PHYSIOLOGY

Accumulation of milk increases the width of tight junctions in the epithelium of mouse mammary alveoli

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Abstract

The study of the molecular mechanisms of maintaining the integrity of the epithelium during mechanical stress remains a relevant problem in the physiology of tissue barriers. A methodical approach has been applied which makes it possible to reproduce mechanical pressure on the apical region of cells in vivo and to study the participation and role of tight junctions in maintaining the integrity of the epithelial structure. Mammary gland tissue specimens from lactating control mice and animals after a 20-h interruption of suckling were prepared and the width of the tight junction of the secretory epithelium was analyzed. At the ultrastructural level, it was shown that accumulation of milk caused a significant increase in the width of the tight junction between epithelial cells. In the control group, the width of this structure was 2.1 ± 0.1 μm; in the experimental group — 4.2 ± 0.1 μm. The marked increase in the width of tight junctions between epithelial cells is in accordance with an observed increase in the level of claudin-1 and -3 in the secretory epithelium and can be interpreted as adaptive changes aimed at maintaining the structure of the alveoli.

Keywords: mammary gland, alveolus, epithelium, tissue barrier, tight junction, claudins, electron microscopy.

Introduction

During mechanical stress on the epithelia, adaptive changes occur which contribute to preserving the integrity of tissue barriers (Markov et al., 2017). When milk accumulates in the alveolar cavity and when the mechanical pressure of the milk is supported by contraction of myoepithelial cells, the secretory cells of the alveoli are exposed to strong and repeated vectorial forces (Markov, 2001). Mechanosensitivity of murine mammary gland alveoli has been reported previously (Tolkunov and Markov, 1997). These results were confirmed in HC11 mammary gland epithelial cell monolayers when defined hydrostatic pressure was applied (Mießler et al., 2018a, b). To study this process in the mammary gland of mice, a model was established to create hydrostatic pressure in the alveolus by milk accumulation in its cavity. In in vivo experiments, it was shown that the pressure gradient in the alveolar cavity causes an increase in the expression of barrier-strengthening claudin proteins in the epithelium (Markov et al., 2012).

The tight junction (TJ) protein family of claudins represents the structural correlate of epithelial barrier function (Amasheh et al., 2010). Different members of this family and the resulting junctional complexes provide selective paracellular transport in the epithelium. According to their physiological properties, they are divided into two groups: mediating selective permeability, or reducing the

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permeability of the epithelium (Amasheh et al., 2002; Furuse et al., 2002). In addition, it is assumed that claudins can provide a mechanical connection of cells while maintaining the integrity of the alveoli structure (Markov et al., 2017). The accumulation of secretion in the alveoli of the mammary gland can lead to disintegration of the cells of the alveoli. In this case, the adaptation of the epithelium to the mechanical pressure of the secretion will be accompanied by adaptive changes in the dense contacts of the epithelial layer. The purpose of this study was to study the width of the TJ of the secretory epithelium in a model for creating accumulation of milk in the cavity of the alveoli of the mammary gland of the mouse by transmission electron microscopy.

Materials and methods

In the experiments, outbred female white mice were used ($n = 6$). All animals were kept in vivarium on a standard diet with free access to food and water. Each female with offspring was in a separate cage. The tissue was examined during steady-state lactation 10–12 days after birth. The experiments were carried out in accordance with the standards adopted by organizations for working with laboratory animals FELASA and RusLASA. In the control group ($n = 3$), mammary gland epithelial was taken immediately after feeding the pups. In the experimental group ($n = 3$), this procedure was performed 20 hours after the female stopped feeding the pups, which was necessary for the accumulation of milk (Markov et al., 2012). In the experiments, inguinal mammary gland was used. To prepare the necessary solutions, reagents from Sigma Aldrich (Germany) were used.

Mammary gland epithelial fragments were prefixed in a 2.5% glutaraldehyde solution on 0.1 M phosphate buffer (pH 7.4) with calcium chloride (1%) (120 min, 4 °C) and postfixed in a 2% solution of OsO_4 on 0.1 M phosphate buffer (pH 7.4) (120 min, 4 °C). Then, washing and dehydration were carried out in a series of alcohols of increasing concentration and poured into Epon hardening resin. To identify areas that are optimal for electron microscopy, semi-thin sections were prepared on a Leica EM UC7 ultra-microtome (Leica, Germany) and stained with toluidine blue. Ultrathin sections were prepared on an ultra-microtome Leica EM UC7 (Leica, Germany). Sections were stained with uranyl acetate and lead citrate. The preparations were examined using a JEM-1400 transmission electron microscope (Joel, Japan), which had a maximum accelerating voltage of 120 kV.

