Structural proteins of plasmolemma of the jejunum absorbing enterocytes of cattle fetus in early fetal period

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1. Introduction

At present, the polarity of epithelial cells has shifted to the vanguard of cell biological research. The plasma membrane of the cell epithelium is divided into two main domains. The apical surface contacts the lumen of the organ, and the basolateral surface contacts the adjacent epithelial cells. The apical and basolateral surfaces perform very different functions and therefore have very different protein and lipid compositions. The apical surface is specialized in the exchange of materials with the cavity, while the basolateral surface is specialized for the interaction with other cells and the bloodstream (St Johnston & Sanson, 2011).

The epithelium provides the relationship between the outside world and the internal environment of the body (Yeaman & Nelson, 1999). Epithelial cells are different from other cell types in their organization, which provides a number of unique physiological properties. Most importantly, the closed epithelium allows you to regulate the metabolism of nutrients and end products of metabolism between internal and external media (Cereijido & Shoshani, 2004).

Polarized epithelial cells have a characteristic apical-basal polarity axis for vector transport of ions and dissolved molecules. During development, mesenchymal cells are transformed into the epithelium by clumping into aggregates that undergo epithelial differentiation (Nelson, 2009).

The apical and basolateral membrane domains have different morphologies due to their functions. The apical membrane domain of enterocytes is characterized by a brush border consisting of microvilli that enlarge the cell surface and improve tissue absorption and exchange properties (Aroeti et al., 1998). On the basis of their functional features, the apical and basolateral domains of membranes are composed of different proteins and lipids (Rodriguez-Boulan & Powell, 1992). On the apical plasma membrane of the intestine cells, for example, many hydrolases, while integrins concentrate in the basolateral domain and facilitate the formation of cell contacts. The composition of mem-
brane lipids is also slightly different, in particular cholesterol and sphingolipids are concentrated mainly on the apical part, whereas phosphatidylethanolamine is on the basolateral membrane (Simons & van Meer, 1988). This polarized architecture is stabilized in epithelial cells by a dense complex of compounds that acts as a barrier against protein or lipid diffusion from one membrane domain to another. Differences in the protein and lipid composition of the two membrane domains are provided by highly specific mechanisms (Caplan, 1997). The mechanisms that transport proteins from TGN to the plasma membrane can be divided into several steps: protein segregation or sorting, budding and transport of vesicles arising from TGN, along intracellular pathways, docking and fusion with membrane (Delacour & Jacob, 2006). The heterogeneity of epithelial cells can be explained by a flexible phenotype, they target proteins to different membrane domains based on cell type, their cell localization and specialization (Muth & Caplan, 2003; Rodriguez-Boulan & Musch, 2005).

The functional properties of absorptive enterocytes are due to the polarization of these cells with the formation of macromolecular plasma membrane – apical (AM) and basolateral (BM) membranes, which, accordingly, requires some difference in the chemical composition of their bilayer. The data obtained hypothetically indicate stimulation with lycopene by the elongase-desaturase system of fatty acids (Buhai & Tsvilikhovskyi, 2010).

The transport function of polarized epithelial cells requires that membrane proteins be sorted and stored in the desired apical or basolateral membrane domain. The main sorting site for newly synthesized plasma membrane proteins is the Golgi trans complex (TGN) (exocytic pathway) (Nejsum & Nelson, 2007; Folsch, 2008). Additionally, in the endocytic pathway, protein distribution occurs between TGN domains, the plasma membrane (Gravotta & Rodriguez-Boulan, 2007) and different membrane domains (the transcytic pathway) (Casanova & Mostov, 1990) Some vesicle migration between the TGN and the plasma membrane occurs along the microtubules (Lafont & Simons, 1994; Jaulin & Kreitzer, 2007). Joining and fusion of vesicles with the correct membrane domain requires specific binding of vesicles (exocyst) and SNARE complexes (Mellman & Nelson, 2008).

Epithelial cell polarization is not limited to the separation of two domains of plasma membranes, but also involves the orientation of cytosolic organelles and the cytoskeleton. Highly organized actin microfilaments stabilize microvilli in the apical part of epithelial cells (Tilney & Mooseker, 1973).

