Relationship between Protein Deficiency in the Ration of Rats during Early Ontogeny and Function of Enzyme Systems of Digestive and Non-Digestive Organs in Adult Life

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Low protein content in the ration of rat pups during transfer from mixed to definitive nutrition (days 21-30 of life) has a negative impact on digestive function of the small intestine and trophic and barrier functions of the large intestine, liver, and kidneys and increases (sucrase, glycyl-L-leucin dipeptidase) or decreases (alkaline phosphatase, aminopeptidase M, glycyl-L-leucine dipeptidase) enzyme activities in these organs in 6-month-old rats. Protein deficiency during the early ontogeny modulates functioning of the enzyme systems in digestive and non-digestive organs in adult life, which can lead to the development of not only gastrointestinal, but other visceral diseases.

Key Words: *protein deprivation; ontogeny; digestive enzymes; intestine; liver*

It was previously shown that feeding of low-protein diet by female rats during gestation and lactation (critical periods of pre- and postnatal development of the progeny) led to appreciable changes in the structural and functional characteristics of digestive (small and large intestine) and non-digestive (liver, kidney) organs of their offspring not only during the early ontogeny, but also during adult life [3,4,11,13]. These changes in adult progeny most often presented as appreciable reduction and sometimes as increased activity of intestinal digestive enzymes realizing membrane digestion and in activities of relevant hydrolases of the liver and kidneys responsible for the realization of the trophic and barrier functions. Changes in body weight and weights of various organs were observed, which was in line with previous reports [8].

We concluded that such a stress factor as protein deprivation during gestation and lactation is imprinted in biochemical memory of the progeny promoting functioning of enzyme systems in a different mode. It is therefore interesting whether malnutrition during the early ontogeny can affect programming of enzyme systems in digestive and non-digestive organs. It seems to be important to clear out the role of the quality of nutrition during the early postnatal period in the formation and functioning of hydrolytic enzyme systems of the gastrointestinal organs, liver, and kidneys in adult animals; it is also essential for understanding of the molecular mechanisms of metabolic/alimentary programming of these systems.

We studied delayed aftereffects of protein deficiency in the nutrition of rat pups during the early ontogeny (during transfer from mixed to definitive feeding) and its effects on activity of digestive enzymes realizing membrane and intracellular hydrolysis of nutrients in the small intestine, and on activity of hydrolases in the large intestine, liver, and kidney playing a critical role in body detoxification.

MATERIALS AND METHODS

Experiments were carried out on 6-month-old male Wistar rats (n=5). The animals received isocaloric diet with 2.5-fold reduced protein for 10 days (on days 21-30 after birth) during transition from mixed to definitive feeding. Starting from day 31 of life the animals

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were transferred to full-value balanced feeding. Rats of the same age (n=5) receiving normal diets served as controls. The control diet contained 22% casein, 53% starch, 10% glucose, 0.2% methionine, 5% mineral oil, 5% vitamins and salts, and 4.8% cellulose. Low-protein diet was isocaloric in comparison with the control and contained 9% casein, 63.8% starch, 12.4% glucose, 0.08% methionine, 5% mineral oil, 5% vitamins and salts, and 4.72% cellulose [4,8]. Casein (protein component of these diets) is a full-value high-quality protein containing complete set of amino acids; it is as a rule used in studies of the effects of food quality on some body functions [8]. Activities of membrane enzymes sucrase (EC 3.2.1.48), maltase (EC 3.2.1.20), alkaline phosphatase (EC 3.2.1.1.), aminopeptidase M (EC 3.4.11.2), and predominantly cytosolic glycyl-L-leucin dipeptidase (EC 3.4.13.2) were measured in homogenates of the duodenal, jejunal, ileac, and large intestinal mucosae, liver, and kidneys. Enzyme activities were measured in the membrane and cytosol fractions of jejunal and ileac mucosa (from 5 animals) obtained by isolation of the membrane vesicles from enterocyte brush border by ultracentrifugation [12]. Maltase activity was measured by the glucose oxidase method [7], sucrase activity as described previously [5], aminopeptidase M activity as described elsewhere [10], dipeptidase activity by the glycin method [6], alkaline phosphatase activity by using 0.6 mM p-nitrophenylphosphate, and protein was measured by the method of Lowry. The data were processed using Student's t test.

