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Ultrastructure of the Submandibular Gland in the Korean Spider Shrew, *Sorex caecutiens*

Soon-Jeong Jeong, Jiyun Yoo[1] and Moon-Jin Jeong*
Department of Oral Histology, College of Dentistry, Chosun University, Dong-Gu, Seosuk-Dong, Gwangju 501-825, Korea
[1]Department of Microbiology, Division of Life Sciences/Research Institute of Life Science, Gyeongsang National University, Jinju 660-701, Korea
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뒤쥐, *Sorex caecutiens* 악하선의 미세구조

정 순 정, 유 지 윤[1], 정 문 진*
조선대학교 치과대학 구강조직학교실
[1]경상대학교 생명과학부 미생물학과/생명과학연구소

ABSTRACT

The ultrastructure of submandibular gland was examined in the Korean spider shrew, *Sorex caecutiens*. The submandibular gland was composed of acini and salivary ducts. A submadibular acinus was a mixed gland having serous demilune cells and mucous cells that were filled with well developed rER, mitochondria and large amount of dense secretory granules. Serous acinar granules were oval shape without distinct limiting membrane on the border and it had only coarse specks with various density. Mucous acinar granules were oval shape without distinct limiting membrane and had a variety pattern with several thin or transparent bands into the homogeneous dense matrix. Thus submandibular acinar granules of *S. caecutiens* belonging to subfamily Soricinae were distinct from the other mammalian species including Crocidurinae, because of the absence of limiting membrane of acinar granules and specific pattern of mucous acinar granules. Granular duct cells had large amount of small granular vesicles and several characteristic structures of granule which were covered with stratified limiting membranes and filled with coarse serous-like granules or homogeneous matrix.

Keywords : Mucous acinar granule, Serous acinar granule, *Sorex caecutiens*, Submandibular gland, Ultrastructure

INTRODUCTION

Insectivora including Soricidae is a primitive mammalian group, thought to be ancestral to many groups of mammals, exhibiting characteristics of specific interest for the study of mammalian evolution (Churchfield, 1990; Carson & Rose, 1993). Soricidae is generally

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* Correspondence should be addressed to Dr. Moon-Jin Jeong, Department of Oral Histology, Department of Conservative Dentistry, College of Dentistry, Chosun University, Gwangju 501-759, Korea. Ph.: (062) 230-6895, FAX: (062) 224-3706, E-mail: mjjeong@chosun.ac.kr

considered to comprise two subfamilies, Soricinae and Crocidurinae. Subfamily Soricinae includes the red-toothed shrews, the genus *Sorex* having pigmentation of the tips of the teeth which is commonly reddish-brown (Jones & Johnson, 1960; Won, 1967; Churchfield, 1990). These red-toothed shrews, genus *Sorex* inhabiting Korea thought to include two species, the little spider shrew, *S. minutus* and the Korean spider shrew, *S. caecutiens* (Jones & Johnson, 1960; Won, 1967). However these species are very rare in the wild and their population also have been decreased, they have never been studied with exception of the brief comments and taxonomical reviews in Korea (Jones & Johnson, 1960; Won, 1967).

Ultrastructural differences between the homologous cells in salivary glands represent the potential value of the possible evolutionary significance at cellular level (Hand, 1980; Phillips & Tandler, 1987; Phillips et al., 1987a, b, 1993; Tandler et al., 1990, 1994; Tandler & Phillips, 1993) as well as hold much promise in systematic and comparative ultrastructural analysis which can serve as a foundation for molecular comparisons (Phillips & Tandler, 1987; Tandler et al., 1990). Although recently salivary glands has been examined in various species (Hand, 1980; Phillips & Tandler, 1987; Phillips et al., 1987a, b, 1993; Tandler et al., 1990, 1994; Tandler & Phillips, 1993), studies on this organ have been restricted largely to a few commonly available laboratory animals (Tandler et al., 1990). In case of Soricidae, salivary glands of a few members, European water shrew *Neomys Fodiens* (Schaffer, 1908), the house shrew *Crocidura russula* (Raynaud, 1964), the black and Rufous elefant shrew *Rhynchocyon chrysopygus* (Mineda, 1981), the musk shrew *Suncus murinus* (Mineda, 1985), the big white-toothed shrew *C. lasiura* (Jeong & Jeong, 2005; Jeong et al., 2005a) and the lesser white-toothed shrew *C. suaveolens* (Jeong & Jeong, 2005; Jeong et al., 2005b) have been reported.

