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Title of the doctoral dissertation:

„Study of the expression of proteins related to the brain α1 adrenergic receptor transduction pathway in animal models of depression"

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Summary

Explaining the pathomechanisms of depressive disorders continues to be a major challenge for neurobiology because the pathophysiological changes involve many related and interacting systems in the brain. One of them is the noradrenergic system, part of which is the signal transduction pathway from α1-adrenergic receptors to various effector proteins inside the cell, including proteins involved in pro-life processes. Adrenergic receptors (ARs), of which α_1-ARs are a subfamily, are mainly responsible for the activity of neurons of the noradrenergic system. In this subfamily the α_{1A}, α_{1B} and α_{1D} subtypes are specified, all related to the $G_{q/11}$ protein-dependent signal transduction pathway, and the stimulation of the formation of secondary messengers, consequently leading to an increase in the level of intracellular calcium ions and activation of protein kinase C. In studying the mechanisms of depression and its treatment, animal models of depression are used. Especially important are stress-based models, which at least partially represent the changes seen in patients, including the axial symptom of depression, which is a state of anhedonia, i.e. avoidance of experiencing pleasure.

The goal of this study was to investigate in two animal models of depression whether depressive disorders lead to adaptive changes within the receptor system of the α_1-ARs in the brain at 3 levels: (1) assessment of the density and expression of α_1-AR receptor subtypes; (2) expression of G proteins bound directly to this receptor; (3) effector proteins bound only indirectly to the α_1-AR receptor system and involved in processes to counteract the effects of cellular stress – the activity of

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ERK1 / 2 kinases and the expression of heat shock proteins of the Hsp70 and Hsp90 families and Bcl-2 family proteins that regulate apoptosis. The first model was a mouse model of the stress of repeated short-term postnatal manipulation (PSM), in which mouse neonates were subjected to daily isolation from their mother and her scent after birth, and symptoms of anhedonia were studied in adult (90-day-old) mice. The second model was the rat model of chronic mild stress (CMS), in which adult rats were subjected to unpredictable, repeated stress stimuli, and symptoms of anhedonia were studied in parallel. The CMS model also examined the effect of treatment with the antidepressant drug, imipramine (IMI), on CMS-induced adaptive changes. In both models, the applied stress induced a state of anhedonia in the test animals. In addition, the CMS model identified rats that did not respond with anhedonia to the administered stress, and among those responding to stress, those that did not respond to IMI treatment.

Based on the obtained results, it was found that both PSM and CMS stress-induced changes in the amount of brain α_1-AR receptors, as well as changes in the expression of proteins related to the regulation of neuroprotective processes.

The PSM stress caused a decrease in the density of the total α_1-AR pool and the α_{1B}-AR subtype in some thalamic nuclei and the hippocampus in adult mice. Three-week CMS stress caused a decrease in the density of the α_{1B}-AR subtype in the rat thalamic nucleus, which normalized after 8 weeks of CMS. Treatment with IMI reduced the density of the α_{1B}-AR subtype in the thalamic nucleus, which remained at the level of untreated rats. In the hippocampus, on the other hand, 3-week CMS caused an increase in the density of the α_{1B}-AR subtype, which in turn decreased after 8 weeks of CMS. IMI treatment restored the density of the α_{1B}-AR subtype to the values of the control group.

The PSM stress decreased Hsp72 mRNA expression in the prefrontal cortex and increased in the thalamus of adult mice, altering their protective effects. In contrast, the PSM-induced decrease in Hsp90α mRNA expression in the prefrontal cortex and thalamus may result in the attenuation of glucocorticoid receptor-dependent signaling. The increase in anti-apoptotic Bcl-2/Bax and Bcl-xl/Bax mRNA ratios in the hippocampus observed after PSM may reflect the enhancement of neuroprotective mechanisms in adult mice. In contrast, 3-week CMS reduced Hsp90α mRNA expression in the HIP of stress-responsive rats, which may result in attenuated signaling from the receptor for glucocorticoids. In contrast, after 8 weeks of CMS, there was an increase in Hsp72 protein in both the hippocampus and

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prefrontal cortex indicating the existence of a stress response at the cellular level. Treatment with IMI extinguished this response, but in untreated animals there was an induction of Hsp72 mRNA expression, suggesting the existence of an unquenched cellular stress response. It was also found that 3-week CMS induced an increase in anti-apoptotic Bcl-2/Bax and Bcl-xl/Bax mRNA ratios in the hippocampus, which may be a compensatory enhancement of neuroprotective processes. In contrast, these ratios were elevated in untreated rats, which also indicates the induction of neuroprotective compensatory mechanisms. In addition, in the 8-week CMS model, a decrease in the level of pERK1/2 phosphorylation protein was observed in the hippocampus of untreated rats, which may indicate impairment of learning, memory and plasticity mechanisms in these rats.

In conclusion, the study revealed an important role of α_1-AR subtypes, including α_{1B}, in the mechanisms of action of both types of stress, as well as in the process of response to treatment with IMI. The induction of neuroprotective compensatory mechanisms by PSM and CMS, as well as the cellular stress response by CMS, which was extinguished by IMI treatment, was also demonstrated. These results enrich our knowledge of the pathomechanisms of depression helpful in developing more effective antidepressant therapies.

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