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# Proteomic Characteristics of Blood Serum in Rats with Different Behavioral Parameters after Acute Stress

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We studied proteome profile of blood serum of Wistar rats with different behavioral activity immediately and in 1 and 3 days after acute stress on the model of 12-h immobilization during the nighttime. Comparative analysis of 2D-electrophoretograms revealed differences in the expression of serum proteins in non-stressed (control) and stressed (experimental) rats. We found 22 protein spots that characterized the proteomic features of blood serum in rats with different prognostic resistance to stress. Mass-spectrometry of isolated spots identified 6 functional proteins. Persistent proteome changes in the blood of animals at different stages after acute stress were determined. The specificity of proteomic characteristics of blood serum was shown in behaviorally passive and active rats during the post-stress period. These data extend the concept on specific protein markers for the formation of a negative emotional state and adaptive-and-compensatory processes in mammals with different sensitivity to stressogenic factors.

**Key Words:** *proteomics; blood; immobilization stress; behaviorally passive and active rats*

Emotional stress is a nonspecific response of the body to exogenous and endogenous factors leading to complex systemic changes in various physiological functions. There is convincing evidence of marked differences in the individual resistance of mammals to the development of negative consequences of stressful effects. This manifests in behavioral disorders [3], changes in stress-marker organs [6], shifts of biochemical parameters of tissues [4], and other physiological parameters during stress. The open-field test is widely used for prediction of the sensitivity of experimental animals to stress. It was shown, in particular, that rats demonstrating active behavior in this test are prognostically resistant, and passive rats are predisposed to the formation of negative consequences of emotional stress [2].

Many pathological conditions in mammals develop not during, but after stress exposure. This post-stress period is critical for the development of the entire complex of compensatory reactions aimed at limiting the negative consequences of extreme factors. It is important that the course of rehabilitation period after stress exposure differ in individuals with different behavioral activities demonstrating different sensitivity to stress. For instance, we revealed specificity of changes in metabolic and behavioral parameters reflecting shifts in endogenous biological rhythms in open field passive and active rats at different time periods after negative emotional effects [5].

Thus, the development of new methods and approaches to prevent or reduce the severity of pathologies caused by stress should be based on the results of a detailed analysis of physiological and biochemical processes unfolding in the mammalian organism during the post-stress period. In view of the latest achievements in molecular medicine, the search for new proteomic markers of the formation of a negative emotional state during stress exposure and implementation of adaptive-compensatory reactions at different stages after the stress seems to be a promising trend and peripheral blood seems to be promising object, because it is easily available, studied parameters are

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highly informative, and the results are well reproducible. It should be noted that the formation of stress reactions in mammals should be performed with consideration of individual characteristics of systemic organization of physiological functions.

Here we analyzed proteomic profiles of blood serum in rats with different behavioral activity at different time after acute stress exposure.

## MATERIALS AND METHODS

The work was performed on male Wistar rats ($n$=48; body weight 181.3±16.1 g) with strict adherence to "Regulations for Conduction of Animal Experiments" approved by Ethical Committee of P. K. Anokhin Research Institute of Normal Physiology (protocol No. 1, September 3, 2005), the requirements of the WSPA, and the European Convention for the Protection of Animals used for Experiments or for Other Scientific Purposes.

The rats were kept in cages (6 animals per cage) in rooms with artificial illumination (light from 09.00 to 21.00) at 20-22°C and had free access to water and food. After delivery to the laboratory, the rats were allowed to adapt to laboratory conditions for 5 days.

The initial behavioral characteristics of rats were determined by 3-min open-field testing [2]. To calculate the activity index of animals, the total number of crossed peripheral and central sectors, peripheral and central rearing postures, as well as explored objects was divided by total duration of latent periods of the first movement and visits to center of the open field. Depending on the initial parameters of behavior, the rats were divided into passive ($n$=24) and active ($n$=24) that differed by open field activity index (0.47±0.02 and 4.47±0.47 respectively).

Acute emotional stress was modeled by 12-h immobilization in individual plastic boxes during the nighttime (21.00-09.00). The control (non-stressed) animals remained in "home" cells during this period.

Behaviorally passive and active rats were divided into 8 groups (6 animals per group): non-stressed active and passive rats (control groups 1 and 2, respectively); active and passive rats subjected to acute emotional stress (stress groups 3 and 4, respectively); active and passive rats that recovered for 1 day after stress (rehabilitation groups 5 and 6, respectively); active and passive individuals that recovered for 3 days after stress (rehabilitation groups 7and 8, respectively).

The animals of the control and experimental (stressed) groups were decapitated, the blood collected during decapitation was centrifuged for 15 min at 1500$g$, and serum was collected. The obtained serum samples were frozen and stored -20°C.

Before 2D electrophoresis, serum samples were processed using ProteoMiner Protein Enrichment Small-Capacity Kit (cat.# 163-3006; Bio-Rad). Proteomic mapping, hydrolysis of protein with trypsin, and recording of mass-spectra were conducted as described previously [7].

