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Effect of Taurine on the Proteomic Profile of the Cytosolic and Microsomal Fractions of Rat Hepatocytes during Ontogeny

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The proteomic features of the cytosolic and microsomal fractions of rat hepatocytes were studied during long-term dietary consumption of taurine (12 months) as a modulator of energy homeostasis. We identified proteomic markers of the effect of taurine on regulation of cell homeostasis. A protein with unknown biological function was revealed.

Key Words: *proteomics; taurine; ontogeny*

Taurine is a sulfur-containing amino acid, which is not incorporated into the peptide chain during ribosomal protein synthesis. This compound performs some physiological functions (*e.g.*, fatty acid conjugation and osmotic regulation) and has the antioxidant properties. Taurine serves as a chemical transmitter in ursodeoxycholic acid conjugation to reduce stress of the endoplasmic reticulum and is essential for protein synthesis in mitochondria [14]. Taurine decreases blood glucose concentration in patients with type 2 diabetes mellitus [8]. Experiments on rats showed that taurine can prevent or delay the development of insulin resistance caused by fructose excess [11]. Taurine stimulates the secretion of growth hormone, contributes to the decrease in cholesterol level, and increases the concentrations of creatinine and urea in blood plasma of rats [4,10]. It was hypothesized that aging is accompanied by taurine deficiency, which requires taurine supplementation of the ration [7].

Here we studied proteomic features of the cytosolic and microsomal fractions of rat hepatocytes during long-term dietary consumption of taurine (12 months) as a modulator of energy homeostasis. This work was designed to reveal a possible nutriproteiomic effect of taurine.

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MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 80-110 g. The animals were divided into 2 groups of 32 specimens each. They fed a complete semisynthetic diet. The rats of the treatment group additionally received 1% aqueous solution of taurine *ad libitum*. Eight rats of each group were decapitated 1, 3, 6, and 12 months after the start of the study. The blood was collected to obtain the plasma. The liver was excised during autopsy.

Microsomal and cytosolic fractions of rat hepatocytes were routinely isolated by differential centrifugation. Proteins were separated by the method of two-dimensional electrophoresis and stained with silver nitrate [1]. Protein hydrolysis with trypsin and recording of the mass spectra were performed as described previously [2].

Mass spectrometric study was conducted at the Center of Collective Use "Human Proteome" (V. N. Orekhovich Institute of Biomedical Chemistry).

The significance of differences was evaluated by Student's test. The differences were significant at $p<0.05$.

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RESULTS

In series I, we derived the proteomic maps of rat blood plasma and identified 300±61 protein spots. Comparative study of electrophoretograms after 2D electrophoresis of blood plasma allowed us to identify 12 protein spots for the early age period in control animals, as well as 9 distinctive spots in specimens of the treatment group. Age-related changes in animals of both groups were associated with the presence of 5 characteristic proteins. Mass spectrometry of blood plasma identified 4 characteristic proteins (Table 1). Protein phosphatase-1 (PP1) and unknown protein rCG25855 were identified in 6- and 12-month-old animals of the treatment group aging. PP1 is a wide-specificity enzyme, which plays an important role in the regulation of glycogen metabolism. This enzymes catalyzes dephosphorylation of phosphorylase a, phosphorylase kinase, and glycogen synthase b. Moreover, the SH2 domain of protein 1B that plays a role in signal transduction through a variety of cytokines and growth factor receptors (including somatotropin and insulin-like growth factor) was identified in animals of the treatment (1, 3, and 6 months) and control groups (1 month). Our results indicate that taurine has a modulatory effect on cell energy homeostasis. These data formed the basis for a proteomic study of microsomal and cytosolic fractions of rat hepatocytes, which are characterized by increased lability of the protein composition and high dynamics of posttranslational events.

Comparison of proteomic maps after two-dimensional electrophoresis allowed us to identify 208±30 protein spots in the microsomal fraction of rat hepatocytes. Similarly to control rats, the age-related proteomic variations in animals of the treatment group were observed from the age of 3 months. We found that 18 of 28 proteins for ontogenetic changes are apparent at the age of 3 months. Twenty-three proteins were revealed in 6-month-old animals. The average number of proteins in electrophoretograms of the cytosolic fraction from rat hepatocytes was 294±33. The proteomic spectrum of this fraction differed from that of the microsomal fraction in a low number of proteins for the senior age animals. Twenty-eight proteins were characteristic of the early age; 21 proteins were expressed up to the age of 6 months. Only 3 proteins were typical of age groups.

