한국전자현미경학회지 제 35권 제 2호 2005
Korean J. Electron Microscopy 35(2), 65～72(2005)

Ultrastructure of the Submandibular Gland in the Lesser White-Toothed Shrew, *Crocidura suaveolens*

Soon-Jeong Jeong, Moon-Jin Jeong, Do Kyung Kim[1], Joong-Ki Kook[2], Heung-Joong Kim[3], Myung-Hee Yoon[4] and Joo-Cheol Park*
Department of Oral Histology, [1]Oral Physiology, [2]Oral Biochemistry,
[3]Oral Anatomy, College of Dentistry, Chosun University, Gwangju, 501-759, Korea;
[4]Division of Natural Sciences, College of Sciences, Kyungsung University, Busan, 608-736, Korea
(Received May 4, 2005; Accepted May 20, 2005)

작은땃쥐 *Crocidura suaveolens* 악하선의 미세구조

정순정, 정문진, 김도경[1], 국중기[2], 김흥중[3], 윤명희[4], 박주철*
조선대학교 치과대학 구강조직학교실, [1]구강생리학교실,
[2]구강생화학교실, [3]구강해부학교실, [4]경성대학교 자연과학대학 자연과학부

ABSTRACT

The ultrastructure of submandibular gland was examined in the lesser white toothed shrew, *Crocidura suaveolens*.

The submandibular gland of *C. suaveolens* was a mixed gland composed of serous and mucous acinar cells. Secretory granules from the acini were discharged through the salivary ducts into the oral cavity.

Serous and mucous acinar cells had well developed rough endoplasmic reticulum and mitochondria and large amount of granules. In case of serous acinar granules, an immature granule was formless and had only dense specks, and a matured granule was a complete round type delimiting by a single membrane and had a homogeneous dense center with dense specks on the border. In case of mucous acinar granules, while an immature granule was a round type and had an only homogeneous matrix and an indistinct limiting membrane, a mature granule was an even round type having a variety of pattern with several dense bands into the homogeneous matrix and had a distinct membrane. Therefore, a mature mucous acinar granule of *C. suaveolens* was not only distinct from those of the other mammalian species to have a variety of pattern but also from those of *C. lasiura* to have an even round type. A great serous like secretory granules and Myelin like body were observed in the cytoplasm and lumen of granular duct cells. Myelin like body, a characteristic structure only reported in salivary gland of three shrews, was discharged from secretory cell into lumen by the manner of exocytosis which has little differences from discharging manner of secretory granules.

Key words: *Crocidura suaveolens,* Mucous acinar granule, Myelin like body, Submandibular gland, Ultrastructure

This work was supported by research funds from Chosun University, 2004.
*Correspondence should be addressed to Dr. Joo-Cheol Park, Department of Oral Histology, College of Dentistry, Chosun University, Gwangju, 501-759, Korea. Ph.: 062-230-6874, FAX: 062-224-3706, E-mail: jcapark@chosun.ac.kr

INTRODUCTION

Mammalian salivary glands are a highly complex organ associated with mucosa of the oral cavity, and interspecies ultrastructural differences between the homologous cells in this organ represent the potential value of the possible evolutionary significance at cellular level (Hand, 1980; Phillips & Tandler, 1987; Phillips et al., 1987a, b, 1993; Tandler & Phillips, 1993; Tandler et al., 1990, 1994). Despite the versatility of this organ, studies on salivary glands have been restricted largely to a few commonly available laboratory animals (Tandler et al., 1990). However ultrastructural differences between homologous cells in salivary glands 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), recently this organ has been examined in various species (Hand, 1980; Phillips & Tandler, 1987; Phillips et al., 1987a, b, 1993; Tandler & Phillips, 1993; Tandler et al., 1990, 1994).

Insectivora including Soricidae is a primitive mammalian group, thought to be ancestors to many groups of mammals, exhibiting characteristics of specific interest for the study of mammalian evolution (Eisenberg, 1981; Churchfield, 1990; Carson & Rose, 1993). Soricidae is generally considered to comprise two subfamilies, Soricidae and Crocidurinae (Jones & Johnson, 1960; Won, 1967; Churchfield, 1990). Crocidurinae inhabiting Korea is composed of three species, *C. suaveolens*, *C. dsinezumi* and *C. lasiura* (Jones & Johnson, 1960; Won, 1967). There are studies on salivary glands in a few member of Soricidae (Raynaud, 1964; Mineda, 1981, 1985), recently my colleagues and I reported a study on the submandibular gland of *Crocidura lasiura* inhabiting Korea (Jeong et al., 2005). However, there is not at all study on the salivary glands in *C. suaveolens*.

