Delayed Effects of the Terms of Separation of Rat Pups from Lactating Females and Low-Protein Diet on Enzyme Activity in Digestive and Non-Digestive Organs

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Early and late separation of rat pups from lactating females and combined effects of the terms of separation and low-protein diet are essential for the formation of enzymes (maltase, alkaline phosphatase, aminopeptidase M, and glycyl-L-leucine dipeptidase) in the large and small intestine, liver and kidneys of adult animals. Similarities and differences in the enzyme reactions to early and late weaning and to a combination of untimely weaning and low-protein diet were detected.

Key Words: *time of removal of rat pups from lactating females; low-protein diet; enzymes*

Periods of transition from placental/amniotrophic to lactotrophic and then to definitive nutrition are critical periods in mammalian and human life [3-5,11]. Alimentary imbalance, particularly protein imbalance, during the pre- and early postnatal development of the progeny is associated with a variety of morphofunctional shifts in organs and systems in adult life [2,12-14]. Activities of digestive enzymes in various compartments of the small and large intestine and activities of the corresponding hydrolases in the liver and kidneys, whose role in detoxification is well known, were significantly reduced in the progeny of mothers receiving low-protein diets during pregnancy or lactation [6]. These experiments were carried out on Wistar rats separated from lactating females at normal terms (on day 30 of life). On the other hand, it was shown that the time of separation of young animals from mothers is essential for the formation of small intestinal digestive enzymes [3,11,15].

The significance of early weaning for activities of intestinal enzymes (primarily lactase) was studied previously, while the significance of late weaning is virtually unknown [3,4,9,12]. The significance of the quality of nutrition of animals subjected to early and late weaning for the formation of the enzyme systems of not only gastrointestinal, but also other organs during adult life was never studied.

We studied the delayed effects of protein deficient nutrition of rats separated from lactating females at different age on enzyme activities in the small and large intestine, liver, and kidney during adult life.

MATERIALS AND METHODS

The study was carried out on 4-month-old Wistar rats separated from lactating females on day 21 (early), 31 (normal) and 41 (late) of life and receiving standard (group 1) or low-protein diet (group 2) for 10 days directly after separation. Before the age of 4 months (start of the experiment), the rats of both groups received standard ration. A total of 48 rats (8 per term in both experimental groups) from 6 lactating females were used in the study. The number of rat pups per litter was equalized to 8 on the next day after birth. Females with the pro-

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geny were kept on standard rations with free access to water at 12:12 day:night regimen. Protein content in low-protein diet was 2.5 times reduced in comparison with the control; the diets were described previously [8]. Animals separated from lactating females on day 31 of life served as the control.

Activities of membrane enzymes: maltase (EC 3.2.1.20), alkaline phosphatase (EC 3.1.3.1), aminopeptidase M (EC 3.4.11.2), and predominantly intracellular glycyl-L-leucin dipeptidase (EC 3.4. 13.2) were measured by routine methods [7] in homogenized duodenal, jejunal, ileac, and colonic mucosa and kidney and liver homogenates from rats of both groups. Enzyme activities were calculated in μmol of hydrolysis products per gram tissue per minute.

The data were statistically processed using Student's *t* test.

RESULTS

The results suggest that the time of separation of rat pups from lactating females and 10-day standard or low-protein diet directly after the separation are significant for activities of enzymes realizing the membrane and intracellular digestion in the small and large intestine and of the corresponding hydrolases in the liver and kidneys during adult life (Tables 1, 2). Maltase activity increased (1.4 times) only in the kidneys of group 1 rats separated late from lactating female, while in group 2 rats it decreased (1.2 times) in the jejunum of animals separated early and decreased in the duodenum (1.3 times) and jejunum (1.4 times) and increased (1.5 times) in the large intestine of animals separated late from lactating females.

Activity of alkaline phosphatase in group 1 animals increased 2-fold in the jejunum and decreased 2-fold in the liver of animals weaned early, while after late weaning it decreased in all studied organs except the duodenum: by 1.4 times *vs.* control in the jejunum, by 3.5 times in the ileum, by 2.3 times in the large intestine, by 1.9 times in the kidneys, and 2-fold in the liver. In group 2 animals, enzyme activity increased 2-fold in the ileum and 3.5 times in the large intestine; enzyme activity in the kidneys increased 1.9 times only in late weaned rats.

