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Effect of Acute Emotional Stress on Proteomic Profile of Selected Brain Areas and Lysosomal Proteolysis in Rats with Different Behavioral Activity

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We compared proteome profiles of selected brain areas (cortex, amygdala, hippocampus, and reticular formation) and measured cathepsins B and D activity in liver lysosomal fraction in rats with different behavioral activity under conditions of emotional stress. In passive rats, the expression of some proteins in various brain regions was changed and baseline cathepsin B activity was higher than in active animals. Taken together, the results attest to differences in the adaptive response formation in rats, depending on behavioral features.

Key Words: *brain; emotional stress; proteomics; cathepsin B; cathepsin D*

In conflict situations, emotional stress first leads to disintegration of multiparametric interactions of different homeostatic functional systems. Numerous animal experiments have demonstrated that brain structures, particularly limbic–reticular formations, are first included in the response to stress [6]. Limbic system is a complex of brain structures involved in organization of emotional and motivational behavior and instincts, as well as feeding, sexual and defense behavior and sleep-wake rhythm. Multifunctional limbic formations, such as hippocampus and amygdala are directly linked to emotions, as is the case with reticular formation, which can activate different brain areas and bring new, unusual or biologically significant information to its specific areas [1].

Several studies have explored the role of lysosomal proteolysis in the response to various stress factors [3]. It was found that activity of proteolytic enzymes significantly increased upon exposure to various stimuli, and they are transformed from regulation factor into injury factor [2].

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Here we compared proteome features of selected brain areas (hippocampus, amygdala, reticular formation, and cerebral cortex) and evaluated activity of the two most powerful lysosomal proteases differing by specificity and functional role (aspartate proteinase cathepsin D and cysteine proteinase cathepsin B), in liver lysosomal fraction of rats with different behavior under conditions of emotional stress development.

MATERIALS AND METHODS

The study was carried out on male Wistar rats (n=48) weighing 253.8±3.1 g. The experiments were designed in accordance with "Rules for Studies with the Use of Experimental Animals", approved at the meeting of the Ethics Committee of the P. K. Anokhin Research Institute of Normal Physiology (Record No. 1, September 3, 2005), and requirements of the World Society for Protection of Animals (WSPA) and the European Convention for the Protection of Experimental Animals.

The animals were housed in cages (6 animals per each) in rooms with artificial light (08.00-20.00 h light, 20.00-08.00 h darkness) at 20-22°C with free access to water and food. The rats were adapted to laboratory conditions for 5 days. Acute emotional

stress was modeled by 12-h immobilization of rats in individual plastic houses at night (21.00-09.00 h). During this period, control (non-stressed) animals remained in home cages.

Individual typological characteristics of animals were determined when the rats were tested in the open field for 3 min [4]. Depending on the baseline behavior in the open field rats were divided into active (n=24) and passive (n=24). Then behaviorally active and passive rats were divided into 8 groups (6 animals per each). There were groups of non-stressed (intact) animals, active and passive individuals, active and passive rats, subjected to stress and decapitated immediately after emotional stress, and 1 and 3 days later. After decapitation, the cerebral cortex, amygdala, hippocampus, and reticular formation were isolated.

Preparation of brain samples, proteomic mapping, protein hydrolysis by trypsin and mass spectra recording were carried out according to the previously described protocols [7-9].

Liver lysosomal fraction was isolated by differential centrifugation according to de Duve. Protein content in liver tissue homogenate was determined by the Bradford method.

Cathepsin D activity was determined spectrophotometrically at 280 nm by the increase in optical density of TCA-soluble products released from hemoglobin as a result of 5-min enzymatic reaction. Enzyme activity was expressed in micromoles of tyrosine released as a result of hemoglobin cleavage per 1 mg of protein. Cathepsin B activity was measured fluorometrically. Na-CBZ-Arg-Arg-7-amido-4-methylcoumarin (Arg-Arg-7-AMC) was used as the substrate. 7-Amido-4-methylcoumarin (AMC) was released as a result of enzymatic reaction for 5 minutes and assessed by changes in fluorescence intensity at 440 nm (excitation at 348 nm). Cathepsin B activity was expressed in μmol of AMC, released from Arg-Arg-7-AMC, per 1 mg of protein.