The aim of the present study on the ultrastructure of the submandibular salivary gland in the lesser white-toothed shrew, *C. suaveolens* inhabiting Korea, is to determine ultrastructure and characteristics of submandibular gland cells and secretory granules, to compare these features to the other species and *C. lasiura*.

MATERIALS AND METHODS

Seven adult males of *C. suaveolens* were collected at October 1999 and March, April, July and September 2000 from Mt. Jiri using the Sherman live traps.

To observe ultrastructure of the submandibular gland, 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 microscopy. Thin sections (60～90 nm) were double stained with uranyl acetate and lead citrate. All of the thin sections were examined with a JEOL 100S transmission electron microscope.

RESULTS

The submandibular gland of the lesser white-toothed shrew, *C. suaveolens* was examined with a light and electron microscopy.

From the observation of the microscopic specimens, a submandibular gland of *C. suaveolens* was composed of acini and salivary ducts (Fig. 1). A submandibular acinus was a mixed gland composed of serous and mucous cells, secreted granules from the acinus were discharged through the salivary ducts into the oral cavity.

From the observation of ultrastructure of acinar cells in the submandibular gland, serous cell had well developed rER (rough endoplasmic reticulum) and mitochondria, and large amount of serous acinar granules

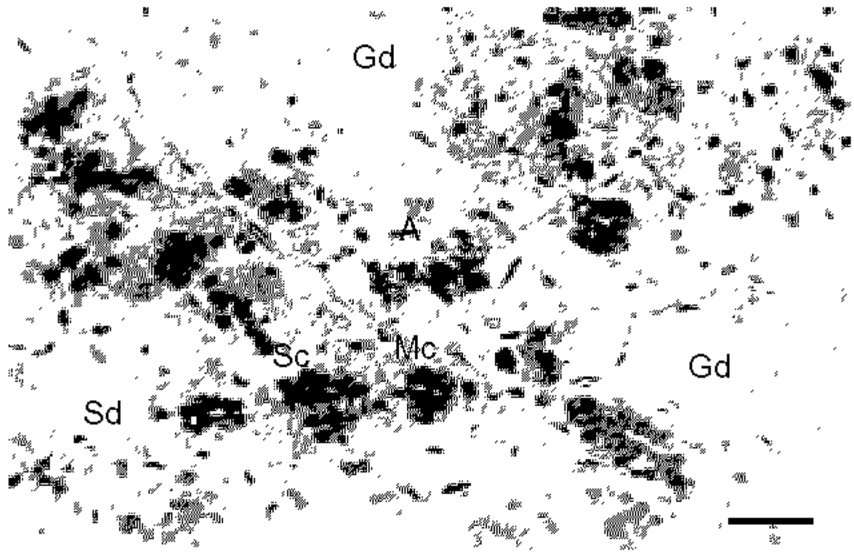

Fig. 1. Light micrograph of the submandibular gland. Acinus (A) composing serous (Sc) and mucous (Mc) cells contain numerous secretory granules. Granular duct (Gd) cells also contain great secretory granules. Sd, striated duct. Scale bar = 1 μm.

with various stages of maturing process (Fig. 2). An immature serous granule of early stage was formless and it had only minute dense specks, according as granules progress, it had became a round type and had a homogeneous dense center. Finally, a matured serous acinar granule was a complete round type delimiting by a single membrane and had a homogeneously dense center with dense specks on the border (Fig. 3). Mucous cells also had well developed rER, mitochondria and large amount of mature and immature round mucous granules (Fig. 4). While an immature mucous acinar granule had a homogeneous matrix and an indistinct limiting membrane, a mature mucous acinar granule had a variety of pattern with several dense bands into a homogeneous matrix and a distinct limiting membrane (Fig. 5). Granular duct cells had great serous-like secretory granules with dense specks and Myelin-like body which is a manifold form of membrane in the cytoplasm (Fig. 6). Secretory granules and Myelin-like body of the granular duct cell were discharged from secretory cell into lumen by the manner of exocytosis (Fig. 7). Namely, droplet and Myelin-like body moved to the luminal border of the cell and the outermost membrane of such droplet and Myelin-like body fused with the plasma membrane. In case of droplet, these fused membranes had disappeared and the content of the droplet flowed into lumen. However, in case of Myelin-like body, fused membrane had not disappear and flowed into lumen.