Aminopeptidase M activity increased significantly in the duodenum, jejunum, ileum (more than 2-fold), in the kidneys (by 1.7 times), and liver (by 1.3 times) of group 1 animals separated early from lactating females. Enzyme activities in the kidneys and liver increased by 1.5 and 1.3 times, respectively, in late weaned rats of this group. Activity of aminopeptidase M in the kidneys and liver increased by 1.5 times in early weaned group 2 rats. In late weaned animals of this group, enzyme activity was higher in the ileum by 1.3 times, in the large intestine by 1.6 times, and in the kidneys by 1.7 times compared to the control values.

In group 1, activity of glycyl-L-leucine dipeptidase decreased only in the duodenum (2.2 times) of animals early separated from lactating females. Enzyme activity in early weaned group 2 animals was by 1.7 times elevated in the duodenum, by 1.2 times in the jejunum, and by 1.3 times in the kidneys in comparison with the control. In late weaned rats of this group, activity of glycyl-L-leucine dipeptidase decreased only in the kidneys (by 1.4 times).

Hence, early and late weaning is a factor essential for the formation of enzyme systems of the digestive and nondigestive organs in adult animals. It is noteworthy that significant changes in alkaline phosphatase activity in each studied organ of adult animals were observed after late weaning, while changes in aminopeptidase M activity were observed after early weaning. Low-protein diet after early or late weaning also caused delayed changes in the functioning of enzymes cleaving the nutrients in the small intestine and of the corresponding hydrolases in the large intestine, liver, and kidneys, involved in the intermediary metabolism and realizing trophic barrier functions. Similarities and differences were noted in the reactions of the enzymes of the digestive and nondigestive organs to early and late weaning and to a combination of untimely weaning and low-protein diet. Maltase activity underwent maximum changes in rats after late separation and low-protein diet. Activity of alkaline phosphatase changed in the jejunum and liver of early weaned rats, while low-protein diet caused no changes in activity of this enzyme in these animals. By contrast, enzyme activities decreased in all studied organs of late weaned animals, while the combination of late weaning and low-protein diet significantly increased enzyme activity in the ileum, large intestine, and the kidneys. It is noteworthy that the increase in enzyme activity in the ileum indicates delayed formation of the adult type of its proximal/distal gradient along the small intestine. In group 1 animals, activity of alkaline phosphatase in the ileum constituted 8% of the maximum activity in the duodenum, while in group 2 it reached 33%.

Analysis of aminopeptidase M activity showed identical changes. It increased in untimely weaned animals and after combination of this factor with protein deficiency. Enzyme activity increased significantly in all studied organs of rats after exposure to one factor (early weaning) and only in the liver

TABLE 1. Enzyme Activities (μmol/min/g tissue) in Various Organs of 4-Month-Old Rays Separated from Lactating Females on Days 21, 31, and 41 of Life and Receiving Standard Ration after Weaning

Enzyme	Day of weaning	Small intestine			Large intestine	Kidneys	Liver
		duodenum	jejunum	ileum			
Maltase	21	46±4	66±5	37±3	7±0.6	23±3	2.3±0.2
	31	47±4	77±7	39±3	8±1	21±3	2.7±0.2
	41	42±2	71±5	33±2	7±1	30±1*	3±0.3
Alkaline phosphatase	21	29±4	20±6	7±2	0.60±0.07	7±1	0.20±0.05*
	31	20±2	10±1	4.6±0.7	0.7±0.1	4.7±0.4	0.40±0.02
	41	17±1	7.0±0.4*	1.3±0.1*	0.30±0.01*	2.5±0.2*	0.20±0.05*
Aminopeptidase M	21	16±4*	26±4*	21±3*	2.2±0.5	29±4*	3.5±0.4*
	31	7.0±0.5	12±1	10.0±0.7	2.0±0.2	17±2	2.6±0.1
	41	8±1	12±1	10±1	1.7±0.2	25±2*	3.3±0.3*
Glycyl-L-leucine dipeptidase	21	50±5*	144±14	136±14	57±7	215±28	78±4
	31	111±18	155±15	149±11	59±6	280±26	90±8
	41	147±8	172±12	151±15	66±5	276±25	97±5

Note. Here and in Table 2: *$p<0.05$ compared to the control.

