats N=35) undifferentiated and differentiated (novelty test at baseline conditions) in terms of locomotor activity level/stress reactivity into high-responders (HR) vs. low-responders individuals (LR). At baseline conditions (before cannula implantation and injections), the level of spatial memory (Morris water maze test - MWM), anxiety-like symptoms (the elevated plus

maze test - EPM and open field test - OF) and the level of anhedonia - a basic indicator of depressive disorders (the sucrose preference test - SPT) were determined in these rats. Subsequently, a sAD model was induced by administering ICV-STZ injections (3 mg/kg), or an injections of ICV-IGF-1 neurotrophin (2 μg) were also co-administered with the induction of the sAD model. Control groups of animals were created by intracerebroventricular administration of citrate buffer (ICV-VEH, STZ solvent) and by injections of physiological saline solution (ICV-SAL, IGF-1 solvent). At three stages (very early phase from 7th day of sAD, early phase from 45th day of sAD, late phase from 90th day of sAD) of sAD progression the spatial memory (the MWM test), anxiety-like disorders (the EPM, OF tests) and depressive-like disorders (the SPT test) were measured again. In the early and late phases of sAD, blood of rats was collected by cardiac puncture in order to determine the levels of peripheral markers of inflammation (number and percentage of different leukocyte populations - haematological counter/flow cytometry; concentration of interleukin-6 (IL-6) and interleukin-10 (IL-10) - ELISA; corticosterone (CORT) concentration - radioimmunoassay - RIA) as well as haematological parameters related to the red blood cell and platelet systems (haematological counter). At the end of the late phase of sAD, after sacrifice of the animals, the brain, spleen and thymus were collected. In various regions of the CNS, the number of activated microglia cells ($CD68^+$) and the number of βA deposits were determined using immunohistochemistry (IHC). The spleen and thymus were weighed to determine the relative weight index of these organs.

The obtained results allowed us to conclude that the HR individuals with the sAD model developed more severe spatial reference memory impairments (48-50, 92, 94-95 day sAD) measured in the MWM test and stronger anxiety-like disorders (45-46, 90 day sAD) determined on the basis of measurements in the EPM maze and in the OF test compared to LR rats. However, the level of anhedonia measured in the SPT test did not reach a statistically significant level between HR and LR animals with the sAD model at any of the sAD stages. In turn, the central administration of IGF-1 in rats with the sAD model reduced deficits of the reference and working spatial memory (7-14, 45, 48-52, 90, 94-97 day sAD), anxiety-like disorders (15, 53, 98 day sAD) and depressive-like disorders (52, 97 day sAD). ICV injections of IGF-1 in HR animals with the sAD model reduced spatial memory disorders more strongly (MWM test, 16-19, 54-55, 103-106 day sAD) compared to LR rats. In turn, ICV-IGF-1 applications in LR individuals with sAD model reduced stronger anxiety-like disorders (EPM test, 53 and 98 day sAD) and depressive-like disorders (SPT test, 14 and 52 day sAD) than in HR animals.

Memory deficits and anxiety-like disorders in individuals from the STZ HR group were accompanied (99 days sAD) by a higher number of βA oligomers in the hippocampus (regions: CA1-CA3, DG) and in the shell of the nucleus accumbens (NAcS) compared to the STZ LR animals. In turn, ICV-IGF-1 administration in the STZ IGF-1 group of rats resulted in a lower number of βA deposits in the CA1 region of the hippocampus and a lower number of activated microglial cells in the CA2 part of the hippocampus (98 days sAD) compared to STZ SAL animals. The improvement of spatial memory in STZ IGF-1 HR individuals was associated with a reduction of the number of activated microglial cells (107 sAD days) in the CA2, CA3 and DG regions of the hippocampus, as well as with a reduction of the number of βA oligomers (107 day sAD) in the CA3 and DG parts of the hippocampus compared to STZ IGF-1 LR rats. In contrast, the reduction of neuropsychiatric disorders in STZ IGF-1 LR animals was associated with a decrease in the number (day 107 sAD) of βA deposits in the NAcS.

Furthermore, in the STZ HR group, suppression of the peripheral immune system, understood as a lower number of NK cells, monocytes and granulocytes in the blood (54 day sAD) was observed in compare to the STZ LR animals. Intracerebroventricular administration of IGF-1 neurotrophin to rats with the sAD model resulted in a reduction of peripheral inflammation (99 days sAD), i.e. a lower absolute number of leukocytes, lymphocytes (including T and Th lymphocytes), monocytes, granulocytes, and lower plasma interleukin-6 (IL-6) concentration compared to STZ SAL animals. In addition, intracerebroventricular administration of IGF-1 in STZ IGF-1 individuals resulted in a reduction of plasma concentrations of the peripheral stress response indicator - corticosterone (day 99 of sAD) compared to STZ SAL animals. Analyzing the effect of individual differences in stress sensitivity (HR vs. LR), we noticed that ICV-IGF-1 injections in STZ IGF-1 LR rats reduce peripheral inflammation more strongly (lower absolute number of leukocytes and lymphocytes, lower plasma IL-6 concentration) compared to the STZ IGF-1 HR group.

In summary: (1) in the streptozotocin model of sAD in high-responder rats (HR) with greater sensitivity to stress, there are more severe memory deficits and anxiety-like symptoms compared to low-responder rats (LR). These changes are associated with increased βA deposition in the hippocampus and NAcS in HR animals compared to LR rats; (2) induction of sAD in rats, combined with simultaneous intracerebroventricular IGF-1 injections, reduces spatial memory deficits and depressive/anxiety-like symptoms accompanying sAD progression. This positive effect of IGF-1 neurotrophin is associated with a reduction of the number of βA deposits in the CA1 region of the hippocampus, lower levels of neuroinflammation, measured by the number of $CD68^{+}$ cells in the CA2 region of the hippocampus, and a peripheral anti-

inflammatory effect; (3) central administration of IGF-1 to rats with sAD and varying stress sensitivity (HR vs. LR) reduces memory deficits and anxiety/depressive-like symptoms in a manner dependent on the behavioral characteristics of the individual. In HR animals, a stronger reduction of spatial memory deficits is observed, associated with a reduction in the number of βA deposits and $CD68^+$ cells in the hippocampus. In contrast, in LR individuals with the sAD model, IGF-1 injections have a stronger antidepressant and anxiolytic effect compared to rats with high behavioral activity and greater sensitivity to stress (HR). Restoring impaired insulin signaling in the early stages of sporadic Alzheimer's disease through central administration of the neurotrophin IGF-1 may represent a new approach to the treatment of this disease and related neuropsychiatric disorders. However, it should be remembered that the therapeutic effect of IGF-1 depends on individual differences in behavioral and stress reactivity.