DISCUSSION

Matured serous acinar granules of the lesser white-toothed shrew, *Crocidura suaveolens* were similar to those of the other mammal species including *C. lasiura* to have a single membrane and a homogeneous center with dense specks on the border. However, the other characteristics in serous acinar granules of *C. suaveolens* which have a complete round type and a dense center, were different from those of *C. lasiura* having an oval type and a pale center (Jeong et al., 2005). Also, matured

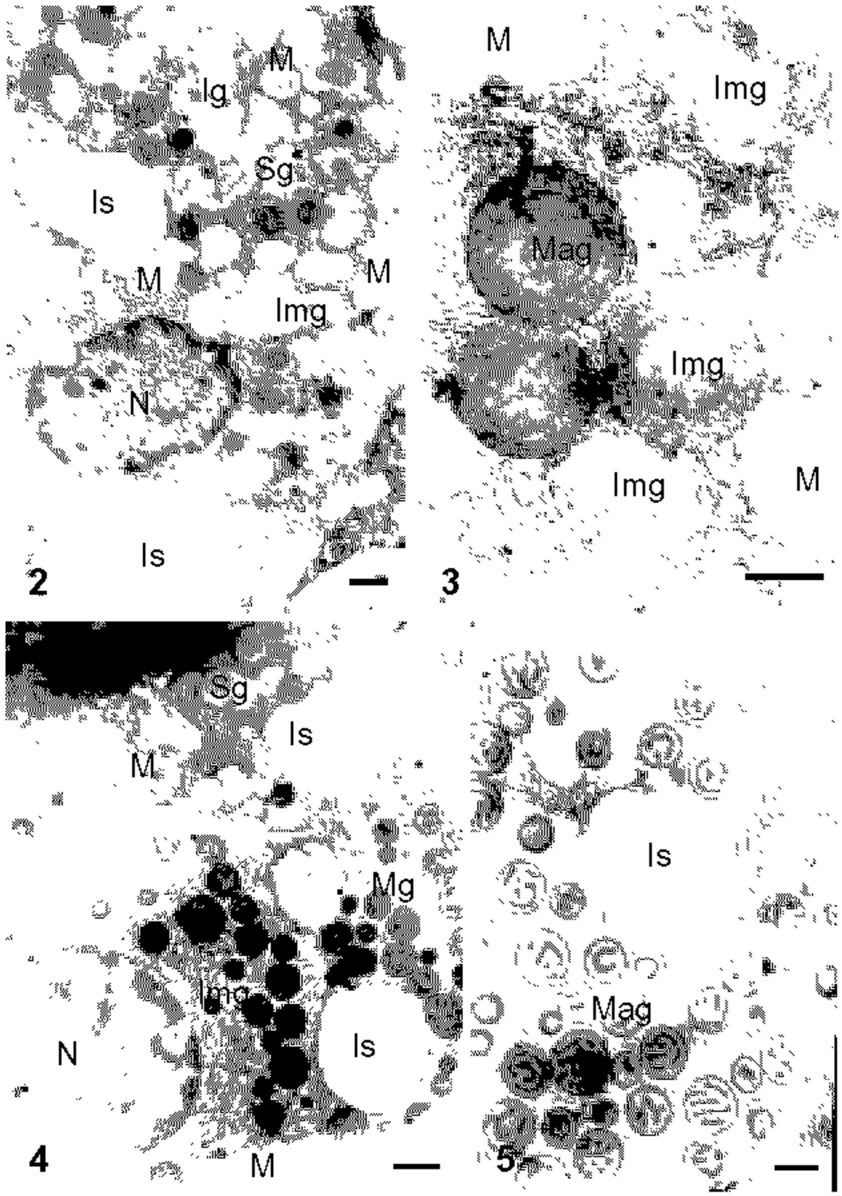

Fig. 2. Electron micrograph of the serous acinar cell in the submandibular gland. The cytoplasm contains well developed rER and mitochondria (M), and large amount of serous acinar granules (Sg) with various stages of the maturing process. Img, immature granule; Is, intercellular space; N, nucleus. Scale bar = 0.1 μm.

Fig. 3. Higher magnification of the serous acinar granules (Sg) in the submandibular gland. According to the maturing process, a formless immature granule (Img) has became a round type, and has become to have a complete homogeneous round dense center and a distinct limiting membrane on the border. M, mitochondria; Mag, mature granule. Scale bar = 0.05 μm.

Fig. 4. Electron micrograph of the mixed acinus composing mucous and serous acinar cells in submandibular gland. The cytoplasm of the mucous and serous acinar cells contains well developed rER, mitochondria (M) and large amount of granules. Img, immature granule; Is, intercellular space; Mg, mucous granule; N, nucleus; Sg, serous granule. Scale bar = 0.1 μm.