The enterocyte membrane undergoes molecular differentiation during fetal development that expresses several basic stage-specific polypeptides (Masiuk et al., 2008; Tsvilikhovskyi & Yakymchuk, 2014). Immunoblotting revealed that polypeptides with molecular weights of 120, 100, 87, 75, and ~24 kD of the apical membrane of the small intestine epithelium at birth (age 1 hour, before the first colostrum feeding) and the 110 kD polypeptide of the apical membrane of enterocyte enterocytes 3 days, showing the ability to selectively bind immunoglobulins. On the basis of the obtained data, the hypothesis of receptor-endocytotic mechanism of colostrum immunity formation in cattle was proposed (Tsvilikhovskyi, 1998). However, data on the structural proteins of the plasmalemma of the absorptive enterocytes of the cattle fetus in the early fetal period are absent. The purpose of the study is to investigate the ratio of structural proteins of plasmalemma of the jejunum absorbing enterocytes of the cattle fetus in the early fetal period.

2. Materials and methods

The research material was selected from 80 cattle, from 2 to 4 months old, obtained from clinically healthy cows, during forced slaughter in a meat processing establishment. The slaughter of animals was carried out in accordance with the requirements of the “European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes” (Strasbourg, 1985) and the decisions of the First National Congress on Bioethics (Kyiv, 2001). After euthanasia of the fetus, the abdominal section was dissected and the jejunum was isolated. An intestinal area was selected in the early fetal period (2, 3, 4 months) with an average length of 0.8 m, which was cut lengthwise and divided into small segments of 1.5–3 cm and washed thoroughly (4–5 times) with cold (4–6 °C) medium of the following composition: 120 mM NaCl and 1 mM HEPES, the pH was 7.4 with a dry Trice. The method of cutting the intestine from the fetus of 2–4 months of age was used instead of rotation due to its small diameter. The basis for the selection of intestinal cells was the chemical (citrate/EDTA) method, on the basis of which an author’s modification of the method was developed (Masiuk et al., 2008) production of isolated enterocytes of the jejunum intestine of cattle. To obtain apical membranes and basolateral membranes from the suspension of isolated enterocytes of the cattle fetus, we used the basic method of differential centrifugation (Tsvilikhovskyi, 1998) in our modification (Masiuk et al., 2008). The efficiency of obtaining fractions of the plasma membrane was carried out by the output of the membrane material by the amount of protein. Studies on the content and composition of the structural proteins of plasmalemic enterocytes were performed by polyacrylamide gel electrophoresis of 1 mm thickness (Laemmli, 1970). Acrylamide gradient T = 7 – 18 % was formed in the protein separation gel.

The results were analyzed using parametric and non-parametric statistical criteria for small samplings: Student’s t-test. Changes in indicators were considered significant at P < 0.05–0.001, after testing the hypotheses about the normality of distribution and the difference between the general dispersions.

3. Results and discussion

The analysis of the obtained results shows that in the early fetal period (from two to four months of age of the fetus), 27 and 25 protein fractions were detected in the apical and basolateral membranes of the plasmalemma enterocytes of jejunal intestine, respectively. It should be noted that most of the detected protein fractions of the apical membrane of the jejunum enterocytes of two-month-old cattle fetus were of low and medium molecular weight, in particular, proteins with a molecular weight of 9.6 – 14.2 kDa were 9.06 ± 0.08 %; 15.5 kDa – 5.91 ± 0.05 %; 17 kDa – 2.47 ± 0.03 %; 21 kDa – 8.51 ± 0.04 %; 22.5 kDa – 6.10 ± 0.05 %; 26 kDa – 5.15 ± 0.02 %; 29 kDa – 2.78 ± 0.03 %; 31 kDa – 3.28 ± 0.02 %; 33 kDa – 4.15 ± 0.02 %; 35 kDa – 6.02 ± 0.02 %; 37 kDa – 3.33 ± 0.01 %; 39 kDa – 6.04 ± 0.02 %. Proteins with higher molecular weight were found significantly smaller: 43 kDa – 3.33 ± 0.02 %; 46 kDa – 3.99 ± 0.02 %; 52 kDa – 6.16 ± 0.03 %; 57 kDa – 4.23 ± 0.02 %;
63 kDa – 4.19 ± 0.02 %; 72 kDa – 3.02 ± 0.01 %; 75 kDa – 2.77 ± 0.01 %; 87 kDa – 3.32 ± 0.02 %. It should be noted that protein fractions with molecular weights of 100 kDa and more were found only: 100 kDa – 1.15 ± 0.01 %; 120 kDa – 2.07 ± 0.01 %; 155 kDa – 2.11 ± 0.02 %; 170–185 kDa – 1.45 ± 0.01 %; 205 kDa – 2.11 ± 0.01 %. There were no high molecular weight proteins in the apical membrane of the jejunum enterocytes of two-month-old cattle fetus with a molecular weight greater than 205 kDa (Fig. 1).

