REGULATION OF HOMEOSTASIS IN THE PROCESS OF PROTEIN ABSORPTION FROM SMALL INTESTINE TO BLOOD

Electron microscopic and immunofluorescent study in rats aged 1 and 3 days after birth allowed establishing a process of absorption of protein from the small intestine into the lymph and blood. Blood homeostasis was provided by the proteins filtrated from glomerular capillaries of nephrons and reabsorbed by the epithelial cells in canaliculi of nephrons. The absorbed natural heterologous protein was depleted by lysosomes of epithelial cells of intestine and kidneys and macrophages. It supported not only blood homeostasis but also prevented loss of protein by an organism, formed sites for its digestion in the organism.

Key words: Small intestine, kidney, protein, absorption, reabsorption, homeostasis.

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Introduction

Studies have shown that assimilation and dissimilation processes of food products of different quality and volume are essentially correlated with mechanisms of regulation and adaptation for an organism, maintenance of homeostasis. The nutrition factor has direct influence on the structural and functional characteristics of organs and systems, homeostasis and adaptation; food may either prevent or stimulate the development of certain diseases (Leibach and Ganapathy, 1996; Luft, 2003; Zufarov and Yuldashev, 1999; 2001; Titov, 2007; Yuldashev et al., 2009а; 2009b). The study, taking into consideration both the nutrition factor and processes of intensive development of organs and systems in mammals after birth, persuaded the morphologic examination of homeostasis regulation mechanisms at the process of protein absorption from small intestine into blood in the early postnatal ontogenesis.

Material and methods

One and three days old white rats were the objects of investigation. Cytophysiology and mechanisms regulating the protein absorption in the small intestine and its re-absorption in the proximal canaliculi of nephron were studied by electron microscopy in dynamics after per os introduction a dose of 0.2-0.3 ml gamma-globulin (Globulins Human, Sigma, mol. mass 180.000) via special pipette or 7% solution of bovine serum albumin (Albumin bovine, Sigma, mol. mass 60.000).

Before introduction of each protein the rats were starved for three hours. Anesthetized animals were killed by decapitation in 10, 30, 60 minutes, as well as 3, 6, 9 hours after a single feeding. Every experimental group consisted of three rats. Specificity of the protein, administered per os and revealed in all absorbing cells of villi of the jejunum mucosa or in cells of the proximal canaliculi of nephrons, was identified by immunofluorescent method using gamma-globulin and its anti-serum, as well as yellow fluorescent protein. (Burmakin et al., 2005; Seliverstova et al., 2007).

For electron microscopic investigation the specimens of mucosa, obtained from small intestine and kidney cortex, were fixed in 2.5% buffer solution of glutar-aldehyde and 1% solution of OsO4. The specimens were fixed in alcohol and then embedded in araldit. Ultra-thin slides contrasted in uranil acetate and lead citrate were examined under electron microscope JEM - 100S.
Results

Albumin or gamma-globulin, administrated per os to one and three days old rats, were found in 10 minutes within endocytotic vesicles, in tubulovesicular structures beneath microvilli, in cisterns of smooth and rough endoplasmic reticulum and structures of the Golgi complex in the apical zone of the absorbing cells of the jejunum villi. These proteins were identified by electron microscopy as substrates of moderate electron density being transported only by endocytotic formations. However, it is noteworthy that 3-5 minutes after the first signs of absorption by the bases of microvilli the tubulo-vesicular formations were not detected. That is why these endocytotic structures between the bases of microvilli were considered as morphological equivalent of nutrients transported into the absorbing cells of microvilli.

**Figure 1.** Protein absorbed by villi enterocytes of small intestine and accumulated in the Golgi structures (GS), intercellular spaces (IS) and interstices (IS). Thirty minutes after administration of a single dose of protein to 1 and 3 days old rats. Mag.8000

Vesicles with the protein of moderate electron density were trans-located from the base of microvilli to the supra-nuclear zone and fuse with structures of the Golgi complex. Thirty minutes after the first signs of absorption, the Golgi complex showed a sharp increase in volume. Its numerous vesicles, vacuoles, enlarged cisterns were found to contain the absorbed protein (Figure 1).

In 10 minutes, simultaneously with accumulation process, the vesicles of the Golgi complex began to release the absorbed protein into intercellular space on the level of the upper pole of nucleus. Thirty minutes later the main volume of the transported protein was within the structures of the Golgi complex and in the spaces between epithelial cells of the villi, interstices (Figure 2). No protein absorption was observed in the epithelial cells of the developing crypts.

