THE DISTRIBUTION OF COLLAGEN AND ELASTIC FIBRES IN THE LACTATING BOVINE MAMMARY GLAND

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ABSTRACT

Collagen and elastic fibres are generally present in organs whose normal function requires great resistance and elasticity. The aim of this study was to localize the collagen and elastic fibres in the stroma of the bovine lactating mammary gland and to determine their role in the process of milk ejection. For this purpose, the histochemical staining for collagen and the immunohistochemical method for the detection of elastin were used. The accumulation of scattered collagen fibres was observed between and inside the lobules where they formed distinct septa. Between secretory alveoli, the collagen fibres were found to be concentrated into two incomplete layers surrounding the blood capillaries. Bundles of elastic fibres in high density were located in the interlobular spaces. A dense network of elastic fibres was located between adjacent alveoli. Elastic membranes were located beneath the secretory epithelium. The high concentration of the collagen and elastic fibres indicated, that both types of fibres play a significant role in the resistance during the secretory stage and in the recoil of the mammary gland after milk ejection.

Keywords: collagen; elastic fibres; mammary gland; bovine

INTRODUCTION

Fibrillar collagen in the stroma has been described to be the most abundant extracellular matrix constituent which maintains the structure of most interstitial tissues and organs, including skin, gut, and exocrine glands. The stroma surrounding secretory units and interlobular septa of the mammary gland is composed of supporting and free cells and fibrous elements embedded in the extracellular matrix. The scanning electron microscopy image of mammary acini showed individual mammary epithelial cells surrounded by oriented collagen fibres [1]. The ultrastructural localization of V-type collagen revealed that it is mainly located on the basal surface of epithelial, but not beneath the myoepithelial cells. In the 100-day-old virgin and lactating rat mammary glands V-type collagen was mainly present in the interstitial connective tissue and in association with blood vessels. It was not present in the basement

membrane region surrounding the ducts in mature virgin glands, but it was present in this region in neonatal and lactating glands [20].

It has been well demonstrated, that the extracellular fibrillar structures composed of various types of collagens, such as collagen fibrils and basement membranes, have a supportive function in various tissues and organs [4]. Fibrillar collagen deposition has been quantified in the rat mammary gland across the pregnancy, lactation, and involution cycle, and the ratio of collagen to epithelial cells differed by an order of magnitude depending on the developmental state [14]. The density and spatial alignment of the three-dimensional collagen architecture define mechanical tissue properties, i.e. stiffness and porosity, which guide or oppose cell migration and positioning in different contexts, such as morphogenesis, regeneration, immune response, and cancer progression [21]. Several other proteins such as fibronectin and proteoglycans bind to collagens and affect the organization of collagen fibres, and thereby have effects on the mechanical milieu [17].

The elastin is a highly elastic protein in connective tissue and allows many tissues in the body to resume their shape after stretching or contracting. Elastin is also an important load-bearing tissue in the bodies of vertebrates and is used in places where mechanical energy is required to be stored. It is present in large amounts in the organs the elastic properties of which are essential for their function, such as arteries and lungs. The elastic fibres, unlike collagen fibres, were generally not described in the mammary gland. The aim of this study was to localize collagen and elastic fibres in the lactating bovine mammary gland.

MATERIALS AND METHODS

Mammary gland tissue samples were obtained from five lactating cows (Holstein) at a slaughter house. The samples were placed in 0.1 mol phosphate buffered 10% formalin for 24 hours at room temperature, dehydrated and embedded in paraffin. The 5 mm sections were deparaffinised and rehydrated. For collagen fibres the Azan staining method was used. For the detection of elastic fibres, the sections were pretreated with 3 % H_2O_2 in methanol for 30 min and preincubated with 2 % goat serum. Afterwards, the sections were incubated at 4 °C overnight with monoclonal anti-elastin antibody (Sigma), dilution 1:5000. The

sections were washed twice in a phosphate-balanced salt solution (PBS) and incubated with a biotinylated polyvalent secondary antibody for 45 min and incubated with avidin-biotin-peroxidase complex (Vectastain ABC kit; Vector, Burlingame Calif., USA). The peroxidase activity was visualized with 0.05 % 3',3'-diaminobenzidine (DAB) and 0.03 % v/v H_2O_2 . Some sections were counter-stained with Mayer's haematoxylin. The negative controls were performed by omitting the primary antibody.

RESULTS

The light microscopic study of a bovine lactating mammary gland revealed, that connective tissue septa consisted of bundles of fibres with fibroblast and different amounts of free cells. An accumulation of scattered collagen fibres was observed between the lobules where they formed the dominant structure, being denser in the interlobular and less dense in the intralobular connective tissue septa. The blood capillaries located between alveoli and arterioles and venules between lobules were closely surrounded by fibres.

Collagen

In the stained histologic sections on the bovine mammary gland, the collagen fibres in the interlobular space were seen to form a dominant component of the supporting tissue. A high accumulation of the collagen fibres was observed between the lobules where they formed a layer of surrounding blood vessels and ducts (Fig. 1). As the duct diameter increased, collagen fibres size and number also increased. From the gross interlobular tissue, collagen fibres enter the lobes that divided them into smaller compartments. Among the secretory alveoli, collagen fibres were concentrated into two incomplete layers surrounding the blood capillaries.