Proteomic analysis by the mass spectrometric method revealed catalase in the hepatocyte microsomal fraction of rats at the age of 3, 6, and 12 months. This protein was not identified in the control group (Table 2). Similarly to SOD [12], catalase serves as a main primary component of the antioxidant defense system. Identification of this enzyme in treated animals suggests that long-term dietary consumption of taurine is accompanied by activation of antioxidant processes. Cytochrome b5 was revealed in rats of both groups at the late stage of ontogeny (6 and 12 months). This protein plays an important role in the metabolism of endogenous and exogenous compounds by enzymes of the cytochrome P-450 system in various organs and tissues [15]. The presence of this protein in animals of both groups at the same stages of the study indicates that cytochrome b5 serves as a marker for ontogenetic changes in the body. Transthyretin is an acute phase protein, which belongs to the fraction of albumins. The major function of this protein is thyroxin transport from the plasma to the brain [13]. Transthyretin was not identified in 12-month-old control animals. By contrast, this protein was revealed in 6- and 12-month-old rats of the treatment group aging . Probably, taurine has a geroprotective effect.

Proteomic analysis of the cytosolic fraction from rat hepatocytes by mass spectrometry revealed some typical characteristics (Table 3). The expression of C-type lectin that plays a role in immune reactions and apoptosis [3] was observed in control animals at the age of 1, 3, and 6 months. C-type lectin was also

TABLE 1. Proteomic Characteristics of Rat Plasma after Dietary Consumption of Taurine

Age, months	Identified proteins (mass spectrometry)							
	rCG25855		protein phosphatase-1 (PP1)		SH2 domain of protein 1B		rCG48501	
	control	treatment	control	treatment	control	treatment	control	treatment
1					*	*		
3						*		
6		*		*		*	*	*
12		*		*				*

TABLE 2. Proteomic Characteristics of Microsomal Fraction of Rat Hepatocytes after Dietary Consumption of Taurine

Age, months	Identified proteins (mass spectrometry)					
	catalase		cytochrome b5		transthyretin	
	control	treatment	control	treatment	control	treatment
1					*	
3		*			*	
6		*	*	*	*	*
12		*	*	*		*

TABLE 3. Proteomic Characteristics of the Cytosolic Fraction from Rat Hepatocytes after Taurine Supplementation

Age, months	Identified proteins (mass spectrometry)									
	C-type lectin		ras-related protein Rab-14		26S proteosome, isoform CRA_b		cyclin-dependent kinase (CDK5)		rCG33743	
	control	treat-ment	control	treat-ment	control	treat-ment	control	treat-ment	control	treat-ment
1	*		*	*		*	*	*		*
3	*				*	*		*		*
6	*		*		*	*				*
12		*	*		*					

present in 12-month-old rats of the treatment group. These data suggest that taurine is involved in the protective response in the body.

The expression of ras-related protein Rab-14 was typical of animals from both groups at the early stage of ontogeny (1 month). At the age of 12 months, Rab-14 expression was observed only in control rats. Rab proteins belong to a group of low-molecular-weight GTP-bound proteins (family of RAS proteins) and regulate intracellular transport of membrane structures [9]. The ubiquitous ras proteins family (found in mammals, insects, yeasts, and nematodes) regulates various aspects of cell proliferation, differentiation, and morphology.

The maintenance of cell homeostasis (*i.e.*, rate of aging) is determined by several factors, including the proteosome-catalyzed proteolysis of abnormal proteins [6]. Expression of the 26S proteosome was high in 1-month-old animals of the treatment group, which suggests that taurine plays a role in activation of the proteosome complex. Cyclin-dependent kinase 5 (Cdk5) was revealed in 1-month-old rats of both groups. At the age of 3 months, Cdk5 was present only in rats of the treatment group. The major role of Cdk5 is structural-and-functional transformation of cells [5]. These data indicate that taurine modulates activation of differentiation processes.

The majority of proteomic markers identified after long-term treatment with an energy metabolism modulator taurine characterize the regulation of cell energy homeostasis. Therefore, the methods of proteomic diagnostics should be used in nutriproteomic studies. This approach will allow us to evaluate the efficiency and delayed risk of consumption of biologically active food components.

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