PHYSIOLOGY

Proteomic Study of Rat Hippocampus under Conditions of Emotional Stress

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Proteomic differences in the hippocampus of stress-resistant and stress-sensitive rats were detected on the model of emotional stress. Differential expression of some proteins was detected in animals with different behavioral activity initially and after experimental stress exposure. Specific involvement of the hippocampus in the realization of stress response in animals with different sensitivity to emotional stress was demonstrated.

Key Words: *proteomics; emotional stress; hippocampus*

Emotional stress (ES) induces a complex of autonomic disorders associated with changes in the systemic organization of physiological functions, development of immune, hormonal, and mental disorders [1]. The structural and functional components of the brain are highly sensitive to stress factors and play the key role in the realization of systemic response to stress. The hippocampus and other limbic structures of CNS in mammals are actively involved in organization of emotional reactions [4]. Persuasive evidence of the important role of the hippocampus in the formation of response to stress exposure of different kind has been obtained by the present time. For instance, experiments on rats have demonstrated that immobilization stress stimulates acetylcholine release in the hippocampus [10]. Receptors of glucocorticoids (the hormones playing an important role in the feedback mechanisms and inhibiting functional systems of the organism under of extreme conditions) have been detected in this brain structure [13]. In addition, the hippocampus is characterized by high density of specific binding sites for endogenous bioactive substances (for example, melatonin) with antistress properties [15].

Significant genetic and individual differences in the sensitivity of mammals to the negative aftereffects of ES have been revealed [4]. It is essential to predict the resistance or sensitivity of individuals to stress exposure before the emergence of conflict situations. For instance, open field behavior is a reliable indicator of the rat sensitivity to stress [2]. Animals with high orientation and exploratory activity in this test demonstrate better resistance to stress than passive rats.

Despite numerous clinical and experimental data on the role of the hippocampus in the systemic organization of emotional status of mammals, many questions in this sphere remain unanswered. For example, specific involvement of the hippocampus in the formation of stress response in animals with different behavioral characteristics and different predicted resistance to negative aftereffects of typical stress exposure is unclear. Changes in functional activity of the hippo-

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campus in mammals at different stages after exposure to ES were never analyzed.

Here we studied the proteomic characteristics of the hippocampus in rats demonstrating active and passive behavior during different periods after ES.

MATERIALS AND METHODS

The study was carried out on male Wistar rats (n=48) weighing 253.8±3.1 g. Experiments were carried out in accordance with Regulations for Studies with the Use of Experimental Animals approved by the Ethic Committee of P. K. Anokhin Research Institute of Physiology (Protocol No. 1, September 3, 2005), and regulations of the World Society for Protection of Animals (WSPA) and European Convention for Protection of Experimental Animals.

The animals were kept in cages (6 per cage) in rooms with artificial illumination ("day" from 08.00 to 20.00, "night" from 20.00 to 08.00) at 20-22°C with

TABLE 1. Proteins Identified by Mass-Spectrometry in the Hippocampus of Experimental Rats

Protein	Region No.	Comments	Function	Reference
Calreticulin	1	Low expression in groups recovering after stress (days 1 and 3)	Usually presents in ER, where it functions as Ca-binding protein and molecular chaperone, interacts with the majority of monoglycoside glycoproteins produced in ER. Interacts with DNA-binding NR3C1 domain and mediates nuclear export. Involved in gene expression regulation	[8]
gl 256000796+gi 149053421	2	Low expression in stressed passive animals	Not determined	–
α-Synuclein	4	Not identified in stress group	In neurons is presumably associated with vesicular membrane and participates in their transport to the presynaptic terminal. Has no manifest secondary structure and is referred to proteins with nonglobular conformation. These proteins are involved in protein-protein interactions due to which they are structured upon binding to other proteins	[9]
Phosphatidylethanolamine-bound protein	4	Low expression in stressed passive animals	Binds ATP and phosphatidylethanolamine. Characterized by low affinity to phosphatidylinositol and phosphatidylcholine. Inhibits RAF1 kinase activity and acts as MEK phosphorylation competitive inhibitor	[12]
LDH	3	Appears in passive animals on days 1 and 3 after stress	Oxidoreductase enzyme catalyzing at the terminal stage of glycolysis the reversible oxidation of L-lactate to pyruvate	[14]
Thiomorpholine carboxylate dehydrogenase (ketimine reductase)	3	Low expression in stressed passive animals	Specifically catalyzes imide bonds reduction in brain tissue substrates (cystathionine ketimine (CysK) and lanthionine ketimine (LK)). Binds thyroid hormone	[6]
Prohibitin	3	Not detected in control passive animals	Inhibits DNA synthesis. Involved in regulation of proliferation. Presumably participates in the regulation of mitochondrial respiration and aging processes	[5]
F-capping protein subunit α-2	3	High expression only in stress group	Binds to rapidly growing terminals of actin filaments, thus blocking subunit replacement in these terminals	[7]
Cu-Zn-SOD	4	Low expression in active animals of all experimental groups	Plays the important role in cellular antioxidant defense by destroying radicals produced in cells and toxic for the biological system	[11]
Glutamine synthetase	5	Low expression in stressed passive animals	Ligase catalyzing NH3 incorporation into organic compounds in the presence of bivalent metal ions. Located in cell mitochondria and cytoplasm; Mg2+ ions are a cofactor essential for enzyme activity	[16]

