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Comparative Ultrastructure of the Acinar Cell and Secretory Granules of Parotid Salivary Gland in the Lesser White−toothed Shrew, *Crocidura suaveolens* and the Big White−toothed Shrew, *C. lasiura*

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작은땃쥐 *Crocidura suaveolens*와 우수리땃쥐 *C. lasiura*의 이하선 선세포와 분비과립의 미세구조 비교

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ABSTRACT

The acinar cells and secretory granules of the parotid salivary gland were examined in the lesser white toothed shrew, *Crocidura suaveolens* and the big white toothed shrew, *C. lasiura*.

The parotid gland of both species were a serous gland having only one kind of serous acinar cells, and had conventional arrangement of acini and intercalated, granular and striated ducts.

In case of *C. suaveolens*, serous acinar cells had well developed rER, prominent Golgi complex, several large mitochondria and abundant moderate dense secretory granules with various stages of the maturing or fusing process. Immature acinar secretory granules were only or mainly filled with fine strong dense specks and had an indistinct limiting membrane, and mature granules were filled with homogeneous pale large round center and had fine strong dense specks at the periphery of the homogeneous pale center and a distinct limiting membrane. In case of *C. lasiula*, serous acinar cells had well developed rER, prominent Golgi complex, several large mitochondria and abundant dense secretory granules with maturing or fusing process. Immature acinar secretory granules were only filled with pale rough specks and had an indistinct limiting membrane, and mature granules were only filled with rough dense specks and had a distinct limiting membrane. Eventually The acinar secretory granules of *C. suaveolens* were seen moderate at the light and ultrastuctural level, those of *C. lasiura* were strong dense at the light microscopic level and dense at the ultrastructural level.

Key words : *Crocidura lasiura, Crocidura suaveolens,* Parotid salivary gland, Serous acinar granule, Ultrastructure

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INTRODUCTION

Mammalian salivary glands are a highly complex organ, that are associated with mucosa of the oral cavity and secrete digestive enzymes, hormones, antibodies and so forth (Hand, 1980; Tandler, 1972), and interspecific ultrastructural differences between the homologous cells in this organ represent the potential value of the possible evolutionary significance at cellular level (Phillips & Tandler, 1987; Phillips et al., 1987a,b, 1993; Tandler & Phillips, 1993; Tandler et al., 1988, 1990, 1994), and 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 (Phillips & Tandler, 1987; Phillips et al., 1987a,b, 1993; Tandler & Phillips, 1993; Tandler et al., 1988, 1990, 1994).

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 (Carson & Rose, 1993; Churchfield, 1990; Eisenberg, 1981). Soricidae is generally considered to comprise two subfamilies, Soricinae and Crocidurinae (Churchfield, 1990; Jones & Johnson, 1960; Won, 1967). Crocidurinae inhabiting Korea is composed of three species, *C. suaveolens*, *C. dsinezumi* and *C. lasiura* (Jones & Johnson, 1960; Won, 1967). Although Soricidae has received attention as ancestor to many groups of mammals for the study of the mammalian evolution, there are only a few studies on ecology and histology. Especially, Crocidurinae inhabiting in Korea recently has been studied (Han, 1997; Jeong & Yoon, 2001; Jeong et al., 2005a, b; Yoon & Jeong, 2001) with exception of some brief comments and there is not at all study on parotid salivary gland.

By the present comparative study on the parotid salivary gland in the lesser white-toothed shrew, *C. suaveolens* and the big white-toothed shrew, *C. lasiura*, we determined ultrastructure and characteristics of parotid gland cells and acinar secretory granules, and compared these features to the other small mammalian species.

MATERIALS AND METHODS

Crocidura suaveolens (3 females, 7 males) were collected at October 1999 and March, April, July and September 2000, and *C. lasiura* (5 females, 7 males) of ten adults were collected at June, October and November 1999 and March and April 2000 from Mt. Jiri with the Sherman live traps.

One parotid salivary gland from the sacrificed *C. suaveolens* and *C. lasiura* was dissected in 0.74% saline (physiological saline solution), and fixed in the 4% formaldehyde (phosphate buffer, pH 7.2) for 12 hr. the specimens were dehydrated with a series of the graded ethyl alcohol and embedded in Paraplast (Sherwood Medical Industries, St. Louis). The blocks were cut 6～7 μm sections. Sections were stained with Hematoxylin-Eosin and examined with a light microscopy. The other was fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in Millonig's phosphate buffer (pH 7.4) for 1 hr. The 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. Thin sections (60～90 nm) were double stained with uranyl acetate and lead citrate, and then examined by a JEOL 100S transmission electron microscope.