TABLE 2. Enzyme Activities (μmol/min/g tissue) in Various Organs of 4-Month-Old Rats Separated from Lactating Females on Days 21, 31, and 41 of Life and Fed Low-Protein Ration for 10 Days and Then Standard ration Throughout the Experiment

Enzyme	Day of weaning	Small intestine			Large intestine	Kidneys	Liver
		duodenum	jejunum	ileum			
Maltase	21	44±2	73±3*	36±2	8±0.5	26±2	2.6±0.9
	31	43±2	89±6	35±2	9±1	24±3	3.0±0.5
	41	32±2*	64±4*	37±3	14±2*	24±4	2.0±0.2
Alkaline phosphatase	21	20±3	7±1	2.0±0.3	0.60±0.07	3.00±0.01	0.10±0.03
	31	15±3	9±1	2.6±0.4	0.6±0.1	3.6±0.4	0.3±0.1
	41	15±2	10±1	5±1*	2.1±0.5*	7±1*	0.6±0.2
Aminopeptidase M	21	8.0±0.9	11±1	8.0±0.4	1.8±0.1	22±3*	3.6±0.2*
	31	6±1	11±1	9.0±0.6	2.0±0.2	15±1	2.5±0.1
	41	6±1	12±1	12±1*	3.2±0.3*	25±3*	2.9±0.2
Glycyl-L-leucine dipeptidase	21	169±14*	202±12*	209±10	72±4	335±20*	99±6
	31	99±5	164±12	196±7	87±7	262±17	99±11
	41	105±10	184±13	173±19	72±6	192±25*	83±7

and kidneys after exposure to both factors (early weaning and protein deficiency). In late weaned rats, this activity increased only in nondigestive organs, while the combination of untimely weaning and low-protein diet resulted in increase in enzyme activity in the ileum and large intestine and in the kidneys. Changes in activity of glycyl-L-leucine dipeptidase were more pronounced in animals after early weaning followed by low-protein diet in comparison with animals subjected to early weaning alone.

Analysis of the data indicates that violation of the enzyme system programs in the digestive and other than digestive organs can result not only from protein-deficient diets directly after weaning, but also from early or late weaning of pups from the females. It is known that the time of nursing discontinuation is essential for further development. Early and late weaning can have unpredictable effects on the formation and functioning of enzyme systems of various organs of the progeny not only in the early ontogenesis [3,15], but also in adult

life, which is seen from our findings. This is caused not only by loss of connection with the mother and disorders in the hormonal and immune status, but also by changes in the quality of nutrition. Early separation from mothers results in an increase of the carbohydrate component (starch) in the diets of rat pups, while in case of late separation protein insufficiency is progressing with prolongation of nursing. In some cases, a combination of untimely (early or late) weaning and low-protein diet caused more pronounced changes in activities of enzymes of digestive and nondigestive organs (maltase, alkaline phosphatase, glycyl-L-leucine dipeptidase), while sometimes no changes were observed (aminopeptidase M).

It seems that any modification of the quality of nutrition during the critical periods of the progeny development (pregnancy, lactation, early ontogenesis) can be regarded as a common regularity violating the program by triggering a cascade of metabolic reactions of enzyme systems of the digestive and nondigestive organs. Early metabolic alimentary programming is a multifactorial process. Presumably, enzyme synthesis programming under conditions of improper nutrition during the pre- and early postnatal development is caused by changed expression of the respective genes [10]. It is known that DNA methylation controls all genetic processes and is regarded as the epigenetic control of the genetic functions of the body. It is a principally new mechanism of gene expression and cell differentiation control [1]. This epigenetic phenomenon can include modification of packing and activation of various genes, including that of the genes regulating the synthesis of enzymes in digestive and nondigestive organs. Presumably, DNA methylation is disturbed during the early metabolic/alimentary programming, if the nutrition quality is modified during the critical periods of progeny development.

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