Fig. 5. Electron micrograph of the mature mucous acinar granules (Mag) in submandibular gland. These round granules have a variety of pattern with several dense bands into the homogeneous dense matrix. Is, intercellular space. Scale bar = 0.05 μm.

Fig. 6. Higher electron micrograph of the granular duct cell in the submandibular gland. The cytoplasm contains great serous-like secretory granules (G) with limiting membrane and Myelin-like body (Mb). N, nucleus. Scale bar = 0.1 μm.

Fig. 7. Electron micrograph of great secretory granules (G) and Myelin-like body (Mb) showing the exocytosis processing in the granular duct. The secreted Myelin-like body (SMb) are also seen in the lumen (L) of the granular duct. Scale bar = 0.1 μm.

mucous acinar granules of *C. suaveolens* having a variety of pattern with several dense bands into the homogeneous matrix, were similar to *C. lasiura* which is distinct from the other mammalian species. However, the other characteristics of mucous acinar granules in *C. suaveolens* which have an even round type, were different from those of *C. lasiura* having an uneven type (Jeong et al., 2005). It is considerable that interspecies variation of granule ultrastructure exported by homologous salivary acinar cells (Ball, 1993; Tandler et al., 1994). Because the secretory process appears to be widely conserved ranging from yeast to many in mammals (Rothman & Orci, 1992), this striking interspecies variation in secretory granules implies differences in post-translational modification of relatively small number of basic products (Levine et al., 1987; Tandler et al., 1990). Finally, interspecies 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., 1993), diet (Phillips et al., 1987b; Tandler et al., 1990) and species isolation (Nagato et al., 1984). Although we cannot say precisely which factors were correlated with ultrastuctural differences of salivary acinar granules between *C. suaveolens* and *C. lasiura* or *C. suaveolens* and the other mammalian species, it is clear a variety of pattern with several dense bands into the homogeneous matrix in mucous acinar granules is a characteristic of *C. suaveolens* and *C. lasiura* belong to Crocidurinae. Thus, a variety of pattern in mucous acinar granules of *C. suaveolens* and *C. lasiura* might be used with a key which is classified from the other mammalian species.

A great serous−like secretory granules and Myelin−like body were observed in the cytoplasm and the lumen of granular duct cells in *C. suaveolens*. A great secretory granule in *C. suaveolens* was different from relatively small secretory granule in *C. lasiura*, but granules both of two species were discharged into the lumen by the same manner of exocytosis. Myelin−like body, only reported in the salivary glands of three shrews, *Suncus murinus* and *C. dsinezumi* (Mineda, 1985), and *C. lasiura* (Jeong et al., 2005), is a manifold form of membranes. It is considered a formation at the basal cytoplasm of the granular duct cell and discharge into lumen by the secretory manner of granules, exocytosis (Mineda, 1985). In this study, Myelin−like body moved to the luminal border of the cell and the outermost membrane of Myelin−like body fused with the plasma membrane, and then the fused membrane had not disappear and flowed into lumen. The secretory manner of Myelin−like body had a little difference in those of granules. Thus the manner of exocytosis of Myelin−like body in the granular duct of salivary gland was confirmed in this study.

ACKNOWLEDGEMENT

This works was supported by research funds from Chosun University, 2004.

REFERENCES

Ball WD: Cell restricted secretory proteins as markers of cellular phenotype in salivary glands. In Dobrosielski Vergona K (ed) Biology of the salivary glands. CRC Press Boca Raton pp. 355 396, 1993.

Carson KA, Rose RK: Fine structure of the submandibular salivary gland of the Venomous short tailed shrew, *Blarina brevicauda* (Insectivora: Soricidae). European J Morph 31 : 111 128, 1993.

Churchfield S: The natural history of Shrews. A&C black Ltd London, 1990.

Eisenberg JF: The mammalian radiations an analysis of trends in evolution, adaptation and behavior. University of Chicago Press Chicago IL 1981.

Hand AR: Salivary glands. In Bhaskar SN (ed) Orban's oral histology and embryology. Mosby St Louis pp. 336 370, 1980.

Jeong SJ, Lim DS, Park JC, Kim HJ, Jeong JO, Choi BD, Jeong MJ: Ultrastructure of the submandibular gland in the big white toothed shrew, *Crocidura lasiura*. Korean J Electron Microscopy, 2005. (In Press)

Jones JK, Johnson DH: Review of the Insectivores of Korea. Univ Kansas Publ Mus Nat Hist 9 : 551 578, 1960.