The aim of the present study on the ultrastructure of the submandibular salivary gland in the Korean spider shrew, *S. caecutiens*, is to determine ultrastructure and characteristics of submandibular gland cells and secretory granules, to compare these features to the other small mammalian species.

MATERIALS AND METHODS

Sorex caecutiens of two adults were collected at January and October 1999 from Mt. Jiri (Kyongsangnamdo, South Korea) using the Sherman live traps.

A pair of submandibular gland was fixed in 2.5% glutaraldehyde and 2 % paraformaldehyde in Millonig's phosphate buffer (pH 7.4) for 1 hr. Specimens were post-fixed with 1.3% osmium tetroxide in the same buffer for 2 hr, dehydrated with a series of the graded ethyl alcohol and acetone, and embedded in epoxy resin. Thick sections (0.5 ~ 1 μm) were stained with 5% toluidine blue for light microscopic observation. Thin sections (60 ~ 90 nm) were double stained with uranyl acetate and lead citrate for electron microscopy. All of the thin sections were examined with a JEOL 100S (JEOL Ltd., Japan) transmission electron microscopic observation.

RESULTS

The submandibular gland of the Korean spider shrew, *Sorex caecutiens* was examined with a light and electron microscopy.

From the observation of the microscopic specimens, a submandibular gland of *S. caecutiens* was composed of acini and salivary ducts (Fig. 1). A submadibular acinus was composed of serous demilune cells and mucous cells (Fig. 2).

From the observation of ultrastructure of acinar cells in the submandibular gland, a serous demilune cell had well developed rER, prominent Golgi complex and several mitochondria at the basal cytoplasm of the cell,

and large amount of dense serous acinar granules at the apical cytoplasm (Fig. 3). Serous granules were oval shape without distinct limiting membrane on the border and it had only coarse specks with various density, were also seen at the lumen of the intercellular space (Fig. 4). A mucous acinar cell also had well developed rER, mitochondria and large amount of mucous granules with various stages of the maturing process (Fig. 5). Immature granules were a oval shape and had a homogeneous matrix with thin core, but indistinct limiting membrane was not seen on the border (Fig. 5). Mature mucous granules were oval shape and had a variety pattern with several thin or transparent bands into the homogeneous dense matrix, but distinct limiting membrane also was not seen on the border (Fig. 6). A granular duct cell had many various shaped and well developed mitochondria, large amount of small granular vesicles and several characteristic structures of granule (Figs. 7, 8). The characteristic structures of granule were filled with serous-like granules and covered with stratified limiting membranes, each layer of limiting membrane were not connected, small granular vesicles were surrounded by free ribosome on all sides (Fig. 9). The inside coarse serous-like granules gradually changes into homogeneous (Fig. 10). This characteristic structures of granule seem to be formed by fusion of small granular vesicles.

DISCUSSION

Submandibular acinar granules of the Korean spider shrew, *Sorex caecutiens* belonging to subfamily Soricinae (Jones & Johnson, 1960; Won, 1967) were distinct from the other mammalian species including Crocidurinae, because serous and mucous acinar granules all had not limiting membrane on the border and mucous acinar granules had a specific pattern by several thin or transparent bands into the homogeneous dense matrix. Serous acinar granules of *S. caecutiens* were oval shape without distinct limiting membrane on the border and it had only coarse specks with various density. Mucous acinar granules of *S. caecutiens* were oval shape and had a variety pattern with several thin or transparent bands into the homogeneous dense matrix, but distinct limiting membrane also was not seen on the border. Generally, granules in salivary gland all have limiting membrane and species-specific patterns (Tandler et al., 1990). Previous studies on granules of salivary gland in Soricidae species including Soricinae and Crocidurinae also have limiting membrane and species-specific patterns (Schaffer, 1908; Raynaud, 1964; Mineda, 1981, 1985; Jeong & Jeong, 2005; Jeong et al., 2005a, b). However those in the submandibular grand of *S. caecutiens* was characterized by the absence of limiting membrane of serous and mucous acinar granules as well as the specific pattern of mucous acinar granules by several thin or transparent bands into the homogeneous dense matrix. It is considerable that interspecific variation of granule ultrastructure exported by homologous salivary acinar cells (Ball, 1993; Tandler et al., 1994). Interspecific variation in granule ultrastructure might be not only indicative of functional differences in saliva (Levine et al., 1987; Tandler et al., 1990) but also correlated with genetic history (Phillips et al., 1987a; Tandler et al., 1994), diet (Phillips et al., 1987b; Tandler et al., 1990) and species isolation (Nagato et al., 1984). Thus the absence of limiting membrane of acinar granules and specific pattern of mucous acinar granules of *S. caecutiens* might be used with a key which is classified from the other mammalian species.