Fig. 1. Structural proteins of the apical membrane of the jejunum enterocytes of two-month-old cattle fetus (M ± m; %; n = 6)

The composition of the low molecular weight polypeptides of the apical membrane of the jejunum enterocytes of the cattle from two to three months of age does not change significantly (Fig. 2). One should only note the increase in the percentage of proteins with a molecular weight of 29 kDa (1.21 times; P ≤ 0.001) and a decrease in the percentage of proteins with a molecular weight of 21 kDa, 26 kDa and 43 kDa (respectively 1.27 times; P ≤ 0.001, 1.15 times; P ≤ 0.01 and 1.11 times; P ≤ 0.05). At the same time, not only are the ratios of high molecular weight proteins of the apical membranes of enterocytes up to three months of age are changed, but new fractions are also emerging. Thus, in apical membranes of small intestine enterocytes of three-month-old calf embryos, proteins with molecular weights of 250 kDa and 300 kDa (0.45 ± 0.01 % and 0.88 ± 0.01 %, respectively) appeared, which were not present in the membranes of enterocytes fetus of two months of age. In addition, the content of protein fractions with a molecular mass of 100 kDa, 120 kDa, and 155 kDa from two to three months of age of the fetus increases respectively 1.43 times (P ≤ 0.001), 1.18 times (P ≤ 0.01) and 1. 61 times (P ≤ 0.001), however, the concentration of proteins with a molecular weight of 170–185 kDa decreased by 1.20 times (P ≤ 0.001), and with a mass of 205 kDa by 1.29 times (P ≤ 0.001) during the indicated period of studies.

Fig. 2. Structural proteins of the apical membrane of the hollow enterocytes of three-month-old cattle fetus (M ± m; %; n = 6)
From three to four months of age of cow fetus (Fig. 3), the relative content of low molecular weight polypeptides with a molecular weight of 9.6–14.2 kDa in the apical membrane of the jejunum enterocytes significantly decreased (1.34 times; \( P \leq 0.001 \)). While the content of proteins with a molecular weight of 17 kDa is increased 1.62 times (\( P \leq 0.001 \)).

We also note a significant decrease in the percentage of proteins with a molecular weight of 22.5 kDa (1.09 times; \( P \leq 0.05 \)), 75 kDa (1.18 times; \( P \leq 0.01 \)), 120 kDa (1.14 times; \( P \leq 0.01 \)) and 300 kDa (1.16 times; \( P \leq 0.01 \)). It is evident that the decrease in the proportion of these proteins in the apical membrane of fetal enterocytes is due to the increase in the percentage of polypeptides with a molecular weight of 37 kDa (1.22 times; \( P \leq 0.001 \)), 87 kDa (1.18 times; \( P \leq 0.01 \)) and 100 kDa (1.13 times; \( P \leq 0.05 \)).

In the basolateral membranes of enterocytes of two-month old cattle fetus, 23 protein fractions with a molecular weight from 9.6 to 120 kDa were detected (Fig. 4). It is interesting to note the absence of polypeptide fractions with molecular weights of 22.5 kDa, 37 kDa, 155 kDa and 170-185 kDa, which are present in the apical membranes of these enterocytes. Instead, proteins with molecular weights of 19 kDa, 24 kDa, and 66 kDa are available on the basolateral membranes.

Most of the detected protein fractions were of low and medium molecular weight (9.6–39 kDa – 59.12 %). Proteins with a molecular weight of 40 to 100 kDa revealed only 36.1%, and protein fractions with a molecular weight of 100 kDa or more were found in the amount of only 5.91.

There were no high molecular weight proteins in the basolateral membrane of the jejunum enterocytes of two-month-old cattle fetus with a molecular weight greater than 120 kDa by electrophoresis, unlike the apical membrane.

The composition of basolateral membrane polypeptides of the jejunum enterocyte membranes of cattle fetus from two to three months of age undergoes some changes, but they concern only their quantitative composition, whereas their qualitative composition does not change significantly (Fig. 5).
It should be noted that the increase in the basolateral membrane of enterocytes up to three months of fetus age of the percentage of proteins with a molecular weight of 46 kDa and 52 kDa in 1.16 times ($P \leq 0.001$) and 2.08 times ($P \leq 0.001$), respectively, compared to these indicators in two months. Moreover, the content of proteins with a molecular weight of 15.5 kDa in 1.35 times ($P \leq 0.001$), 24 kDa – 1.15 times ($P \leq 0.001$), 29 kDa – 1.30 times ($P \leq 0.001$), 39 kDa – 1.27 times ($P \leq 0.001$), 63 kDa – 1.17 times ($P \leq 0.01$), 72 kDa – 1.18 times ($P \leq 0.01$) and 75 kDa – 1.18 times ($P \leq 0.01$) are decrease.