Levine MJ, Reddy MS, Tabak LA, Loomis RE, Bergey EJ, Jones PC, Choen RE, Stinson MW, Al Hashimi I: Structural aspect of salivary glycoproteins. J Dental Res 66 : 436 441, 1987.

Mineda T: Histological and histochemical investigations on the salivary glands of the black and refous elephant shrew (*Rhynchocyon chrysopygus*). J Dent Aichi Gakuin univ 19 : 78 85, 1981.

Mineda T: Fine structure of salivary gland and cytomegalovirus of the House musk shrew, *Suncus murinus*. In Oda S, Kitoh J, Ota K and Isomura G (eds.) *Suncus murinus* Biology of the laboratory shrew. Japan scientific societies press (JSSP) pp. 370 388, 1985.

Nagato T, Tandler B, Phillips CJ: Unusual smooth endoplasmic reticulum in submandibular acinar cells of the male round eared bat, *Tonatia sylvicola*. J ultrastruct Res 87 : 275 284, 1984.

Phillips CJ, Tandler B: Mammalian evolution at the cellular level. Current mammalogy 1 : 1 66, 1987.

Phillips CJ, Nagato T, Tandler B: Comparative ultrastructure and evolutionary patterns of acinar secretory product of parotid salivary glands in Neotropical bats. Zoology 39 : 213 229, 1987a.

Phillips CJ, Tandler B, Pinkstaff CA: Uniques salivary glands in two genera of tropical microchiropteran bats An example of evolutionary convergence in histology and histochemistry. J Mammal 68 : 235 242, 1987b.

Phillips CJ, Tandler B, Nagato T: evolutionary diversity of salivary gland acinar cells a format for understanding molecular evolution. In Dobrosielski Vergona K.(ed) The biology of the salivary glands. CRC Press Boca Raton pp. 41 80, 1993.

Raynaud J: Dimorphism sexuel de la gland sous maillarie chez la Musaraigne (Crocidura). Compte Rendu soc biol 158 : 942 947, 1964.

Rothman JE, Orci L: Molecular dissection of the secretory pathway. Nature 355 : 409 415, 1992.

Tandler B, Phillips CJ: Structure of serous cells in salivary glands. Microscopy Res Tech 26 : 32 48, 1993.

Tandler B, Phillips CJ, Nagato T, Toyoshima K: Ultrastructural diversity in chiropteran salivary glands. In Riva A and Motta PM (eds) Ultrastructure of the Extrapariental glands of the Digestive tract. Kluwer academic publishers pp. 31 52, 1990.

Tandler B, Phillips CH, Pinkstaff CA: Mucous droplets with multiple membranes in the accessory submandibular glands of Long winged bats. Anat Rec 240 : 178 188, 1994.

Won PH: Illustrated encyclopedia and fauna and flora of Korea. Vol 7. Korea Ministry of education pp. 259 283, 1967.

＜국문초록＞

악하선의 미세구조를 작은땃쥐 *Crocidura suaveolens*에서 연구하였다. 작은땃쥐의 악하선은 장액선세포와 점액선세포로 구성된 혼합샘이었다. 이 샘포에서 분비된 과립들은 도관을 거쳐 구강으로 분비되었다.

장액선세포와 점액선세포는 잘 발달된 조면소포체와 미토콘드리아 그리고 많은 분비과립을 가지고 있었다. 장액선 분비과립의 경우, 미성숙 분비과립은 무형이면서 전자밀도가 있는 작은 알갱이로만 구성되었고, 성숙 과립은 단일막으로 싸여진 완전한 원형으로 전자밀도가 있는 균질의 중앙부와 전자밀도가 있는 작은 알갱이로 구성된 주변부를 가지고 있었다. 점액선 분비과립의 경우, 미성숙 과립은 원형으로 균질한 기질과 불명확한 경계막을 가지는 반면, 성숙 과립은 균질한 기질 내에 몇 개의 전자밀도가 있는 띠를 가짐으로서 문양의 다양성 가지는 매끈한 원형이었고 명확한 경계막을 가지고 있었다. 즉 작은 땃쥐의 성숙 점액선 분비과립은 문양의 다양성을 가져 다른 포유류와 구분될 뿐만 아니라 매끈한 원형이어서 *C. lasiura*의 그것과도 구분되었다. 거대한 분비 과립과 미엘린소체가 과립관세포의 세포질과 내강에서 관찰되었다. 3종의 땃쥐류 침샘의 과립관에서 만 보고된 특징적 구조물인 미엘린소체는 분비세포에서 내강으로 분비되었으며 분비과립의 배출방식과 약간의 차이점을 가졌다.