ULTRASTRUCTURE OF THE SUBLINGUAL GLAND IN SHEEP (*Ovis aries*)

Punipa Ariyakulkaln

Department of Biology, Faculty of Science,
Silpakorn University, Nakorn Pathom 73000, Thailand

Abstract—The structure of the sheep sublingual gland was studied by light and electron microscopy. The glands were removed from sheeps that had been fasted for 20 hours and fixed by perarterial perfusion. With light microscopy, secretory endpieces were shown to compose of tubulo-acini and demilunes. The acini contained neutral glycoproteins and sialoglycoproteins, but the demilunes were devoid of sialoglycoprotein. The ductal system included intercalated ducts, conspicuous striated ducts and excretory ducts. Ultrastructurally, the acinar cells exhibited a characteristic feature of mucous granules with electron lucent matrix and moderate electron dense bands whereas the demilune contained small, moderately electron-dense, homogeneous granules. The epithelium of the striated duct comprised mainly typical light cells with well-developed basal membrane fold and mitochondria, and dark cells. An apical bleb was evident in the light cell. Non-myelinated nerve terminals were commonly observed around the secretory endpieces. A hypolemmal axon was occasionally found in direct contact with the plasma membranes of acinar cells and the myoepithelial cell process.

Key words: Sheep sublingual gland, Carbohydrate histochemistry, Ultrastructure, Innervation.

Introduction

The importance of saliva in moistening food and lubricating its passage to the stomach has long been recognized. The diversity in general morphology, histochemistry and ultrastructure of mammalian salivary glands is well documented (1-4) as is the variability of their innervation pattern (5-7). This diversity has precluded the development of unified concepts and simple classifications (7). It is necessary to study in detail of salivary glands in animals of biological and economic importance. In ruminant salivary glands, investigations at ultrastructural level had been made only on the parotid glands (8,9) and submandibular glands (10,11) in sheep and cattle. No information on the fine structure of the sublingual gland in these ruminants was reported. The present study is therefore undertaken for the morphological assessment of the sheep sublingual gland in relation to its secretory process.

Materials and Methods

Four crossbred Romney ewes weighing 22-35 kg were used. They were housed indoors and fed on lucerne chaffs. Water and salt was available *ad libitum*. Both sublingual salivary glands were obtained from each sheep that had been withheld from food for about 20 hours.

The animals were anaesthetized with pentobarbitone sodium, 25-30 mg/kg body weight, via a jugular vein. The artificial respiration was maintained with a Palmer respiratory pump via a tracheal cannula during arterial perfusion of fixative through both common carotid arteries. The fixative used was half-strength Karnovsky’s fluid (12) containing 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.3, at room temperature. After perfusion, wedges of tissues were excised from both glands, rapidly transected into 1 mm cubes in the fixative and left in the fresh fixative for 2-4 hours at room temperature. The tissues were washed overnight in 0.05 M cacodylate buffer containing 7% sucrose, pH 7.3, at 4°C prior to postfixing in 1% buffered OsO₄ for 1/2 hours, then dehydrated in graded series of ethanol and embedded in Epon-Araldite (13). Ultrathin sections were obtained using glass knives on a LKB Ultratome, stained with uranyl acetate and lead citrate, then examined under a Philips 200 TEM at 60kV. Semithin sections (0.5 μm thick) were also prepared, stained with 1% toluidine blue and examined under light microscope. From these glands, a wedge of tissue was further fixed in
Figure 1. A light micrograph of the rematoxylin and eosin stained section of a sheep sublingual gland showing the secretory endpieces (Se), long intercalated duct (Id) and several striated ducts (Sd). Note the poorly-stained tubuloacinar cell (A) and the eosinophilic demilunes (D).

Figure 2. The subsequent section of figure 1 stained with PAS-H after diastase pretreatment. Demilunes show stronger PAS-reaction than acinar cells. Note the secretion in the lumen of a striated duct.

Figure 3. An electron micrograph showing part of the secretory endpiece in the sheep sublingual gland. Note the arrangement of acinar cells (A), demilunes (D) including their organelles. (R = rough endoplasmic reticulum, G = Golgi complex, Mi = mitochondria, Sm and Ss = secretory granules of acini and demilune, Ic = intercanaliculi, N = nerve terminal, M = myoepithelial cell).

Figure 4. Higher magnification showing secretory granules within the mucous acinar cell (Sm) and the serous demilune (Ss). Secretion is present in the intercanaliculi of demilune.

Figure 5. The arrangement if myoepithelial cell with its cell processes (M) around a secretory endpiece.
the same fixative for at least 24 hours at 4° C prior to paraffin embedding. Paraffin sections (5 μm thick) were stained with hematoxylin and eosin (H&E), periodic acid Schiff-hematoxylin (PAS-H) either with or without diastase pretreatment, and AB (alcian blue, pH 2.5)-PAS-H.

Results

Light microscopy

The secretory endpieces of the sheep sublingual gland were composed of two different cell types: tubulo-acini and demilunes, while the ductal system consisted of a relatively long intercalated duct, numerous striated ducts and excretory ducts. In these resting glands, the acinar cells were poorly-stained with hematoxylin, but were strongly AB-positive and slightly PAS-positive after the pretreatment with diastase. The latter indicated that the secretory materials in the acinar cells were rich in sialoglycoproteins or acidic mucosubstances (14) as well as a small amount of neutral glycoproteins. The demilunes were eosinophilic with H&E staining, moderately PAS-positive, diastase resistant, and AB-negative, representing the presence of neutral glycoproteins and the absence of sialoglycoproteins in these cells. Some AB-positive or/and PAS-positive materials were evident in the ductal lumen. Goblet cells were observed only in larger excretory ducts.