## RESULTS

Comparison of 2D electrophoregrams using PDQuest 8.0 software (Bio-Rad) revealed differences in the expression of some serum proteins in non-stressed (control) and experimental rats. We revealed 22 protein spots that characterize the proteomic features of blood serum in rats with different prognostic resistance to stress. Mass-spectrometry of isolated spots identified 6 functional proteins (Table 1).

**TABLE 1.** Proteins Identified by Mass Spectrometry in Rat Serum

| Protein | Groups | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | control | | stress | | stress+1 day of rehabilitation | | stress+1 days of rehabilitation | |
| | P | A | P | A | P | A | P | A |
| λ-light chain of immunoglobulin | — | — | — | — | + | + | + | + |
| Rho guanine nucleotide exchange factor 5 | + | + | + | — | + | + | + | + |
| Cytochrome P450, family 2, subfamily E, polypeptide 1, isoform CRA_e | + | — | + | — | + | + | + | + |
| Peroxiredoxin 6 | + | — | — | — | — | — | — | — |
| Prelamin A/C | + | + | + | + | — | — | + | + |

**Note**. P: behaviorally passive rats; A: behaviorally active rats; "+" — increased expression; "—" — reduced expression.

Lambda-light chains of immunoglobulins were absent in the blood serum of rats in the control and immediately after the acute stress, but these proteins were detected on days 1 and 3 of rehabilitation period in both behaviorally passive and active animals. Since immunoglobulins are one of the most important factors of a specific humoral response [14], their presence in the serum indicates strained status of the immune system. Marked activation of the humoral response under these conditions is aimed at the maintenance of homeostasis during the rehabilitation period after emotional stress.

Rho guanine nucleotide exchange factor 5 is responsible for GDP to GTP substitution in proteins of the Rho family. These proteins are involved in the organization of the actin cytoskeleton, intracellular signaling, and positive regulation of the podosomes assembly, thus are responsible for intercellular adhesion [9,10,15]. In rats with different behavioral activity, Rho guanine nucleotide exchange factor 5 was detected in blood serum both in the control and during the rehabilitation period after stress (days 1 and 3). Intergroup differences in this parameter were found in animals immediately after stress. Under these conditions, Rho guanine nucleotide exchange factor 5 was detected in behaviorally passive, but not in active animals. Normal regulation of intercellular interactions is essential for the maintenance of diverse cellular functions, including proliferative activity, differentiation, and development. Reduced expression of this factor in active individuals during the early post-stress period attests to some disorders in the organization of the actin cytoskeleton under conditions of negative emotional exposures.

Lamins are fibrillary proteins that are obligatory components of the nuclear plate. Lamins A and C play an important role in nucleus assembly, organization of chromatin, and dynamics of telomeres, are necessary for the development of the peripheral nervous system and skeletal muscles, and for cell proliferation [1]. Prelamin A/C activates the process of aging in smooth muscle cells by disturbing the mitotic cycle and inducing DNA damage [13]. Suppressed expression of prelimin A/C in rats with different behavioral activity revealed by us on day 1 of rehabilitation period demonstrates possible changes in the regulation of the cell cycle and attests to progression of certain destructive processes in animals during the specified period.

At the initial state (control), expression of CRA_e isoform of cytochrome P450 in serum was reduced in behaviorally active rats, but not in passive rats. These intergroup differences persisted after acute emotional stress. However, during the rehabilitation period (days 1 and 3) after stress, the expression of cytochrome P450 protein in blood serum of active animals increased. This dynamics of cytochrome P450 protein expression in the control and during the post-stress period in behaviorally active rats prognostically resistant to negative effects of stress in comparison with passive animals supplement and agree with the previously published data. In previous studies, not only possible association of cytochrome P450 protein with the development of depressive disorders was detected, but also its effect on the development of obsessive-compulsive symptoms and anxiety [11]. It can be assumed that these changes are pathogenetically related to the systemic response of the body to acute stress.

Peroxiredoxin 6 in mammals plays an important role in intracellular signaling and metabolism of phospholipids and participates in neutralization of peroxide compounds, thus limiting the development of oxidative stress [12]. In the initial state (control), behaviorally passive rats, in contrast to active rats, were characterized by enhanced expression of peroxiredoxin 6 in blood serum. Our findings attest to initially more strained status of antioxidant systems in prognostically predisposed to stress animals.

In previous studies on the model of immobilization stress, we demonstrated changes in the proteomic profile of the hippocampus in rats with different behavioral activity in the open-field test [8]. Proteins differentially expressed in the hippocampus of prognostically predisposed and resistant to stress animals were detected by proteomic mapping with subsequent mass spectrometry. The data obtained earlier and the results of this study demonstrate the development of persistent proteome changes in the brain tissues and blood of animals at different stages after acute stress. The specificity of proteomic characteristics of biological tissues was shown in behaviorally passive and active rats in the dynamics of the post-stress period. These data extend our knowledge on specific protein markers of the formation of negative emotional states and adaptation-compensatory processes in mammals with different sensitivity to stress factors.

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