Granular duct cells of *S. caecutiens* had large amount of small granular vesicles and several characteristic structures of granule. Although small granules in *Sorex caecutiens* were different from relatively big secretory granules in other mammals including Crocidurinae, those of *S. caecutiens* had also a limiting membrane. The characteristic structures of granule were covered with stratified limiting membranes and filled with serous-like granules. The inside coarse serous-like granules gradually changed into homogeneous. Characteristic

structures of granule seem to be formed by fusion of small granular vesicles. This study is the first report about characteristic structure of granule in granular duct cell, further studies will be necessary to determine the process of formation and function. Also Myelin-like body, a manifoldly form of membranes, reported in granular duct cells of subfamily Crocidurinae shrews, *Suncus murinus* and *C. dsinezumi* (Mineda, 1985), *C. lasiura* (Jeong et al., 2005a) and *C. suaveolens* (Jeong et al., 2005b) did not observed in granular duct cell of *S. caecutiens* belonging to subfamily Soricinae.

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<국문초록>

뒤쥐 *Sorex caecutiens*의 악하선의 미세구조를 연구하였다. 악하선은 샘포들과 도관들로 구성되었다. 악하선 샘포는 잘 발달된 조면소포체, 미토콘드리아와 많은 전자밀도가 있는 분비과립으로 채워진 장액선 세포와 점액선 세포를 가지는 혼합샘이었다. 장액선 샘포 과립은 명확한 한계막이 없는 타원형으로 다양한 전자밀도를 가지는 거친 알갱이만을 가지고 있었다. 점액선 샘포 과립은 명확한 한계막이 없는 타원형이고 전자밀도가 있는 균질한 기질 내에 몇 개의 연하거나 투명한 띠를 가져 다양한 문양으로 관찰되었다. 따라서 뒤쥐아과(Soricinae)에 속하는 뒤쥐, *S. caecutiens*의 악하선 샘포 과립은 샘포 과립의 경계막의 부재와 점액 샘포 과립의 특별한 문양으로 땃쥐아과(Crocidurinae)를 포함한 다른 포유류 종들과 구분된다. 과립관세포에서 많은 작은 과립소포와 층으로 된 한계막으로 덮이고 거친 장액성의 분비 과립 혹은 균질한 기질로 채워진 몇 개의 특징적 구조들이 관찰되었다.

FIGURE LEGENDS

Fig. 1. Light micrograph of the submandibular gland composing acini (A) and salivary dusts. Gd, granular duct; Sd, striated duct. Scale bar=100 μm.

Fig. 2. Light micrograph of submandibular acini (A) having serous demilune cells (Sc) and mucous (Mc) cells. Scale bar=50 μm.

Fig. 3. Electron micrograph of the serous demilune acinar cell in the submandibular gland. The basal cytoplasm is filled with well developed rER, prominent Golgi complex (Gc) and several mitochondria (M). The apical cytoplasm is filled with large amount of dense serous acinar granules (Sg). Is, intercellular space; N, nucleus. Scale bar=0.1 μm.

Fig. 4. Higher magnification of the serous acinar granules (Sg) in the submandibular gland. Even if serous granules form a oval shape, there is no distinct limiting membrane on the border. Serous granules are composed of only coarse specks with various density. The secreted serous granules are also seen in the lumen (L). Scale bar=0.05 μm.

Fig. 5. Electron micrograph of the mucous acinar cells in submandibular gland. The cytoplasm is filled with well developed rER and mitochondria (M), and many mucous acinar granules (Mg) with various stages of the maturing process. Ig, immature granule; N, nucleus. Scale bar=0.1 μm.

