Ultrastructure of the Salivary Glands in the Gray Short-Tailed Opossum (*Monodelphis domestica*)

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Article History

Received 28 May 2015

Accepted 2 November 2015

Keywords :

Salivary gland, Ultrastructure,
Gray short-tailed opossum,
Monodelphis domestica

Abstract

The salivary glands help to keep the mouth and other parts of the digestive system moist. There have been only a few reports on the salivary glands of the marsupial gray short-tailed opossum (*Monodelphis domestica*). The aim of this study was to reveal the ultrastructure of the parotid and submandibular glands in the gray short-tailed opossum using transmission electron microscopy (TEM). Using TEM, the parotid glands were found to consist only of serous acinar cells. Secretory granules were highly electron dense. The submandibular glands were composed of both serous and mucous acinar cells, showing a mixture of glands. Also, the submandibular glands possessed special serous cells. Secretory granules were divided into homogeneous density type, two biphasic types and annual ring types. The properties of the granules were different in each cell type. However, some cells contained a mixture of every type of granule. It appeared that the variety of granules in the special serous cells represented stages in the cell cycle and was not a property of the cell. Striated ducts of submandibular glands consisted both of columnar epithelium with or without basal infoldings, although typical striated ducts were found in parotid glands.

Introduction

Saliva is crucial for the maintenance of oral health in that it contains a complex mixture of molecules. These are involved in a multitude of functions such as lubrication of the oral mucosa, defense against infections, and prevention of tooth demineralization and promotion of tooth remineralization (1). Mammals normally have three large salivary glands which include the parotid, submandibular and sublingual glands (2). These major salivary glands are made up of many lobular structures (3). The parotid, sublingual and submandibular glands of mammals produce serous, mucous and mixed secretions, respectively (2). The three major

salivary glands collectively supply approximately 90% of the total salivary flow (4).

Marsupials are mammals whose young are born at a very undeveloped stage and are attached to the mother's nipples, usually in a pouch (5). Despite this, the jaw elements for suckling and the digestive system must develop early (6). Therefore, it was thought that the structure of the salivary glands of marsupials may have some differences compared to other mammals.

There have been many morphological studies of major salivary glands of mammals such as humans (2, 7), mice (8, 9, 10), and rats (2, 4, 11). Some of these have investigated the microstructures using transmission electron microscopy (TEM). However, there have only been a few reports of the salivary glands of the gray short-tailed opossum

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Fig. 1 Ultrastructure of parotid gland

The parotid gland acinar cells are pyramidal, and their nuclei exist at basal side (Fig. 1a). They have numerous mitochondria and rough ER throughout the cells, and myoepithelial cells (arrows) are along the outside of acinar cells (Fig. 1b, c). The secretory granules show homogeneous high electron density (Fig. 1d). The striated ducts are composed of columnar cells (Fig. 1e). The intercalated ducts are lined by cuboidal cells (Fig. 1f). 1a: Secretory unit (bar=2 μ m), 1b: ER (bar=1 μ m), 1c: Myoepithelial cell (bar=1 μ m), 1d: Secretory granules (bar=1 μ m), 1e: Striated duct (bar=5 μ m), 1f: Intercalated duct (bar=1 μ m)

(*Monodelphis domestica*) (12, 13). Furthermore, only light microscopy and not TEM was used in these studies.

The aim of this study was to reveal the ultrastructure of the parotid and submandibular glands in the gray short-tailed opossum using light microscopy and TEM.

Materials and Methods

Materials

The parotid and submandibular glands were obtained from three gray short-tailed opossums (*Monodelphis domestica*) (females, 24 months old). The experimental protocol was approved by Nihon University Animal Care and Use Committee (Nos. AP09MD023, AP12MD015).

Methods

For TEM, the parotid and submandibular glands were sliced into small pieces, and fixed with 2% glutaraldehyde

and 1% osmic acid before being embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate-lead citrate following routine procedures, and were observed and photographed in a JEM-1010 (JEOL, Japan).

Results

Parotid gland

The acinar cells were pyramidal, and their nuclei existed at the basal side of the cells (Fig. 1a). A number of mitochondria and tubular or vesicular ER (endoplasmic reticulum) existed throughout the acinar cells. The secretory granules were observed on the lumen side (Figs. 1a, 1b). Myoepithelial cells were flat and were found along the basement membrane (Fig. 1c). The secretory granules were homogeneous high electron density (Fig. 1d). The striated ducts consisted of columnar cells. The striated epithelium had basal infoldings associated with mitochondria (Fig. 1e).

The intercalated ducts were short segments lined with cuboidal cells (Fig. 1f).

Submandibular gland

The secretory units were composed of both serous and mucous cells, indicating mixed glands. An irregularly shaped nucleus was found in the distal part of the acinar cells (Fig. 2a). A number of mitochondria and tubular or vesicular ER existed throughout the acinar cells. Secretory granules were observed on the lumen side (Figs. 2a, 2b). The myoepithelial cells were flat and represented the abundant myofilaments (Fig. 2c). There were four different types of secretory granule in serous cells: i) homogeneous high electron density type, and biphasic granule type with ii) high electron dense core, iii) low electron dense core, and iv) annular ring type (Fig. 2d). Some serous cells had several types of granules (Fig. 2e). The secretory granules of mucous cells were homogeneous low electron density (Fig. 2f). Striated ducts were composed both of columnar epithelium with or without basal infoldings (Figs. 2g, 2h). The intercalated ducts were short segments lined with cuboidal cells (Fig. 2i).

