The influence of galactoolisaccharides on behaviour and selected peripheral and central components of the stress response induced by the long-term electrical stimulation of the central nucleus of the amygdala

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ABSTRACT

The amygdala (Amg), especially its central nucleus (CeA), is one of the key structures of the central nervous system (CNS) regulating fear, anxiety and stress responses, and is also involved in the processing of intestinal microbiota signals. About 40% of the human population suffers from anxiety disorders, depression or post-traumatic stress disorder (PTSD), which is a serious health, social and economic problem. It has been shown that Amg hyperactivity plays an important role in the pathophysiology of the above mentioned diseases, but the mechanisms underlying these interactions are not fully understood. **The aim of my study was to expand knowledge about the influence of Amg hyperactivity induced by long-term electrical stimulation of the CeA on behavioural, haematological, immunological, and neurochemical components of the stress response, as well as the quantitative and qualitative composition of the intestinal microbiota in rats.** Disorders of the intestinal microbiota are another important factor in the development and course of neuropsychiatric diseases. Microorganisms in the gastrointestinal tract and their neuroactive metabolites, via the gut-brain axis, can modulate the immune system, stress response and CNS functioning, including behaviour. Prebiotics such as galactooligosaccharides (GOS) are non-digestible complex sugars that promote the proliferation and activity of numerous beneficial bacterial species natively found in the gut. Reports about the effects of these prebiotics on CNS functions in the aspect of neuropsychiatric disorders are still few and limited. **Another aim of my work was to verify the therapeutic potential of GOS in the context of alleviating changes caused by long-term electrical stimulation of the CeA, as well as to compare the effectiveness of GOS supplementation with the effects of citalopram (CIT) therapy, an anxiolytic and antidepressant drug.**

My study was performed on male Wistar Han rats (n = 48) divided into 6 experimental groups (n = 8 in each group), among which animals from 3 groups were subjected to a 14-day electrical stimulation of the CeA and received water (Stim), GOS (StimGOS) or CIT (StimCIT) for 21 days, while the remaining 3 groups included rats subjected to sham stimulation (the same procedure with the current flow turned off) and receiving water (Sham), GOS (ShamGOS) or CIT (ShamCIT). GOS supplementation and CIT therapy began 7 days before the first stimulation of the CeA. Examined behavioural parameters included locomotor activity (actometer), anxiety-like behaviour (elevated plus maze test), and social behaviour (three-chamber test). The analysis also included the level of haematological parameters in peripheral blood (haematology analyzer), as well as the plasma level of hormones related to the stress response (epinephrine, norepinephrine and corticosterone), and markers related to inflammation (TNF-α and IL-10) determined by ELISA method. I assessed the norepinephrine and gamma-aminobutyric acid (GABA) concentrations in homogenates of CNS structures – the cerebral cortex, hippocampus, brainstem, and hypothalamus with ELISA. The quantitative and

qualitative composition of rat gut microbiota in samples of large intestine content was assessed with microbiological methods (cultures in solid media) and the MALDI-TOF method.

I demonstrated that long-term electrical stimulation of the CeA resulted in an increase in escape-like locomotor activity, as well as in anxiety-like behaviour, and decrease in sociability and social memory level in rats. Stim animals, compared to the Sham control group, were characterized by a decrease in the values of numerous haematological parameters, excessive endocrine stress response, intensification of pro-inflammatory processes, and weakened anti-inflammatory activity. These changes were accompanied by a decrease in GABA level and an increase in norepinephrine concentration in all examined CNS structures, as well as in quantitative and qualitative disturbances in the gut microbiome, manifested as a decrease in the number of probiotic species with simultaneous increase in the number of potentially harmful bacteria. GOS supplementation contributed to a significant alleviation of changes induced by electrical stimulation of the CeA. StimGOS animals, compared to stimulated and unsupplemented rats, were characterized by a decrease in excessive motor activity and anxiety behaviour, an increase in prosocial behaviour and social memory improvement, haematological parameters normalization, an increase in the anti-inflammatory IL-10 concentration, and a decrease in level of pro-inflammatory TNF-α and hormones related to the stress response. GOS administration also contributed to increased GABA level and reduced norepinephrine level in examined brain structures and alleviating disturbances in the gut microbiota composition with a simultaneous increase in probiotic species number. Additionally, I demonstrated that effectiveness of GOS supplementation was comparable to CIT therapy. In the context of social behaviour, haematological parameters, regulation of peripheral and central norepinephrine levels and beneficial effect on gut microbiota disturbances, GOS had even greater therapeutic potential.

The obtained results indicate the high therapeutic potential of GOS in limiting adverse changes caused by electrical stimulation of the CeA, which may lead to further research on animal models, as well as clinical trials verifying the possibility of using these prebiotics as a supplement to support therapy in patients with anxiety disorders, depression or PTSD. In addition, due to the high level of safety and multifaceted health benefits, the dissemination of the results of research on prebiotic supplementation may also contribute to the use of GOS to prevent the development of disorders related to anxiety and stress.