RESULTS

The acinar cells and secretory granules of the parotid salivary gland were examined in the lesser white toothed shrew, *Crocidura suaveolens* and the big white-toothed shrew, *C. lasiura*.

At the light microscopic level, the parotid salivary gland of both species were a serous gland having only one kind of acinar cells, and had conventional arrangement of acini and intercalated, granular and striated ducts, were filled with abundant secretory granules (Figs. 1, 2). In case of *C. suaveoles*, serous acinar secretory granules had moderate dense and mainly were at the periphery of the nucleus which located mainly in the cener or sometimes in the base of the cell (Fig. 1). In case of *C. lasiura*, granules had strong dense and mainly were at the apical region of the cell. The nucleus located in base or sometimes in the center of the cell (Fig. 2).

At the ultrastructural level, in case of the polygonal serous acinar cell of *C. suaveolens*, the nucleus was located in the middle of the cell. The surrounding cytoplasm of the nucleus contained well developed rER, prominent Golgi complex, several large mitochondria and abundant moderate dense secretory granules with various stages of the maturing or fusing process (Fig. 3). Immature acinar secretory granules were only or mainly filled with fine strong dense specks and had an indistinct limiting membrane, and mature granules were filled with homogeneous pale large round center that is the majority of the acinar secretory granule and had fine strong dense specks at the periphery of the homogeneous pale center and a distinct limiting membrane (Fig. 5). In case of *C. lasiula*, the nucleus was located in the base of the cell. The basal cytoplasm contained well developed rER, prominent Golgi complex and several large mitochondria. The apical cytoplasm was filled with abundant dense secretory granules with maturing or fusing process (Fig. 4). Immature acinar secretory granules were only filled with pale rough specks and had an indistinct limiting membrane, and mature granules were only filled with rough dense specks and had a distinct limiting membrane (Fig. 6).

Eventually, The acinar secretory granules of *C. suaveolens* were seen moderate at the light microscopic level and ultrastructural level, because it had a homogeneous large pale center that is majority of the acinar secretory granule, although it had strong dense specks at the periphery of the pale center. While those of *C. lasiura* were seen strong dense at the light microscopic level and dense at the ultrastructural level, because it had only rough dense specks although density of specks was poor than density of fine specks of *C. suaveolens* locating in periphery of the pale center.

DISCUSSION

Electron dense serous images can be correlated in general with granules rich in enzymes. Such an image is typical of many laboratory rodents and primates for which data are available on granule enzymes and other proteins (Ball, 1993; Jacobsen & Hensten−Pettersen, 1974; Robinoritch & Sreebny, 1969; Tandler et al., 1988; Wallach et al., 1975). Also comparative data in the secretory granules of parotid acinar cells of bats suggest that insectivorous and animalivorous species have enzyme−rich, electron−dense granules, whereas frugivorous have enzyme−poor, pale secretory granules (Phillips et al., 1987a; Tandler et al., 1988). The electron density of parotid serous secretory granules of *C. suaveolens* and *C. lasirua* are similar to the already reported other shrews (Mineda, 1981, 1985) but are low than those of insectivorous bats, and *C. suaveolens* has been shown to moderate dense than those of *C. lasiura*. It present that the parotid serous secretory granules of shrew contain little enzyme than those of insectivorous bats and those of *C. suaveolens* contain little enzyme than those of *C. lasiura*.

Because the secretory process appears to be widely conserved ranging from yeast to many in mammals (Rothman & Orci, 1992), striking interspecific variation in secretory granules from homologuous secretory cells implies differences in post−translational modification of relatively small number of basic products (Levine et al., 1987; Tandler et al., 1990). Finally, the interspecific variations in granule ultrastructure might be not only indicate of functional differences in saliva (Levine et al., 1987; Tandler et al., 1990) but also correlate 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). The mature parotid acinar secretory granules of *C. suaveolens* were filled with homogeneous pale large round center and had fine strong dense of specks at the periphery of the homogeneous pale center. Those of *C. lasiura* were only filled with rough dense specks. The parotid acinar secretory granules of *C. suaveolens* were different from those of *C. lasira* and submadibular seorus acinar secretory granlues of *C. suaveolens* (Jeong et al., 2005b) but were similar to submandibular serous acinar secretory granules of *C. lasiura* (Jeong et al., 2005a). Also the parotid acinar secretory granules of *C. lasiura* were distinct from the other granules of parotid and submadibular gland (Jeong et al., 2005a, b) witrhout homogeneous part in granules. At present it impossible to correlate exactly which chemical components with ultrastructural differences and density in secretory granulels. However it is clear that the nature and electron dense of the parotid acinar secretory granules must relate to these various diets and its occureences is significant to comparision among mammalian species including shrews.