In the future, not only the ratio of high-molecular proteins of basolateral membranes of enterocytes changes from three to four months of age, but also new polypeptide fractions (Fig. 6). Thus, proteins with a molecular weight of 22.5 kDa – 4.55 ± 0.05 % and 155 kDa – 0.43 ± 0.02 % appear in the basolateral membranes of small intestine enterocytes of four-month-old calf embryos.

It is necessary to note, among other things, a significant increase in the basolateral membranes of enterocytes from three to four months of age of the fetus of protein fractions with molecular weights of 19 kDa, 29 kDa and 31 kDa, respectively, in 1.10 times ($P \leq 0.05$), 1.14 times ($P \leq 0.01$) and 1.27 times ($P \leq 0.001$). At the same time, during this research period, the concentration of proteins with a molecular weight of 15.5 kDa in 1.33 times ($P \leq 0.001$), 17 kDa – 1.21 times ($P \leq 0.001$), 24 kDa – 1.17 times ($P \leq 0.01$), 35 kDa – 1.15 times ($P \leq 0.01$), 39 kDa – 1.24 times ($P \leq 0.001$), 43 kDa – 1.11 times ($P \leq 0.05$), 72 kDa – 1.13 times ($P \leq 0.05$), 87 kDa – 1.30 times ($P \leq 0.001$).

Analysis of the results of studies of basolateral membrane proteins of cattle fetus enterocytes in the early fetal period showed dynamic changes in their fractional composition. In the basolateral membranes of enterocytes, there is a slight decrease in the content of low molecular weight protein fractions, and four-month-old calf embryos show high
molecular weight fractions of polypeptides with a molecular weight of 22.5 kDa and 155 kDa, which are absent in basolateral membranes two- and three-month-old fetus.

Analysis of the results of electrophoresis of apical membranes proteins of fetus enterocytes in the early fetal period indicates a decrease in the content of low molecular weight protein fractions and an increase – high molecular weight. In addition, if high molecular weight fractions of 250 kDa and 300 kDa polypeptides appear in the calf embryos from the age of three months, which were absent in the apical membranes of the two-month old fetus enterocytes.

Protein distribution at the apical or basolateral membrane domain supports several mechanisms. First, in order to prevent free diffusion of proteins from one region to another, and paracellular diffusion of ions and solutes, there is a molecular fence at the boundary between the apical and basolateral membrane domains (apical binding complex – AJC) (Shin & Margolis, 2006). Second, several classes of membrane proteins bind to the complex of cytoplasmic scaffolds of ankyrin-spectrin on the basolateral membrane, including ion transporters and channels (Bennett & Healy, 2008). These interactions are important in regulating the transport of membrane protein and its retention in different membrane domains.

The dynamics of the concentration of individual protein fractions at the apical and basolateral membranes of enterocytes significantly changes during the early study period, which indicates the intensive mechanisms of maturation and formation of polar cells in the corresponding stages of the fetal period. As noted earlier, the content of polypeptides is not uniform at the individual poles of enterocytes, so the proteins with molecular weights of 19 kDa, 24 kDa, and 66 kDa are absent in the apical membrane at two months, whereas there are no proteins at the basolateral membrane with weight of 155.5 and 22.5 Da. kDa, 170–185 kDa and 205 kDa. Although the rest of the fractions of polypeptides in different poles of cells are common, their content is significantly different. Thus, in the apical membrane of enterocytes, a significantly higher number of proteins with a low molecular weight of 9.6–14.2 kDa (1.56 times; P ≤ 0.001), whereas in the basolateral membrane a significantly higher concentration of proteins with a molecular weight of 15.5 kDa (2.06 times; P ≤ 0.001) and 17 kDa (3.62 times; P ≤ 0.001).

4. Conclusions

In the early fetal period, there are dynamic changes in the protein composition of the apical and basolateral membranes of enterocytes, characterized not only by changes in their ratio within different cell domains, but also by dynamic redistribution of the number of different fractions of polypeptides between the poles of these cells.

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Ukrainian Journal of Veterinary and Agricultural Sciences, 2019, Vol. 2, N 3
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