The data were processed statistically using Statistica 8.0 software and multivariate analysis of variance test.

RESULTS

The study revealed a number of proteins with lower or higher expression in selected brain areas, which depended not only on the type of behavioral activity, but also on the stage of the experiment (Table 1).

GFAP expression was reduced in the amygdala during acute emotional stress both in active and passive animals. This protein is one of the main immunocytochemical markers of astrocytes, the most important macroglia representatives in mammalian CNS. GFAP accumulation in astrocytes is known to be associated with their barrier function [11,13]. The experiment revealed increased GFAP expression in passive animals on day 1 after stress, in active – on day 3, which was probably associated with recovery of this brain area in animals subjected to stress.

Reduced expression of septin-5 protein in the cerebral cortex of rats of both behavior types during the recovery period is of particular interest (days 1 and 3 after stress). This protein belongs to the family of GTP-bound proteins, which play a significant role in mechanisms of neuronal growth and synaptic transmission [14].

Differences in protein expression in the hippocampus were most numerous. There was a certain similarity in the hippocampus protein profile of rats of the

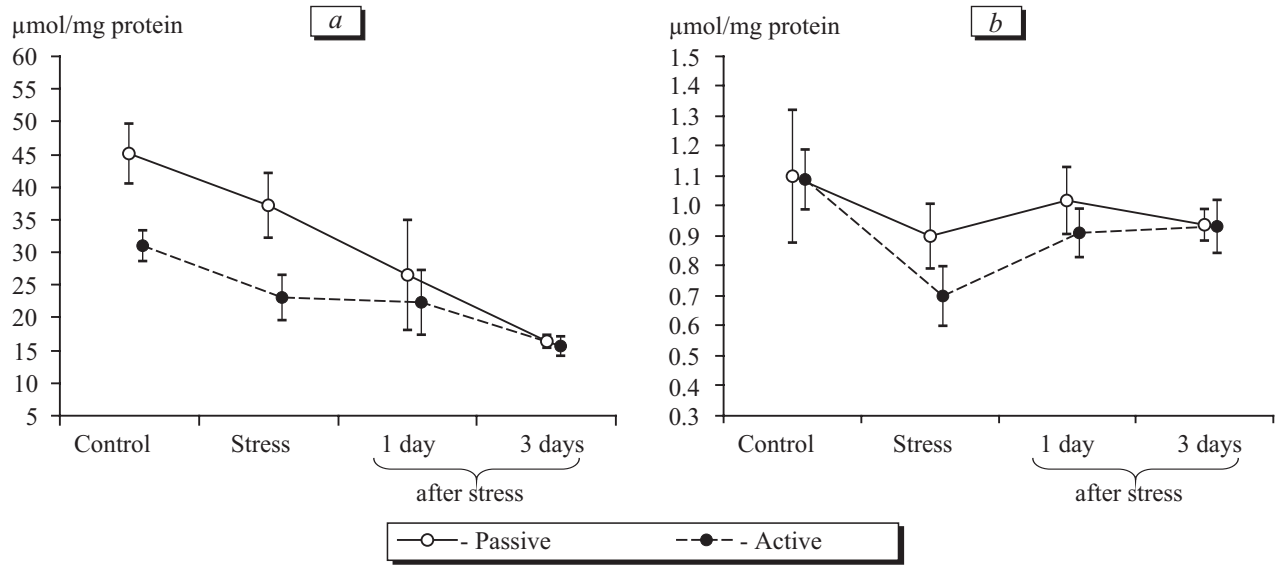

Fig. 1. Activity of cathepsin B (*a*) and D (*b*) in the lysosomal fraction of rat liver under conditions of emotional stress.

