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Transcriptomic response of genes related to the glutamate- and GABA-ergic systems in the hypothalamus of ISIAH and WAG rats exposed to acute restraint stress

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Motivation and Aim: The hypothalamus is the main structure of the brain that implements the body's response to stress by activating the hypothalamic-pituitary-adrenal axis (HPA) and other systems. Glutamate and GABA are the primary neurotransmitters that provide the necessary level of excitation of the hypothalamic neuroendocrine cells. An imbalance of these neurotransmitters can lead to significant changes in HPA axis activity [1]. The aim of the work was to compare changes in the transcriptional activity of genes related to the glutamate- and GABA-ergic systems of the hypothalamus in response to acute restriction stress in ISIAH rats modeling a stress-sensitive form of arterial hypertension and in normotensive WAG rats.

Methods and Algorithms: Male rats of the hypertensive line ISIAH/Icgn and the normotensive line WAG/GSto-Icgn at the age of 3 months were used for the study. Transcriptomic sequencing (RNA-Seq) of the hypothalamus was performed on 4 groups of rats: 1) ISIAH_control; 2) WAG_control; 3) ISIAH_stress; 4) WAG_stress (7 rats in each group). Two groups of rats (ISIAH and WAG) were subjected to restriction stress by placing the rats in a tight wire-mesh cage for 2 hours. After completing the stress procedure, the rats were immediately decapitated, the hypothalamus was isolated, and the samples were homogenized in the ExtractRNA reagent (Evrogen, Russia). Paired-end sequencing of cDNA libraries was performed on the DNBSEQ platform (DNB-SEQ technology) with a read length of 150 bp and a sequencing depth of more than 30 million uniquely mapped reads. All samples were analyzed as biological replicates. The study protocol was approved by the Bioethical Council of the federal research center Institute of Cytology and Genetics SB RAS (Novosibirsk, Russia), protocol No. 115 of December 20, 2021. The quality of the sequencing data was assessed using FastQC software (version 0.11.5). Sequencing data were mapped to the rat reference genome mRatBN7.2/rn7 (Wellcome Sanger Institute, rn7 assembly, November 2020) using the STAR software package, version 2.7.10a. Differential gene expression (DEG) assessment was performed with DESeq2 v1.30.1 using surrogate variable analysis (SVA) to account for unwanted variations in the data caused by possible random biases during sample preparation. The significance threshold for determining DEGs was chosen taking into account correction for multiple comparisons and corresponded to adjusted p-

value < 5%. The results show only DEGs that changed their transcription level under stress by more than 1.5-fold (Log2 fold change ≥|0.585|).

Results: Unidirectional downregulation of the expression of the *Gad2*, *Grik3*, and *Gabrb3* genes in the hypothalamus of both strains of rats indicates a nonspecific (rat strain-independent) change in their expression in response to restraint stress. The *Gad2* gene encodes the enzyme for the conversion of glutamate to GABA [2], and a decrease in the amount of its mRNA can lead to a change in the balance of excitation and inhibition processes. The *Grik3* gene encodes a subunit of the kainate glutamate ionotropic receptor GluK3. Kainate receptors are involved in postsynaptic signal transduction and control of neuronal excitability, and they presynaptically modulate the release of GABA and glutamate [3]. A decrease in *Grik3* gene expression in response to acute stress may lead to changes in GABAergic and glutamatergic transmission in hypothalamus of both ISIAH and WAG rat strains. The *Gabrb2* and *Gabrb3* genes encode metabotropic GABA-B receptors. The amount of *Gabrb3* gene mRNA decreased in rats of both strains under acute stress, while a decrease in the amount of *Gabrb2* mRNA occurred only in the hypothalamus of ISIAH rats. Exposure to stress has shown that the greatest inter-strain differences are observed in changes in the transcriptional activity of the GABA-A receptor gene subunits. The *Gabrq* and *Gabrd* genes encode the δ and θ subunits respectively. This study shows a decrease in *Gabrq* mRNA and an increase in *Gabrd* mRNA after stress in the hypothalamus of WAG, but not ISIAH, rats. The θ-subunit has not been sufficiently studied, while the δ-subunit has been shown to play an important role in the regulation of hypothalamic neurons that synthesize corticotropin-releasing hormone, and knockout of the gene encoding this subunit in these neurons reduces the corticosteroid response to stress, probably due to loss of the disinhibitory effect of GABA following acute stress [4]. Our work also shows a different response to acute stress at the level of transcriptional activity of the metabotropic glutamate receptor gene *Grm5*: a decrease in mRNA in the hypothalamus of ISIAH rats, but no changes in WAG. The contribution of this receptor to synaptic plasticity, the development of long-term potentiation, and the regulation of cell excitability is described in the literature [5].

Conclusion: Short-term (2 hours) restraint stress causes changes in the expression of a significant number of genes related to the functioning of the glutamate and GABAergic systems of the hypothalamus. Downregulation of the expression of the *Gad2*, *Grik3* and *Gabrb3* genes was shown in rats of both strains and, accordingly, does not depend on the genotype. The strain-specific response to stress in the hypothalamus of hypertensive ISIAH rats is associated with a decrease in the transcription of the *Grm5* and *Gabrb2* genes, and in the hypothalamus of normotensive WAG rats – with a change in the level of transcription of the *Gabrq* and *Gabrd* genes. The identified interstrain differences may modulate the activity of synaptic transmission in the hypothalamus and contribute to different stress reactivity in normotensive WAG and ISIAH rats, which are an animal model a stress-sensitive form of arterial hypertension.

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