Two tissue samples were recorded from each animal, on which the width of TJ in the apico-lateral region of the secretory cells of the mammary gland was measured in two different regions. The extension of TJ from the apical surface of the cells to the basolateral membrane was measured, denoted as the width. Statistical processing of the results was carried out using GraphPad Prism 5.0. When processing the results, the non-parametric Mann-Whitney U-test was used. A confidence level of $p < 0.05$ was regarded as statistically significant.

Results and discussion

At the ultrastructural level, significant changes of alveoli structure in the experimental group were observed compared to controls. The accumulation of secretion in the cavity of the alveoli led to a change in cell shape and intercellular space, reflecting milk accumulation (Markov et al., 2012). In the control group, the intercellular space between the epithelial cells in the electron diffraction patterns represented a straight line, which practically did not bend along the entire length of the lateral surface of secretory cells. In contrast, in the experimental group, the lateral membranes of the epithelial cells were curved and not oriented vertically with respect to the alveolar cavity. In the cavity of the alveoli, casein granules were identified, which were absent in the intercellular space, indicating the preservation of TJ integrity in this model. In the apical region of secretory cells, TJs were clearly distinguishable (Fig. 1). No adhesion or desmosome zones were found on the lateral surface of cells. The accumulation of milk, leading to mechanical pressure on the apical membrane of the epithelium, caused a change in the width of TJ. In the control group, the width of this structure was 2.1 ± 0.1 μm (Fig. 1, A). In the experimental group, the width of tight junctions extended twice in comparison to controls, and was equal to 4.2 ± 0.1 μm ($p < 0.01$ $n = 12$; Mann–Whitney U-test) (Fig. 1, B). The TJs of the control and experimental groups differed from each other not only in width, but also in their geometry. It is worth noting that microvilli always could be detected close to TJ areas.

TJs play a major role in the separation of the alveolar cavity from the intercellular space, i.e., in the creation of a tissue barrier, as well as in the cohesion of secretory cells in the alveoli of the mammary gland (Ngyuen and Neville, 1998). It has been suggested that the TJ is a structure that responds to mechanical displacements, in particular, when hydrostatic pressure appears in the alveolus (Mießler et al., 2018a, b; Tokuda and Yu, 2019). By the beginning of lactation, desmosomes and adhesive contacts disappear between epithelial cells, while maintaining TJs (Pietelka et al., 1973). In this situation, TJs become the intercellular structures that ensure the integrity of the mammary alveoli during mechanical stress. The developed and applied method of stretching the alveoli with the accumulation of secretion in it makes it possible to analyze the effect of the mechanical factor on the structure of TJs in the secretory epithelium of the alveoli. The accumulation of milk in the cavity of