Electron microscopy

The fine structure of acinar cells was similar to that of typical mucous cells whereas the fine structure of the demilunes was similar to that of serous cells (Figure 3). The secretory granules of the mucous acinar cells contained flocculent material in electron lucent matrix and (occasionally) a small dense body, including moderate electron dense bands (Figure 4). These granules often showed partial fusion of membrane boundaries. On the contrary, the serous demilunes contained small spherical granules with homogeneous and electron denser materials (Figure 4).

Myoepithelial cells including their processes were usually observed around the tubulo-acini and demilune (Figure 5). The striated ducts were well developed. Their epithelium comprised both light and dark cells (Figure 6). The light cell showed elaborate folds of basal plasma membranes, contained high number of mitochondria and a clear apical bleb.

Non-myelinated nerve fibers were frequently observed in the interstitial spaces with axons containing both granular and agranular vesicles (Figure 7). Basing on neuro-effector sites most of the nerves were classified to be epithelial type (5). Occasionally, hypolemmal nerve terminals were also observed in close relation to the plasma membranes of acinar cells and myoepithelial cell processes (Figure 8).

Discussion

The classification of salivary glands being used in this investigation follows the criteria suggested by Young and van Lennep (3). These are based on cell morphological characteristics of the mature secretory granules studied under LM in paraffin sections stained with H&E and under TEM in ultrathin sections stained with uranyl acetate and lead citrate after aldehyde fixation and post-osmification. Under these conditions the gland cells fall into 3 categories: 1. SEROUS CELLS: LM-granules small, discrete, refractile, usually eosinophilic; EM-granules homogeneous, usually electron dense. 2. MUCOUS CELLS: LM-granules large, closely packed and often ill-defined, poorly stained with H&E; EM-granules often fused, with a homogeneous, fairly electron lucent matrix. 3. SEROMUCOUS CELLS: Intermediates between serous and mucous in appearance. Therefore, the sheep sublingual gland would be classified as a heterocrine gland in which the secretory endpieces contained mucous tubulo-acini and serous demilunes.
It is striking that the secretory granules of the sublingual salivary gland acini not only exhibited the characteristic ultrastructural features of mucous cells, but also had another substructure—that is the moderate electron dense bands. The latter has not been reported in any other mucous granules. This substructure probably represents secretory products of different chemical composition. Some support for the concept of differences in electron densities of secretory granules in relation to different compositions and secretion comes from the work of Ichikawa and Ichikawa (15). They demonstrated that the pale region of the secretory granules of the Mongolian gerbil salivary glands was carbohydrate-rich (mainly sialomucin) and the dense core was protein-rich. Histochemically, the mucous acini of the sheep sublingual gland in this study contained large amounts of sialoglycoproteins apart from some neutral glycoproteins, whereas the demilunes contained only neutral glycoproteins corresponding to moderate dense granules. This is consistent with previous histochemical studies of the same glands in sheep, cattle and pig (1,2). Typical fine structural appearances of mucous acini were also reported in the mandibular glands of sheep and cattle (10,11) with the presence of sialoglycoproteins and neutral glycoproteins (10). These sialoglycoproteins tend to be responsible for the viscosity of the sublingual saliva as was reported in the submandibular saliva (16).

The presence of secretion in the intercanaliculi within the demilune, acinar lumen and some ductal lumen supports the observations described by Kay (17) that a slow continuous flow occurred at rest (when the gland was not stimulated). In anaesthetized sheep, saliva collected from the sublingual gland was very thick mucous, hypotonic and weakly buffered resembling the saliva from the mandibular glands (17). In addition, both glands were active during eating but not during rumination (10,17). Therefore, the major importance of sublingual glands is no doubt to moisten and lubricate food as was proposed by Kay. However, information concerning biochemical studies on the compositions of saliva from sublingual glands is yet lacking. Detailed studies on chemical compositions of their secretory products in comparison to morphological changes of the glands with association to various stimulation are essential for further understanding on their functions.

The findings of this study agree with what was reviewed by Young and van Leenep (3) and Garrett and Emmelin (18) that the arrangement of myoepithelial cells around secretory endpieces is consistent with their role in supporting the underlined cells during salivary secretion as well as the role in promoting the flow of highly viscous saliva. Abundant myoepithelial cells were reported in other salivary glands that secrete very viscous saliva, such as in the sublingual gland of monotreme echidna (3), the mandibular glands of sheep (10) and cattle (11), as well as the cat zygomatic gland (19).

The innervation of myoepithelial cells was evident as hypolemmal type (Figure 8) although was observed only occasionally. This probably provides some direct control of the cells. No direct physiological evidence of myoepithelial cell activity was available in the sheep sublingual gland. Epilemmal nerve terminals were frequently observed near secretory endpieces including myoepithelial cell processes and other effectors (Figure 7). Since a dual sympathetic and parasympathetic innervation of salivary glands was well established, it is unable to identify whether the nerve terminals observed in this study are cholinergic or adrenergic due to the EM procedures employed here could not adequately preserve the small dense core vesicles within the nerve terminals. Further studies using special techniques would prove profitable.

Acknowledgement

The author wish to express her deep appreciation to Dr.D.H. Carr, Department of Physiology and Anatomy, Massey University, New Zealand for his assistance in dissecting the specimen. This work was supported by the research grant from the Faculty of Science, Silpakorn University, Nakorn Pathom 73000, Thailand.
References


Figure 6. An electron micrograph showing striated duct epithelium consisting of electron light cell and dark cell. Note the apical bleb (b) in a light cell.

Figure 7. An electron micrograph showing a non-myelinated nerve terminal (arrow) in the interstitial space near the secretory endpiece. (C = capillary).

Figure 8. A hypolemmal nerve terminal (arrow) in close relation to the plasma membranes of the acinar cell (A) and a myoepithelial cell process (M).