Note. ER: endoplasmatic reticulum.

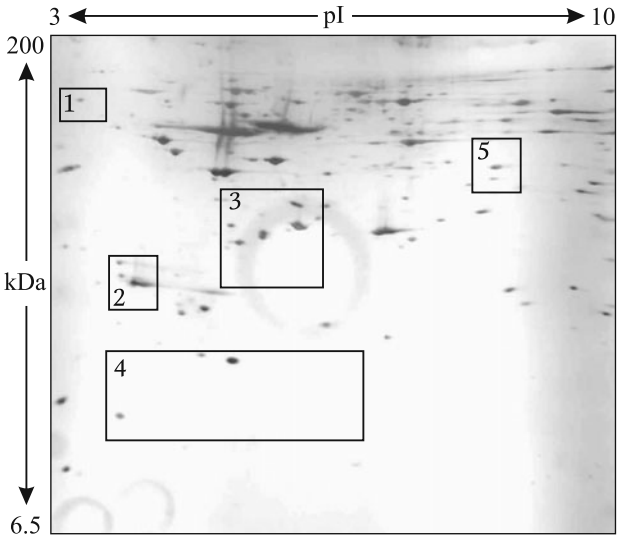

Fig. 1. Electrophoregram of rats' hippocampus. Protein regions 1-5.

free access to water and food. The rats were adapted to laboratory conditions for 5 days. Twelve-hour immobilization of rats in individual plastic boxes during the night hours (21.00-09.00) served as the model of acute ES. Control (unstressed) animals stayed in their "home" cages during these hours.

Individual typological characteristics of rats were evaluated in the open field test for 3 min [2]. By their initial behavioral parameters, the rats were divided into active (n=24) and passive (n=24) animals differing by the mean index of activity: 0.47±0.02 in passive rats and 4.47±0.47 in active rats. The active and passive rats were then divided into 8 groups, 6 animals per group. Groups 1 and 2 consisted of unstressed (intact) active and passive animals. Active and passive rats exposed to stress were decapitated directly after ES (groups 3 and 4), 24 h after stress (groups 5 and 6), and after 3 days (groups 7 and 8). The hippocampus was isolated after decapitation.

For proteomic analysis, the hippocampus (whole structure, 18±2.4 mg) was placed in 250 µl buffer for sample dilution (8 M urea, 2 M thiourea, 80 mM dithiotreitol, 5% ampholytes, 16.7% of 30% CHAPS solution, and 10% NP40), containing Protease Inhibitor Cocktail P8340-1ML (Sigma) in a 100:1 ratio. The sample was exposed in an ultrasonic bath (USV-12, Sapphire) for 10 min, after which it was centrifuged for 10 min at 20,000g. The supernatant was analyzed by 2D electrophoresis. Proteomic mapping, trypsin hydrolysis of protein, and registration of mass spectra were carried out as described previously [3].

RESULTS

Electrophoregrams of the rat hippocampus at different stages of experiment showed different expressions of proteins. Significant differences in the expression were detected for 5 regions of the electrophoretic map (Fig. 1).

The results of mass-spectrometry of the detected spots identifying several functional proteins and one protein with unknown function are presented in Table 1.

The data indicate different expression of some proteins, for example, Cu-Zn-SOD in animals with different behavioral parameters initially and after stress exposure. Differences in α-synuclein expression between active and passive animals were detected after experimental stress exposure.

The results demonstrate specific involvement of the hippocampus in the realization of stress response in animals with different sensitivity to ES. Animals with active and passive behavior are characterized by pronounced differences in the hippocampal proteomic features during different periods after acute emotional stress. These data indicate the significance of an individual approach to the analysis of physiological mechanisms underlying the resistance or sensitivity of individuals to the negative aftereffects of stress exposure.

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