Discussion

Salivary glands have an important role in animals, providing lubrication for eating and vocalization, aiding digestion and taste, and acting as a pH buffer (14). Mammalian salivary glands mainly consist of two epithelial cell types, the acinar cells that secrete salivary fluid and most of the salivary proteins, and the ductal cells which secrete protein and modify the ionic composition of the saliva as they convey it to the mouth (15). There have been a few reports on the structure of salivary glands of marsupials: opossum (16-19), koala (20, 21), and wallaby (22). In these marsupials, the secretory units of the submandibular glands, except for those of koala (20, 21), are composed of serous and mucous acinar cells. This indicates that the submandibular glands of almost all mammals consist of mixed glands. Only the submandibular glands in koalas are reported to be composed of serous acinar cells (20, 21).

In our study, the parotid glands consisted only of serous acinar cells. The submandibular glands were composed of a mixture of both serous and mucous acinar cells. Striated ducts of submandibular glands consisted both of columnar epithelium with or without basal infoldings, although typical

striated ducts were found in parotid glands. Shackelford et al. reported a resemblance to rodent submandibular glands, known as glandular tubules, in which intercalated ducts terminate (23).

Using TEM, according to the research of Wilborn et al. (16), submandibular secretory units of American opossum (*Didelphis virginiana*) contained special serous cells, which differed from typical serous cells. Shackelford et al. (23) reported that special serous cells, which had little or no detectable mucosubstance, differ structurally from other serous cells. Special serous cells, as a group, appear more heterogeneous than serous, mucous, or seromucous cell types. The irregular shaped nucleus was characteristic of opossum special serous cells. Wilborn et al. (16) reported that the most notable difference was that special serous cells did not possess the closely packed lamellae of ER that was reported in the serous cells described by others. Less striking differences included fewer basal infoldings and more mitochondria in special serous cells than reported in the serous cells of human submandibular glands. Special serous cells occurred among mucous cells, extending from the ends of some tubules which constituted the lumen of acinar cells, or encircling other tubules as demilunes. Thus, the apices of some special serous cells did not reach the mucous tubule lumen. Spaces were occasionally seen between cells which possibly served as avenues for secretion of the peripherally located special serous cells to reach the lumen of the mucous acini. Shackelford et al. (23) reported that special serous cells were present in dog sublingual glands and ruminant (cow and sheep) parotid glands. Our observation is focused on the submandibular gland of *Monodelphis domestica*, although the previous studies were about an American opossum (16-18). It is suggested that the special serous cell has a wide distribution among the many Didelphids, including the so-called "opossum". Opossum have been recognized as a primitive lineage based on some features including dentition (24). Marsupials and placentals diverged from a mammalian common ancestor in the late Cretaceous period (25). Special serous cells appear to have a vestigial character in mammals, because they also remain in some placentals (dog, cow etc.) irrespective of food habits.

Blood et al. (19) reported that the secretory granules in acinar cells of Australian brush-tail possums (*Trichosurus vulpecula*) have filamentous material and two types of denser inclusions. Ichikawa et al. (26) reported that the

Fig. 2 Ultrastructure of submandibular gland

The secretory units are mixed glands, and possess the special serous cells (Fig. 2a). The acinar cells have number of mitochondria and tubular or vesicular ER throughout the cells (Fig. 2b). The myoepithelial cell (arrows) contains abundant myofilaments (Fig. 2c). Secretory granules of serous are divided into i) homogeneous density type, ii, iii) two biphasic types and iv) annual ring types and these secretory granules of mucous cells are homogeneous low electron density (Fig. 2d-f). The striated ducts of submandibular glands are composed of columnar epithelial cells with or without basal infoldings, although those of the parotid glands were consisted of typical columnar epithelial cells with the infoldings. (Fig. 2g, h). The intercalated ducts are lined by cuboidal cells (Fig. 2i). 2a: Secretory units (bar=2 μ m), 2b: ER (bar=2 μ m), 2c: Myoepithelial cell (bar=1 μ m), 2d: Serous secretory granules (bar=500nm), 2e: Serous secretory granules (mixed type) (bar=500nm), 2f: Mucous secretory granules (bar=1 μ m), 2g: Striated duct (bar=5 μ m), 2h: Striated duct (bar=5 μ m), 2i: Intercalated duct (bar=2 μ m)

granules in serous acinar cells of monkey submandibular glands have a central dense spherule and a rim of lower dense matrix. The granules in serous acinar cell of the

Mongolian gerbil salivary glands have a high density of cores and bipartite structures. Takada et al.(27) reported that the granules in mucous cells of the mouse parotid

glands in the early postnatal period have low electron density and bipartite structures.

Wilborn et al.(16) reported that the cell base lacks the infoldings observed in light cells of striated duct. Two types of basal cells of striated duct contain numerous filaments and lack basal infoldings. Tamarin et al.(28) reported that dark cells and basal cells of striated ducts have been observed in excretory ducts of rat submandibular glands.

The results of this study demonstrated that the submandibular glands of the gray short-tailed opossum possess special serous cells. Secretory granules were divided into homogeneous density type, two biphasic types and annual ring types. The properties of the granules were different in each cell. However, there were some cells that contained a mixture of every type of granule. It appears that the variety of granule in the special serous cells represents the stage in the cell differentiation and is not the specific character of the cell.

In conclusion, the gray short-tailed opossum shows morphological accordance with and difference from the salivary glands of mammals, in relation to the marsupial phylogenetic position.

Acknowledgments

We thank Mr. Takashi Matsumoto for TEM technique and Ms. Noriko Uemura for opossum care. This study was supported in part by a grant to Kunihiro Suzuki from JSPS (KAKENHI Grant Number 26440127), and a grant to Division of Companion Diagnostics, Department of Pathology of Microbiology, Nihon University School of Medicine from the Health Sciences Research Institute, Inc., Yokohama, Japan.

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