Since bovine serum albumin or gamma-globulin is heterogeneous for rats, a certain amount of protein was digested by secondary lysosomes of villi enterocytes 30 minutes after they were administrated per os (Figure 3). Accumulation, selection and regulation of intracellular transport stage of the absorbed protein are specific functions of the Golgi
complex. Similar opinion concerning the function of the Golgi complex was also supported by Rothman and Wieland (1996), Allan et al. (2004), Snigerevkaya et al. (2006).

**Figure 2. Exocytosis of the protein absorbed by the Golgi Structures (GS) into the spaces between enterocytes of the jejunum villi. Mag 8000**

The absorbed proteins pass from the intercellular space via basement membrane into villi interstices. Then these macromolecules move to and penetrate into the lymphatic
capillaries via their inter-endothelial gaps. The total period of the absorbed proteins transition from intercellular spaces and interstices into the lymphatic capillaries of villi in jejunum lasted about 6 hours.

Twenty minutes after revealing signs of albumin or gamma-globulin absorption by jejunum they were also found in capillaries of kidney glomeruli and, after filtration, in the cells of proximal canaliculi of nephrons (immunofluorescence – after administration of gamma-globulin or yellow fluorescence – using green fluorescent protein). These proteins were filtrated via pores in endothelium of capillaries, basement membrane and interstices between cytopedicles of podocytes and re-absorbed by cells of the proximal canaliculi of nephrons. They, similar to that in jejunum, were absorbed by endocytotic vesicles. Having separated from microvilli bases they moved to structures of the Golgi complex. Simultaneously with protein accumulation, its vesicles transported protein to primary and secondary lysosomes (Figure 4), where they were depleted to amino-acids. After a single feeding, the period of filtration, re-absorption and digestion of the protein absorbed by intestine took about 6 hours in total.

Thus, the mechanism performing regulation of homeostasis in blood in the process of protein absorption from small intestine covers: transportation of the proteins via its mucosa, which take about 6 hours; simultaneous filtration and reabsorption of them by cells of the proximal canaliculi of nephrons; digestion in lysosomes of epithelial cells in every involved organ.

**Discussion**

According to our data (Yuldashev et al., 2009), as well as those of other authors (see e.g. Burmakin et al., 2005; Seliverstova et al., 2007) a structural and functional system regulating homeostasis was found to be complex; it progress gradually at the prenatal and postnatal periods of life in mammals, and became perfect by the period of maturation. Harmoniousness in the development of the regulatory systems in small intestine in suckling, mixed and definitive nutrition periods correlates closely with similar processes in kidneys. Development of the functional system of digestion, absorption and regulation of homeostasis in the small intestinal mucosa occurred simultaneously with the processes of integration of the nephrons subpopulations, interconnected differentiation of spatially distant nephrons and juxtaglomerular apparatus (JGA), and dynamic development of interconnection with small intestine, optimization of filtration processes, re-absorption, and proteolysis. Primary urine filtrated by kidney glomeruli was reabsorbed by the proximal canaliculi cells of nephron, thus preventing a loss of protein being an important nutrient for an organism. But according to Remuzzi and Bertani (1999), Seliverstova et al. (2007), Panina et al. (2007), etc., the prolonged proteinuria triggers a number of reactions leading to the development of interstitial nephritis and renal fibrosis. At the artificial feeding, as it was revealed by us (Yuldashev et al., 2009a) and Titov (2007), absorption of protein from small intestine into blood initiated inflammatory reaction not only in kidney but also in other internal organs.

Hence, at physiological conditions the conjugated functioning of small intestine and kidneys at the protein absorption provided stable homeostasis of blood, prevented loss of protein by an organism, and developed a system for proteolysis of absorbed protein and site of its digestion. Digestion filtration and reabsorption in the intestine and kidneys during 6 hours digestion in lysosomes of their epithelial cells provided stable homeostasis in blood and prevented protein loss by the organism.

**Conclusion**

1. Endocytotic structures localized in the microvilli of the absorbing cells of small intestinal mucosa participate in the protein absorption. It lasts about 6 hours.
2. The Golgi complex provides accumulation of the absorbed proteins, their selective
transport to lysosomes and intercellular spaces and interstices.

3. In 20 minutes transport of the proteins, absorbed by enterocytes of the villi to the lymphatic capillaries, provokes their filtration by glomerular capillaries and endocytotic re-absorption by cells of the proximal canaliculi of nephrons and digestion within lysosomes.

4. The conjugated function of small intestine and kidneys, correlation of time of protein absorption, filtration, reabsorption and digestion in them regulates blood homeostasis.

References


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