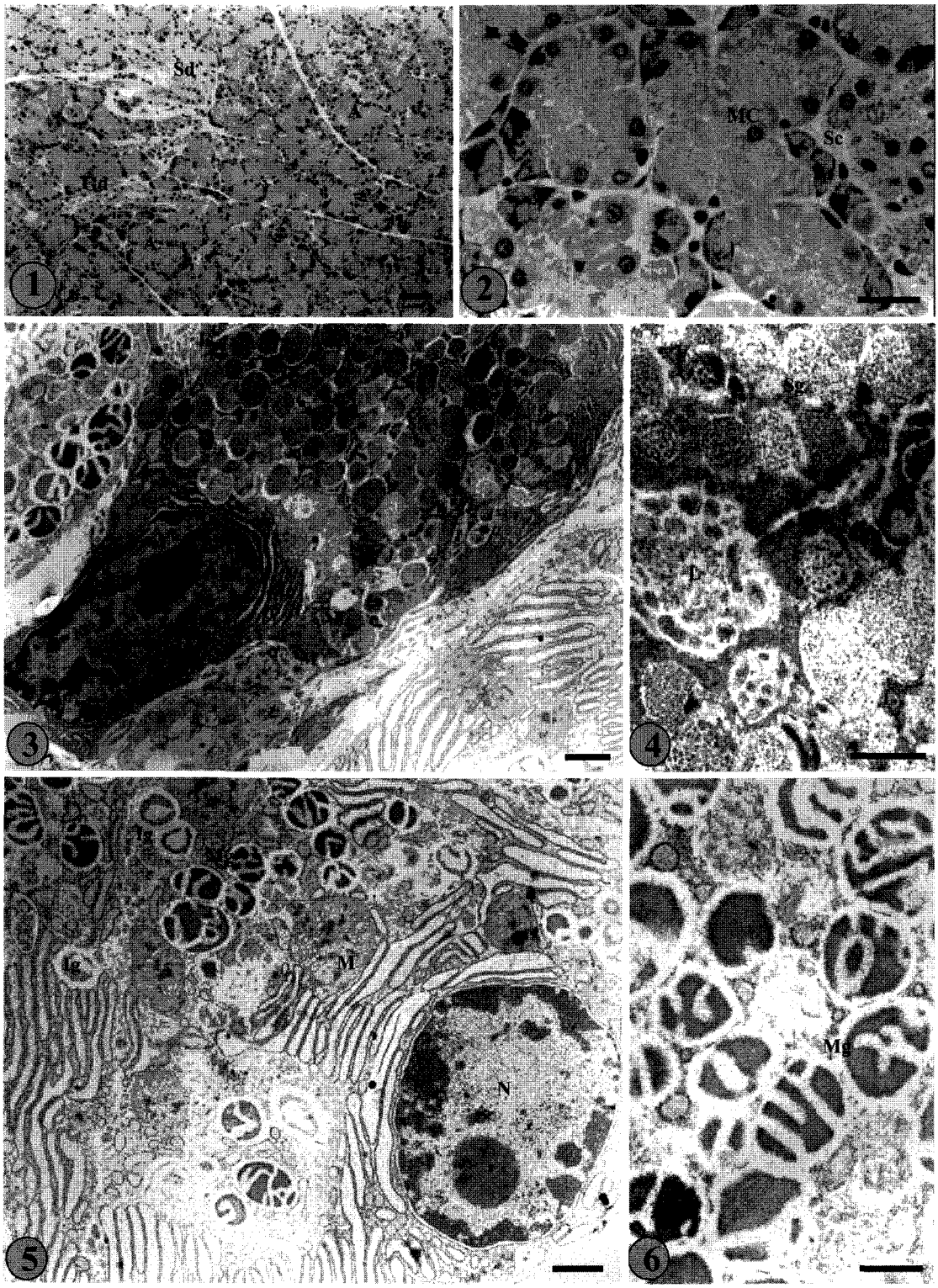
Fig. 6. Higher magnification of the mucous acinar granules (Mg) in submandibular gland. These oval mucous granules also have no distinct limiting membrane on the border. Mucous granules have a variety of pattern with several thin or transparent bands into the homogeneous dense matrix. Scale bar=0.05 μm.

Fig. 7. Electron micrograph of the granular duct cell in the submandibular gland. The cytoplasm contains many mitochondria (M), large amount of small granular vesicles (Gv) and several characteristic structures (Cs) of granule. L, lumen; N, nucleus. Scale bar=0.1 μm.

Fig. 8. Electron micrograph of various shaped and well developed mitochondria (M) and characteristic structures (Cs) of granule contained serous-like granules (G) in the granular duct cell. Gv, granular vesicles; N, nucleus. Scale bar=0.1 μm.