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＜국문초록＞

이하선의 선세포와 분비과립을 작은땃쥐 *Crocidura suaveolens*와 우수리땃쥐 *C. lasiura*에서 연구하였다.

두 종 모두의 이하선은 장액선세포 한 종류만을 가지는 장액선이었고 선세포, 사이관, 과립관 그리고 줄무늬관들은 평범하였다. 작은땃쥐의 경우, 장액선세포는 잘 발달된 조면소포체, 현저한 골지체, 몇몇의 큰 미토콘드리아와 성숙 혹은 융합하고 있는 많은 중간 정도의 전자 밀도 과립들을 가지고 있었다. 미성숙 선세포의 분비과립들은 미세하고 강한 전자 밀도의 알갱이만으로 혹은 알갱이들이 주가 되었고 불분명한 경계막을 가지고 있었고, 성숙 분비과립들은 균일하고 옅은 중심부를 가지고 그 주변에 미세하고 강한 전자밀도의 알갱이들과 명확한 경계막을 가지고 있었다. 우수리땃쥐의 경우, 선세포는 잘 발달된 조면소포체, 현저한 골지체, 몇몇의 큰 미토콘드리아 뿐 아니라 성숙 혹은 융합하고 있는 많은 진한 전자 밀도의 분비과립들을 가지고 있었다. 미성숙 선세포 분비과립들은 옅고 거친 알갱이들만으로 채워져 있었으며 불명확한 경계막을 가지고 있었고, 성숙 분비과립들은 진하고 거친 알갱이들만으로 채워져 있었고 명확한 경계막을 가지고 있었다. 결국, 작은땃쥐의 이하선 분비과립은 광학과 전자현미경 수준에서 중간 정도의 전자밀도로, 우수리땃쥐의 분비과립은 광학 현미경 수준에서는 아주 진하게, 전자현미경에서는 진하게 관찰되었다.

FIGURE LEGENDS

Fig. 1. Light micrograph of the parotid salivary gland of *C. suaveolens*. Acini (A) are composed of one kind of cells, serous acinar cells. Acinar cells are filled with abundant moderate dense secretory granules. Gd, granular duct; Sd, striated duct. Scale bar = 100 μm.

