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DIGESTION OF MILK PROTEINS AND LYSOSOMAL PROTEINASES OF THE ILEAL MUCOSA OF YOUNG RATS

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To study whether lysosomal proteinases of the ileal mucosa can participate in intraluminal digestion, the proteolytic activity of lysosomal and pancreatic proteinases was determined both in the chyme and in a homogenate of ileal and jejunal tissues from rats aged 12 and 30 days. In the period of milk feeding the proteolytic activity of acid (lysosomal) proteinases was shown to be three times higher in the ileum than in the jejunum, and this was shown to be true both for the mucosa and for the contents of these parts of the small intestine. In rats which had changed over to a definitive diet, acid proteinase activity in the jejunum and ileum was almost unchanged both in the mucosa and in the contents. The results are evidence that pancreatic proteinases adsorbed from the chyme of the small intestine can participate in contact digestion.

KEY WORDS: early postnatal period; lysosomes; acid proteinases; milk proteins; pancreatic proteinases.

Data have recently been published to show that giant lysosomes are located in the brush border of the enterocytes in the distal portion of the small intestine in the early postnatal period of development in rats. The change from milk to definitive feeding is accompanied by disappearance of these lysosomes. It has been suggested that lysosomal enzymes participate in intracellular digestion during the period of milk feeding [6, 7].

The problem of the mechanisms lying at the basis of the high efficiency of utilization of milk proteins at an early age has not yet been solved. It is claimed that some milk proteins during this period can be absorbed in the unhydrolyzed state [9]. There is also evidence of the alimentary specificity of milk proteins, which determines the high efficiency of their utilization [1]. Some workers have noticed that the digestive system in the period of milk feeding is immature [7, 8], although the opposite views have also been expressed, namely that at an early age the level of development of the proteolytic system of digestion is relatively high [10].

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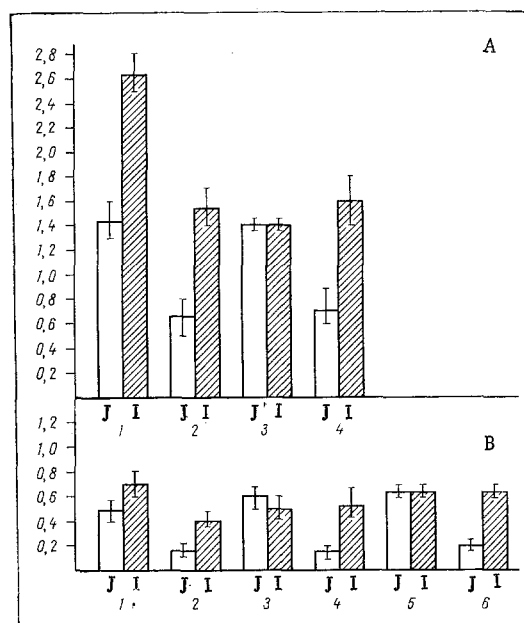


Fig. 1. Specific proteolytic activity in chyme (A) and homogenate of jejunal and ileal tissues of rats (B) at different pH values. 1, 2) pH 7.8; 3, 4) pH 5.0; 5, 6) pH 6.4. 1, 3, 5) rats aged 30 days; 2, 4, 6) rats aged 12 days.

The writers previously obtained evidence to show that in the period of milk feeding activity of pancreatic proteases is high and that intensive digestion of milk proteins can take place. The change from milk to definitive feeding and the associated intensification of the gastric phase of digestion are accompanied by a shift of intraluminal digestion of milk proteins from the distal to the proximal portion of the small intestine.

To study the particular features of digestion of milk proteins in the period of milk feeding and whether lysosomal proteases in the mucosa of the ileum can participate in intraluminal digestion of milk proteases, the proteolytic activity of lysosomal and pancreatic proteases was studied both in the chyme and in a homogenate of jejunal and ileal tissues from rats.

EXPERIMENTAL METHOD

Experiments were carried out on Wistar rats aged 12 and 30 days. The animals aged 12 days were deprived of food for 3 h, then returned to their mothers for natural milk feeding for 1 h. The rats aged 30 days, on a definitive diet, were deprived of food for 6 h and then placed with a lactating femal for milk feeding for 1 h. The animals were killed and the contents of the jejunum and ileum obtained by perfusion with physiological saline.

The proteolytic activity of the proteases (cathepsins) was judged from hydrolysis of hemoglobin [2, 5].

Protein concentrations were determined by Lowry's method [11].

EXPERIMENTAL RESULTS

As a result of determination of proteolytic activity in the chyme from the jejunum and ileum of rats aged 12 and 30 days it was shown that activity of pancreatic proteases in the chyme of these parts of the intestine of animals aged 30 days was almost twice as high as in the rats aged 12 days (Fig. 1A). The proteolytic activity of these proteases in the mucosa of the jejunum and ileum also was more than twice as high in rats aged 30 days than in animals aged 12 days (Fig. 1B).

The results are in agreement with earlier determinations of the activity of trypsin and chymotrypsin in the chyme from the jejunum and ileum of rats aged 3, 12, 20, and 30 days, and also with data in [4]. It follows from these results that pancreatic proteinase activity was more than twice as high in the ileum than in the jejunum, as was also demonstrated for both the mucosa and the content of the small intestine of animals aged 12 days (Fig. 1A, B).