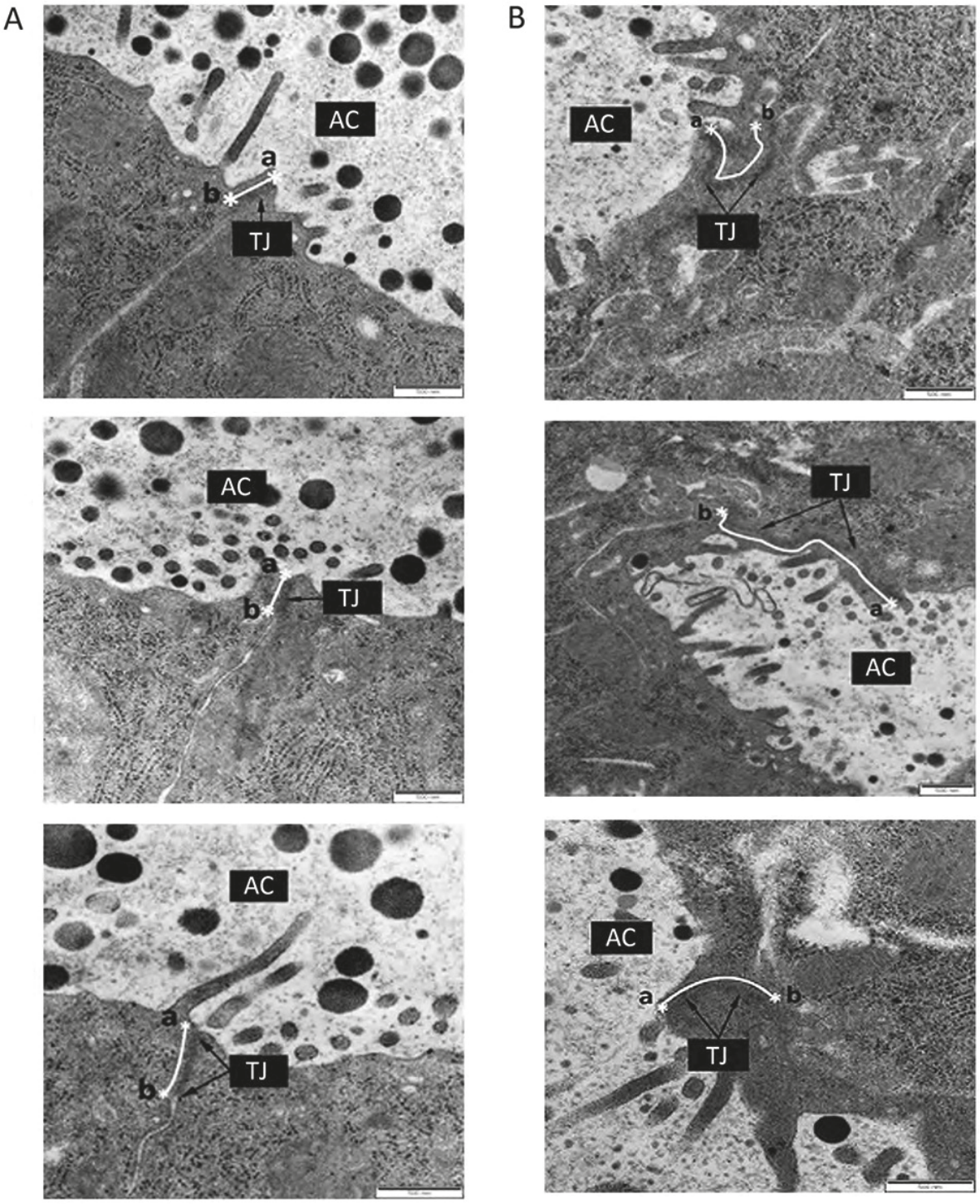

Fig. 1. Ultrathin structure of the apical part of the epithelium of the alveoli of the mammary gland: A — the control and B — experimental group of animals; AC — cavity of the alveoli, TJ — tight junction; [a* < — > b*] — tight junction width line. The line is located next to and repeats the geometry of the tight junction.

the alveoli implies the appearance of such a mechanical effect on the epithelium. Electron microscopy studies confirmed that the integrity of TJs was maintained, ensuring a tight fit of the apical portions of the membranes of neighboring cells.

When the alveoli of the mammary gland are overstretched, there is a mechanical stress on TJs, and protein granules can be detected in the intercellular space. In our experiments, casein granules were not observed outside the alveolar lumen. Under these conditions, it was shown for the first time that there is a significant increase in the width of the TJ between epithelial cells, which can be interpreted as adaptive changes aimed at maintaining the structure of the alveoli. The change in

the width of the TJ is in accordance with the observed increase in the level of claudin-1 and -3 in the secretory epithelium during milk accumulation in the alveoli (Markov et al., 2012).

It is known that the TJ is a dynamic structure that is rearranged under various influences. Thus, by freeze-fracture electron microscopy, it was found that the density of the linear structures that form the TJ, their branching, and the depth of the mesh network correlate with changes in the level of claudins in the TJ (Demehri et al., 2016). In the implementation of mechanical influences on the cell, the apical actomyosin complex is considered the main cellular structure that ensures the perception and transduction of the signal. Claudins are associated within this complex, and with the cytoskeleton via scaffolding proteins. To date, the participation of cytoskeletal microtubules in the transduction of a mechanical signal in interaction with the TJ has not been studied in detail, though (Citi et al., 2019). In this regard, the detection of microvilli close to TJ complexes is interesting. The question of molecular sensors and mechanisms of regulation of the apical actomyosin complex also remains open. The focus is on myosin light chain kinase, which induces a contraction of the apical actomyosin ring and is considered one of the key regulators of the permeability of the TJ of the epithelium (Cunningham, Turner, 2012). In MLCK-deficient mice, an increase of the main scaffolding protein ZO-1 and claudin-15 was reported in intestinal epithelium (Lorentz et al., 2017). These studies indicate that the apical actomyosin complex is included in dynamic changes in the molecular structure of TJs during mechanical stress on epithelial cells.

Conclusion

Mechanical stretching of the alveoli leads to a marked increase in the TJ width. This change of the TJ can be interpreted an increase of the width of the structure, which provides adhesion of cells under mechanical stress, as present during the accumulation of milk.

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