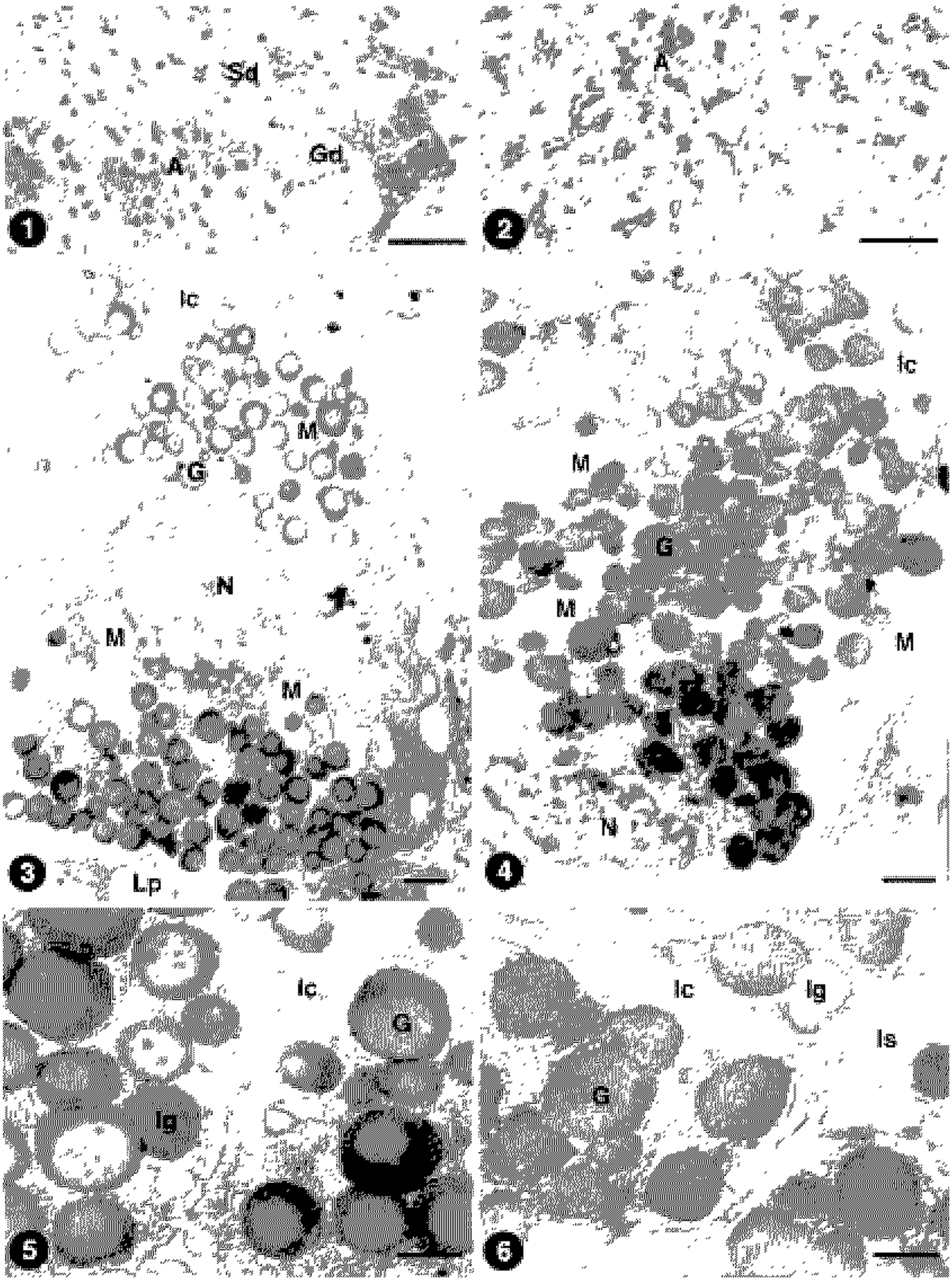
Fig. 2. Light micrograph of the parotid salivary gland of *C. lasiura*. Acini composing serous acinar cells are filled with strong dense secretory granules. Scale bar = 100 μm.

Fig. 3. Electron micrograph of the serous acinar cell of the parotid salivary gland in *C. suaveolens*. The nucleus is located in the middle of the cell. The surrounding cytoplasm of the nucleus contains well developed rER, prominent Golgi complex, several large mitochondria (M) and abundant moderately dense secretory granules (G) with various stages of the maturing or fusing process. Intercellular canaliculi (IC) are present between acinar cells. Lp, lipid droplet. Scale bar = 1 μm.

Fig. 4. Electron micrograph of the serous acinar cell of the parotid salivary gland in *C. lasiura*. The nucleus (N) is located in the base of the cell. The basal cytoplasm contains well developed rER, prominent Golgi complex, several large mitochondria (M). The apical cytoplasm is filled with abundant dense secretory granules (G) with maturing or fusing process. Ic, intercellular canaliculi. Scale bar = 1 μm.

Fig. 5. Higher magnification of the serous acinar secretory granules of the parotid salivary gland in *C. suaveolens*. Immature granules (Ig) are only or mainly filled with fine strong dense specks and had a indistinct limiting membrane. Fusing immature granules also are seen. Mature granules (G) are filled with homogeneous pale large round center and had fine strong dense specks at the periphery of the homogeneous pale center and a distinct limiting membrane. Ic, intercellular canaliculi. Scale bar = 0.5 μm.

Fig. 6. Higher magnification of the serous acinar secretory granules of the parotid gland in *C. lasiura*. Immature granules (Ig) are only filled with pale rough specks and had a indistinct limiting membrane. Mature granules (G) are only filled with rough dense specks and had the distinct limiting membrane. Ic, intercellular canaliculi; Is, intercellular space. Scale bar = 0.5 μm.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