RESULTS

Dietary protein deprivation during the early ontogeny had a strong impact on structural and functional parameters of the digestive and non-digestive organs of 6-month-old rats (Table 1, Fig. 1). Body weight of these animals decreased by 31% (from 448±17 to 309±19 g, p<0.05), weight of the jejunal mucosa decreased by 26%, and the weight of the liver and kidneys by 28 and 39%, respectively, compared to control rats of the same age (Table 1).

Significant differences were detected in activities of enzymes realizing membrane and intracellular hydrolysis of nutrients in different portions of the small intestine and in activities of the relevant hydrolases in the large intestine, liver, and kidneys. Changes in enzyme activities were oppositely directed: activities of sucrase and glycyl-L-leucin dipeptidase increased, while activities of alkaline phosphatase, aminopeptidase M, and glycyl-L-leucin dipeptidase decreased compared to controls.

The data on the distribution of activity of each enzyme along the small intestine and its levels in other organs were in line with previous findings [1]. Sucrase

TABLE 1. Weights of the Mucosa in Different Compartments of the Small Intestine, Large Intestine, Kidneys, Liver and Enzyme Activities in 6-Month-Old Rats Which Received Normal (Control) and Low-Protein (Experiment) Rations on Days 21-30 of Life (M±m)

Enzyme, series		Duodenum	Jejunum	Ileum	Large intestine	Kidneys	Liver
Weight, g	control	0.60±0.05	1.90±0.03	1.4±0.1	0.7±0.1	3.2±0.2	17.0±1.5
	experiment	0.50±0.04	1.40±0.09*	1.2±0.1	0.50±0.05	2.3±0.2*	10.4±0.5*
Sucrase, μmol/min/g protein							
	control	62.4±8.9	57.5±5.9	19.8±3.9	7.2±1.1	0	6.7±1.3
	experiment	94.8±10.5*	112.5±10.6*	50.8±5.1*	13.2±1.7*	4.6±0.9*	15.9±3.0*
Maltase, μmol/min/g protein							
	control	389.6±51.4	684.1±14.6	428.0±46.3	51.2±0.5	197.8±15.8	24.2±4.4
	experiment	403.3±37.5	655.6±60.6	427.0±48.4	71.0±17.5	217.0±10.6	17.0±2.4
Alkaline phosphatase, μmol/min/g protein	control	183.6±26.4	99.2±20.2	23.7±3.0	5.7±0.4	24.9±0.6	1.4±0.2
	experiment	114.3±4.7*	84.0±8.6	12.2±2.4*	6.7±1.3	19.7±1.7	1.5±0.1
Aminopeptidase M, μmol/min/g protein	control	48.2±4.4	75.6±7.0	79.6±9.4	13.7±3.7	164.2±12.5	14.6±2.8
	experiment	43.0±3.3	84.0±6.3	69.6±5.4	9.6±2.3	122.3±12.4*	7.3±1.3*
Glycyl-L-leucin dipeptidase, μmol/min/g protein	control	158.2±20.1	380.9±69.8	488±82	155.3±18.4	1091±62	167.8±12.8
	experiment	289.4±44.2*	440.9±58.9	388.3±62.0	299.9±37.3*	509.2±63.6	218.0±48.1

Note. Each series consisted of 5 experiments. *p<0.05 compared to the control.