TABLE 1. Functional Proteins, Identified by Mass Spectrometry in the Brain Areas under Study in Active and Passive Rats after Emotional Stress

Group	Type of behavioral activity	Cortex	Amygdala	Hippocampus	Reticular formation
Control	Active		↑Proteasome p45	↓Cu-Zn-SOD	
	Passive			↓Prohibitin	
Stress	Active	↓Dynactin	↓GFAP	↓Alpha-synuclein	
				Subunit alpha-2 of the F-capping protein	
				↑Cu-Zn-SOD	
	Passive		↓GFAP	↓Alpha-synuclein	↓Distonin
				↓Phosphatidyl ethanolamine-binding protein	
				↓Thiomorpholin-carboxylate dehydrogenase (ketimine reductase)	
				↓Glutamine synthase	
				↑Subunit alpha-2 of the F-capping protein	
Day 1 after stress	Active	↓Ribonuclease inhibitor isoform b		↓Calreticulin	
		↓Septin 5		↓Cu-Zn-SOD	
		↓Enoyl-CoA hydratase			
	Passive	↓Septin-5	↑GFAP	↓Calreticulin	↓Distonin
				LDH	
Day 3 after stress	Active	↓Septin-5	↑GFAP	↑Calreticulin	↓Guanine nucleotide-5 exchange factor of the Rho family
				↓Cu-Zn-SOD	
	Passive	↓Excision reparation protein RAD23		↓Calreticulin	
		↓Septin-5		↓LDH	
		↓Complex of actin-binding protein $^{2}/_{3}$			

Note. ↑, enhanced expression, ↓, lower expression.

two behavior types during stress: lower α-synuclein (participates in membrane vesicles transport to the presynaptic terminal) expression and higher expression of the α2 subunit of the F-capping protein (actin-binding protein). However, in stressed passive rats there was also observed a decrease in expression of phosphatidyl ethanolamine-binding protein (RAF1 kinase activity inhibitor), thiomorpholine carboxylate dehydrogenase (specific catalyst of imine bond reduction in brain tissue substrates). During the recovery period (days 1 and 3 after stress), expression of calreticulin (calcium-binding protein and a molecular chaperone) involved in gene expression regulation was decreased in the hippocampus of all animals. In addition, passive animals had altered expression of basic cellular enzymes, such as glutamine synthase (decreased during acute stress) and lactate dehydrogenase (increased during the recovery period), at the same time, a decrease in the expression of Cu-Zn-SOD, a central element of the cellular antioxidant defense system, was observed in active animals of all experimental groups, which reflected differential expression of not only specific functional proteins, but also common systemic proteins, in response to stress depending on animal behavior. These findings can be illustrate differences in the initiation of adaptation mechanisms in rats depending on initial behavioral activity [5].

The synergistic decrease in activity of cathepsins B and D during acute stress in all animals (in passive rats at average by 18%, in active rats - by 38%, $p<0.05$) can be viewed as logical development of lysosomal membrane permeabilization (LMP) accompanied by cathepsin B and D release into the cytosol. At the same time, in contrast to cathepsin D, activity of cathepsin B progressively decreased until post-stress day 3 and reached the common level of animals of both behavioral types. Taking into account indirect participation of cathepsin B in signaling and mitochondrial apoptosis pathways, there is a reason to suggest that tissue-specific intracellular proteolysis can influence proteomic changes at the level of target organs upon emotional stress.

In the analysis of total activity of cathepsins B and D, the difference in initial activity of these enzymes in liver lysosomes of passive and active animals should be regarded as the most significant result (Fig. 1). While activity of cathepsin D was almost identical in the two groups, cathepsin B activity in passive rats was by 30% ($p<0.05$) higher than in active animals. These findings show that the system of liver intracellular proteolysis, which to a great extent determines protein renewal intensity, participates in formation of stress adaptation mechanisms [12].

The results also supplement the data on presence of individual features of specific (neurospecific proteins) and central (glutamine synthase, Cu-Zn-SOD) metabolic mechanisms in stress response formation in mammals [10]. It determines the need for system research at different metabolic levels for development of modern concept of homeostasis preservation mechanisms both under normal conditions and in case of negative emotiogenic influences.

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