Fig. 9. Higher magnification of characteristic structures (Cs) of granule. Inside is filled with coarse serous-like granules (G) and outside is covered with stratified limiting membranes which is not connected. Small granular vesicles (Gv) are surrounded by free ribosome (Fr) on all sides. Scale bar=0.05 μm.

Fig. 10. Higher magnification of a complete characteristic structure (Cs) having homogenous matrix. Scale bar=0.05 μm.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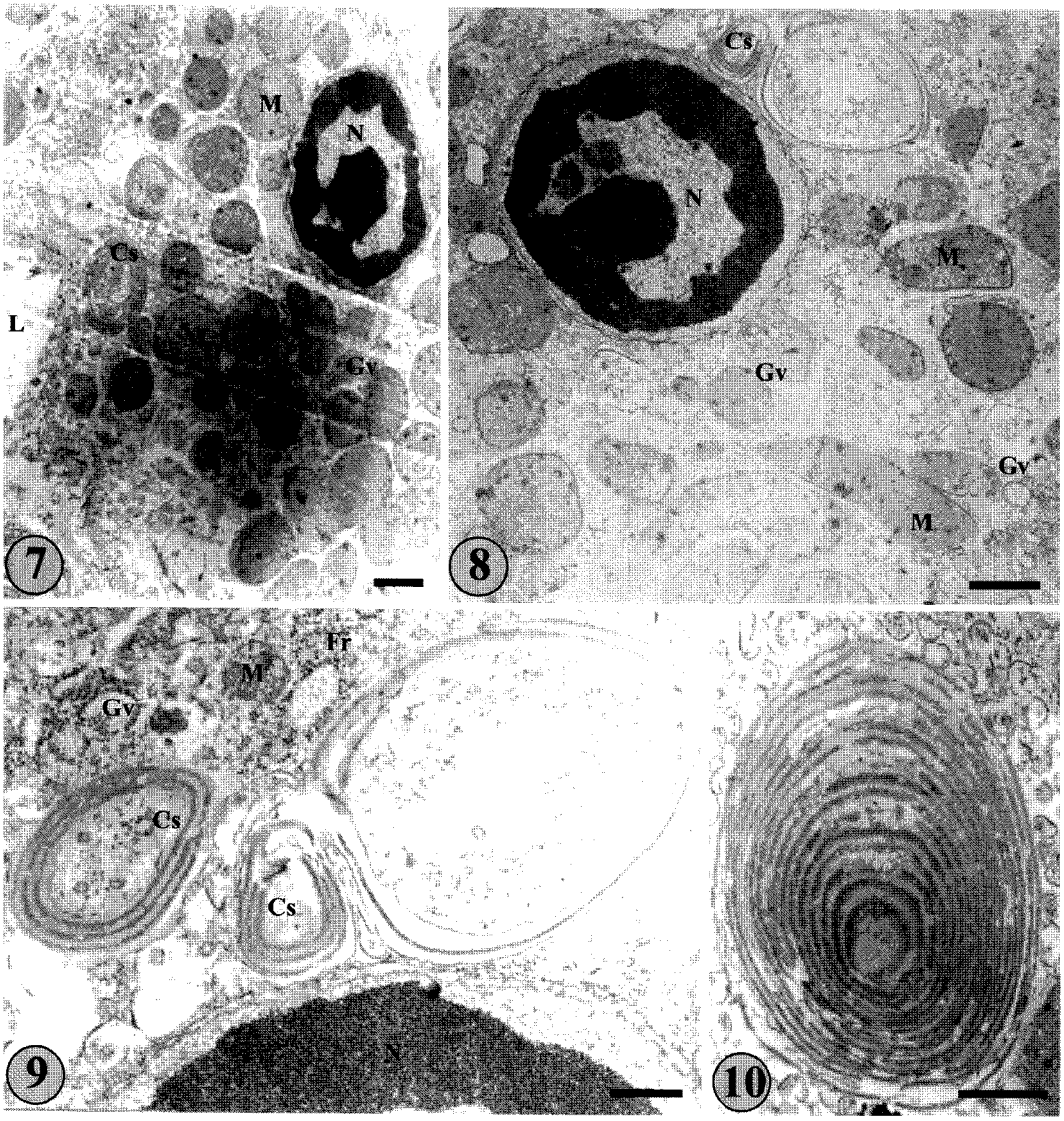
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