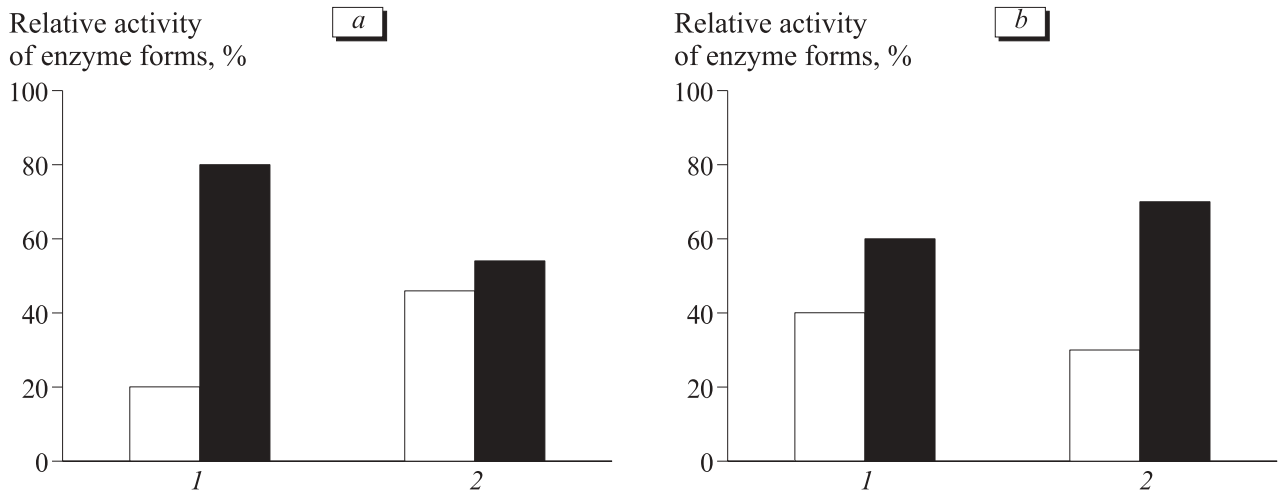

Fig. 1. Activities of soluble (light bars) and membrane (dark bars) forms of glycyl-L-leucin dipeptidase in the jejunum (*a*) and ileum (*b*) of 6-month-old Wistar rats receiving normal (*1*) and low-protein (*2*) rations on days 21-30 of life.

activity increased appreciably in experimental animals: 1.5 times in the duodenum, 2-fold in the jejunum, and 2.6 times in the ileum, while sucrase activity in the liver increased 2.4 times *vs.* the control. Activity of alkaline phosphatase in the duodenum and ileum of experimental animals decreased 1.6- and 1.9-fold, respectively, and remained at the baseline level in other organs. Activity of aminopeptidase M in experimental animals was lower than in the controls (1.3-fold in the kidneys and 2-fold in the liver). Activity of glycyl-L-leucin dipeptidase in experimental animals increased in the duodenum and large intestine (1.8 and 1.9 times, respectively) and decreased in the kidneys (2.1 times).

Study of the ratio of membrane and soluble forms of membrane-bound and predominantly intracellular enzymes isolated from the membrane and cytosol fractions of the jejunal and ileac mucosa showed that the membrane-bound enzymes were presented mainly by the membrane form (92-99% of the total activity of both fractions) both in control and experimental animals. Activity of intracellular dipeptidase was very high in the soluble form, particularly in the ileum, its share in experimental animals being markedly increased in the jejunum and decreased in the ileum in comparison with the control (Fig. 1).

Hence, it was shown that protein deficiency in the ration of rat pups during transfer from mixed to definitive feeding was associated with significant changes in activities of digestive enzymes involved in the cleavage of complex biopolymers and in activities of large intestinal, hepatic, and renal hydrolases responsible for the realization of trophic and barrier functions in adult animals. Changes in activities of different enzymes in the organs were not universal. These results suggest that as a result of changed level of enzyme activities, functioning of some enzyme systems of digestive and non-digestive organs is realized in a different mode than in animals receiving normal balanced ration. Increase or decrease in enzyme activity can be attributed to changed rates of enzyme protein synthesis and degradation; the sensitivity of these proteins to carbohydrate, protein, and lipid metabolites forming during digestion can also change [14]. It seems that these processes are programmed at the genetic level, which is in line with the opinions of the authors, who observed changes in activities of enzymes involved in the maintenance of glucose homeostasis in the liver [9]. Presumably, alimentary modulation during the early ontogeny, similarly as during gestation and lactation [2], modulates the metabolic pathways, in particular through the digestive system, and determines the health status of adult animals.

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