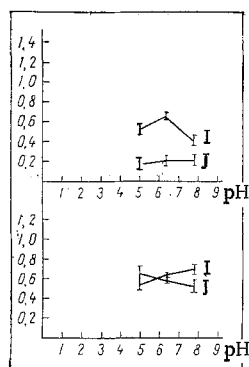


Fig. 2. Specific proteolytic activity of homogenates of jejunal (J) and ileal (I) tissues of rats aged 12 days (a) and 30 days (b) pH. Abscissa, pH; ordinate, specific activity ($\Delta U/h/mg$ protein).

The observations showing that the proteolytic activity of the pancreatic proteinases was present not only in the chyme but also in the mucosa of the small intestine are evidence that pancreatic proteinases absorbed from the chyme participate in contact digestion [3].

Comparison of the proteolytic activity of a homogenate of the jejunal and ileal tissues at different pH values showed that in rats aged 12 days with a change in pH from 5.0 to 7.8 a peak of proteolytic activity was observed at pH 6.4 in the ileum (Fig. 2a). In animals aged 30 days the highest proteolytic activity in the wall of the ileum was observed at pH 7.8 (Fig. 2b). Activity in the wall of the jejunum, on the other hand, increased a little with a change in pH from 5.0 to 7.8 in rats aged 12 days (see Fig. 2a) and it fell very slightly in the animals aged 30 days (Fig. 2b).

Curves describing the dependence of pancreatic protease activity of pH are bell-shaped with an optimum at pH 7.8 [12]. Compared with pancreatic proteases, the optimum of cathepsin activity is shifted into the acid pH zone [13]. Cathepsin activity at pH 8.0 has been shown to be much lower than at the pH optimum.

The results are evidence that during the period of milk feeding activity of lysosomal proteases in the distal portion of the small intestine is relatively high in animals aged 12 days, and a proximo-distal gradient of lysosomal activity is observed. In animals on a definitive diet (rats aged 30 days) no proximo-distal increase in acid proteinase activity was observed (Fig. 1: A3, B3).

Determination of acid proteinase activity in rat jejunal and ileal tissue homogenates showed that the activity of these proteinases is equal in animals aged 30 days. Acid proteinase activity in rats aged 30 days was unchanged in the content of the jejunum and ileum (Fig. 1: A3).

In the period of milk feeding, proteolytic activity of acid proteinases was three times higher in rats aged 12 days in the ileum than in the jejunum, and this was true both for the mucosa and for the contents of the jejunum and ileum (Fig. 1: A4, B4). These results show that in the early postnatal period of development there is a proximo-distal gradient of lysosomal proteinase activity both in the mucosa (Fig. 1: B4) and in the contents of the small intestine (Fig. 1: A4); whereas in animals aged 30 days, on a definitive diet, no such proximo-distal increase in acid proteinase activity could be observed either in the chyme or in the mucosa of the small intestine.

These findings confirm earlier results [6] showing that in the postnatal period of development from the first day to the end of the second week there is an increase in the number of giant lysosomes in the enterocytes of the ileal mucosa. The change to definitive feeding is accompanied by the appearance of a new epithelial population with no accumulation of lysosomes in the ileal mucosa. It can thus be tentatively suggested that during the period of desquamation of the epithelium of the ileal mucosa lysosomes pass into the chyme, where the proteinases evidently take part in intraluminal digestion of milk proteins.

The writers showed previously that the period of milk feeding is characterized by fairly intensive digestion of milk proteins, which has shifted toward the distal portion of the small intestine; the pH of the chyme of the jejunum in the postnatal period of development of rats is 6.4, whereas the pH of the ileal chyme is 6.5-6.7, i.e., the pH of the contents of the small intestine is shifted a little into the acid zone compared with that in animals aged 30 days, in which the pH of the chyme in both the jejunum and the ileum is 7.0. The shift of pH of the chyme of the small intestine toward the acid side ensures maximal efficiency of utilization of the milk

proteins, which is also the responsibility of lysosomal proteinases which participate in intraluminal digestion in the distal portion of the small intestine.

It can be concluded from the results that in the period of milk feeding there is a proximo-distal gradient of activity of lysosomal proteases both in the mucosa and in the contents of the small intestine. The change from milk to definitive feeding is accompanied by disappearance of the proximo-distal gradient of lysosomal protease activity. It can be tentatively suggested that lysosomal proteases of enterocytes in the distal portion of the small intestine participate in intraluminal digestion at an early age.

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EFFECT OF β -PHENYLETHYLAMINE ON EVOKED POTENTIALS IN THE RAT NEOSTRIATUM

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The effect of intraperitoneal (70 mg/kg) and local (39 μ g) injections of β -phenylethylamine (β PPEA) on evoked potentials (EP) in the caudate nucleus during stimulation of the compact zone of the substantia nigra in the frontal cortex was investigated in rats. For local application of β PPEA, glutamate, and haloperidol a push-pull cannula system with simultaneous recording of EP was used. Definite specificity in the action of the drugs on EP of cortical and nigral origin was found. Intraperitoneal injection of β PPEA caused a faster and stronger decrease in amplitude of the N_2 - P_2 component in the response to stimulation of the substantia nigra than local application, but had very little effect on the amplitude of EP in response to stimulation of the frontal cortex. It was shown by the use of haloperidol that the N_2 - P_2 component of EP in response to stimulation of substantia nigra is dopaminergic in nature. It is suggested that endogenous β PPEA may be a regulator of the function of dopaminergic neurons in the nigro-neostriatal system of the rat brain.

KEY WORDS: β -phenylethylamine; dopamine; evoked potential; caudate nucleus; substantia nigra; frontal cerebral cortex.

It can be postulated on the basis of experimental data [2, 3, 11, 13] that β -phenylethylamine (β PPEA) is an endogenous regulator of synaptic transmission effected by catecholamines. It is considered that β PPEA acts

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