Elastin

An immunohistochemical study revealed strong positive reaction for elastin confined to elastic fibres in the connective tissue stroma. The bundles of elastic fibres forming bands of different thickness were located in the broad interlobular space (Fig. 2). Regularly, a distinct layer of the elastic fibres was also seen in the intralobular septa. Between more separated alveoli, the elastic fibres formed

Fig. 1. Section of lactating mammary gland. Azan staining. Thick connective tissue septa (CTS) rich in collagen fibres are in the interlobular and in intralobular space. Fine layer of collagenous fibres is located between the secretory alveoli (AL)

Fig. 2. Immunostaining for elastin. Elastic fibres are accumulated in the interlobular septa (ILS). A dense subepithelial plexus of elastic fibres was among most alveoli (arrows). Inside the parenchyma elastic fibres form a dense plexus (x)

Fig. 3. Immunostaining for elastin. Positive reaction was confined to elastic fibres accumulated in the interlobular interstitial septa (ILS). A dense subepithelial plexus of elastic fibres was also observed among alveoli (arrows)

loose network. High concentration of elastic fibres were found between the alveoli where elastic fibres were often concentrated into membranes located beneath the secretory epithelium (Fig. 3). In this area the elastic fibres were closely associated with the basal lamina that separated the epithelium from the less underlying elastic-rich stromal compartment.

DISCUSSION

Collagen is a component of trabecules in exocrine glands. In the lactating mammary gland collagen fibrils were dominantly present in the interlobular space, whereas between individual acini a small amount of collagen fibres was found. Periductal connective tissue and distinctive populations of collagen fibrils were associated with ductal epithelia of the mammary gland and other exocrine glands [3]. Collagen was not present in the basement membrane region surrounding the ducts in mature virgin glands but was present in this region in neonatal and lactating glands [20]. Fibrillar collagen deposition has been quantified in the rat mammary gland across the pregnancy, lactation,

and involution cycle, and the ratio of collagen to epithelial cells differed by an order of magnitude depending on the developmental state [14]. It seems that the differences coincide with different secretory activity of exocrine glands. Following weaning, high levels of fibrillar collagen are deposited between involuting acini. Ultrastructural localization of V-type collagen reveals that it is mainly located on the basal surface of epithelial, but not beneath myoepithelial cells. In addition, V-type collagen is located on some interstitial collagen fibres that are in close proximity to the basal surface of both epithelial and myoepithelial cells [20].

The fibroblast is the most common cell that creates collagen. Despite the fibroblasts, other types of cells like macrophages were found in the connective tissue stroma of the mammary gland. Studies by Ingman et al. [6] revealed a role of macrophages in collagen fibrillogenesis and in the organization of the structure of terminal end buds. According to the authors, macrophages promote collagen fibrillogenesis around terminal end buds of the developing mammary gland [6]. It is probable that these cells play the same role in the adult body in the process of milk production. Collagen fibres increased deposition in regressing mammary lobules in comparison to lactating lobules. Collagen-

associated proteins known to influence cross linking are similarly regulated including elastin, fibrillin 1, decorin, lumican, and biglycan [18].

Despite their mechanical function, collagen fibres are dynamic structures in the mammary gland. The fibrillar collagens and stromal extra cellular matrix are the major structural proteins that affect the mechanical environment of mammary epithelial cells. In addition to providing a biochemical ligand for several receptors, collagen provides structural support for the gland, which is appreciated when one sees the relationship of collagen fibres to the epithelial cells [17]. Collagen fibres among the alveoli are in close contact with secretory cells and the mammary epithelial cells respond to the stiffness of a collagen matrix [13, 17]. Cross-linking of collagen fibres and collagen arrangement will affect the mechanical properties of the extracellular matrix [15].

The interstitial connective tissue of several exocrine glands revealed that the intra- and extraglandular ducts were surrounded by a specialized connective tissue for the mechanical support of the ducts and glands. The increase of connective tissue has been observed in mice, rats and humans, suggesting that associated tensional changes are required for the massive remodeling that occurs with involution [2, 5, 7].

The elastic fibres in the mammary gland were seen to be much more dense than those observed in other exocrine glands [9, 10, 11] as was previously supposed. Elastic fibres form bridges between cells and their matrix [12]. In the rat submandibular salivary gland, elastic fibres in the ductal system were found to separate from several layers of longitudinal elastic fibres by a capillary-rich zone sparse in elastic fibres except for fine angular ones [9]. In the bovine mammary gland, elastic fibres occurred as individual, branching and anastomosing fibres localized near the basement membrane of the alveoli where they may form an elastic membrane. In the intralobular and in the larger extralobular ducts such membranes were not observed and elastic fibres were present in dense network. The high number of elastic fibres and the high microfibril elastin ratio indicated that most of the elastic fibres were secreted, probably by fibroblasts and myofibroblasts which have been seen in the interalveolar and in interlobular connective tissue. Light microscopic studies indicate that elastin is made up of repeating self-similar structures at many length-scales [19]. The main components of elastic fibres elastin and fibrillin-

containing microfibrils play a structural and mechanical role in the arteries and their essential function is to provide elasticity and resilience to the tissues [8].

It is supposed that in mammary glands, the myoepithelial cells contractions are the principal factor concerned with the “let-down” and the ejection of milk [16]. According to Foschini et al. [1] other factors such as interlobular smooth muscle contraction, vascular changes, and elastic recoil of the stroma appear to play minor roles, if any, in this phenomenon. Our results indicated, that smooth muscle cells are altered in the lactating mammary gland with collagen and elastic fibres. The presence of elastic fibres in the mammary gland reflects their utility in the process of secretion, and mainly after ejection of milk. We assume that the high density of elastic fibres may play an important role—that is to allow proliferation and expansion of the alveoli and consequently the whole gland during lactation and after milk let-down to allow the mammary gland to return to its original state.

In conclusion, the fibrous elements of the lactating mammary gland consist of collagenous and elastic fibres located mainly in intralobular and interlobular septa. Along the adjacent alveoli, elastic fibres form elastic membranes. Elastic and collagen fibres, along with myoepithelial cells, may participate in the recoil of the gland after milk ejection.

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