INTRODUCTION

Soricidae, belonging to Insectivora, is a primitive mammalian group that is believed to be ancestral to many groups of mammals, and exhibits the characteristics of specific interest for a study of mammalian evolution (Eisenberg, 1981; Tsuchiya, 1985; Churchfield, 1990; Carson & Rose, 1992). Soricidae is generally considered to consist of two subfamilies, Soricinae and Crocidurinae. Soricinae includes red-toothed shrews (genus *Sorex*), and Crocidurinae includes white-toothed shrews (genus *Crocidura*) (Jones & Johnson, 1960; Won, 1967; Churchfield, 1990). Genus *Sorex* inhabiting Korea is believed to include two species, the little spider shrew, *S. minutus* and the Korean spider shrew, *S. caecutiens* (Korean
Society of Systematic Zoology, 1997). Genus Crocidura inhabiting Korea is composed of three species, the lesser white-toothed shrew, Crocidura suaveolens, Japanese white-toothed shrew, C. dinszumi and big white-toothed shrew, C. lasiura (Korean Society of Systematic Zoology, 1997). All species belonging to Soricidae are not only useful environmental indicators with sensitivity to pollutions (Diamond & Sherburne, 1969; Braham & Neal, 1974; Robert et al., 1978; Andrew et al., 1984), but are also an important mediator controlling the population of the invertebrates and small vertebrates in the ecosystem (Holling, 1959; Buckner, 1969; Churchill, 1990). All species belonging to Soricidae, however, are quite rare in the wild and their population has decreased.

Interspecies ultrastructural differences between homologous cells in the salivary glands hold much promise in systematic and comparative ultrastructural analysis, which can serve as a basis for molecular comparisons (Phillips & Tandler, 1987; Tandler et al., 1990). This organ has been examined in a range of species (Hand, 1980; Phillips & Tandler, 1987; Phillips et al., 1987a, 1987b; Tandler et al., 1990; Phillips et al., 1993; Tandler & Phillips, 1993; Tandler et al., 1994). In case of Soricidae, the salivary glands of a few members have been reported. These include the European water shrew, Neomys Fodiens (Schaffer, 1908), house shrew, Crocidura russula (Raynaud, 1964), black and Rusous elephant shrew, Rhynchocyon chrysopygus (Mineda, 1981), musk shrew, Suncus murinus (Mineda, 1985), tree shrew, Tupia glis (Suzuki et al., 1995), big white-toothed shrew, C. lasiura, (Jeong & Jeong, 2005; Jeong et al., 2005a), lesser white-toothed shrew, C. suaveolens, (Jeong & Jeong, 2005; Jeong et al., 2005b) and Korean spider shrew, Sorex caecutiens (Jeong et al., 2007).

This study examined the ultrastructural characteristics of the submandibular gland in the Korean Spider Shrew (S. caecutiens), the Lesser White-Toothed Shrew (C. suaveolens) and big white-toothed shrew (C. lasiura), particularly the newly reported characteristic serous acinar granule of C. lasiura, and compared these characteristics with those of previous studied shrews.

MATERIALS AND METHODS

Experimental Procedure

Two adult Korean spider shrews, Sorex caecutiens (1 female, 1 male) were collected in January and October 1999, ten adult lesser white-toothed shrews, Crocidura suaveolens (3 females, 7 males) were collected in October 1999 and March, April, July, and September 2000, and twelve adult big white-toothed shrews, Crocidura lasiura (5 females, 7 males) were collected in June, October and November 1999 and March and April 2000 from Mt. Jiri (Gyeongsangnam-do, South Korea) using Sherman live traps. All animal study was approved by the ‘Institutional Animal Care and Use Committees’ at the Chosun University, and animal care was carried out according to ‘Guide for the Care and Use of Laboratory Animals’. All captured animals were measured for body weight and lengths of head and body, tail, hind of foot and ear. Each specimen was autopsied.

To observe the ultrastructures of the submandibular gland, a pair of submandibular salivary glands was fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in Millonig’s phosphate buffer (pH 7.4) for 1 hr. The samples were post-fixed with 1.3% osmium tetroxide in the same buffer for 2 hr, dehydrated with a graded series of ethyl alcohol and acetone, and embedded in epoxy resin. Thick sections (0.5~1 μm) were stained with 5% toluidine blue for light microscopy. The stained tissues were observed by optical microscopy (Carl Zeiss, Oberkochen, Germany). Histological analysis was performed using Axio vision LE reased 4.6 (Carl Zeiss). Thin sections (60~90 nm) were double stained with uranyl acetate and lead citrate. The section was observed by transmission electron microscopy (JEOL 100S; JEOL Ltd., Tokyo, Japan) at 80 kV.

RESULTS

Sorex caecutiens

At the ultrastructural level, the pyramidal shaped mucous acinar cells were grouped in an acinus-like fashion (Fig. 1A). Well developed and numerous rough endoplasmic reticulum were arrayed in parallel arrays adjacent to the nucleus in the basal cytoplasm of the mucous acinar cell. Several mitochondria were scattered among the rough endoplasmic reticulum, and electron dense mucous acinar granules with various stages of maturing were located in the apical cytoplasm near the secretory canaliculi (Fig. 1A). The serous acinar cells were positioned as demilunes adjacent to the mucous acinar cells (Fig. 1B). The rough endoplasmic reticulum was perinuclear and dispersed more widely in the cytoplasm of the serous acinar cells. Few mitochondria were observed among the rough endoplasmic reticulum, and electron dense serous acinar granules were located the lateral cytoplasm near the secretory canaliculi (Fig. 1B). The mucous acinar granules had a border of lucid corona and several lucid bands into the homogeneous dense matrix producing a complex structure (Fig. 1C). The serous acinar granules had a border of lucid corona and matrix composed of only dense coarse specks (Fig. 1D). The granular duct cells had various shaped mitochondria and many serous like granules composing of specks (Fig. 1E). The granular duct cell also contained a myelin-like body composed of a layer of unit membranes with the center of homogeneous matrix (Fig. 1F).
The mucous acinar cells had the typical appearance of mucous cells, as well as well developed rough endoplasmic reticulum. Few mitochondria were scattered among the rough endoplasmic reticulum and electron dense mucous acinar granules were located in the cytoplasm (Fig. 2A).
Ultrastructure of Submandibular Gland in *S. caecutiens*, *C. suaveolens*, *C. laisura*

Fig. 2. Transmission electron microscopy images of the submandibular gland of the lesser white-toothed shrew, *Crocidura suaveolens* belonging to Crocidurinae. (A) Mucous acinar cells with numerous mucous granules. Scale bar=10 μm. (B) Serous acinar cell with numerous serous granules. Scale bar=2 μm. (C) Mucous acinar granules with a dense lateral border and several dense bands producing a variety of patterns in the homogeneous and moderate dense matrix. Scale bar=2 μm. (D) Serous acinar granules with moderate dense matrix composed of specks and fine dense core often having moderate dense inclusion. Scale bar=0.5 μm. (E) Granular duct cells with big fine dense granules. Scale bar=2 μm. (F) Myelin-like body containing smooth endoplasmic reticulum in cytoplasm of granular duct cells. Scale bar=2 μm. N, nucleus; M, mitochondria; Mg, mucous granule; Sg, serous granule; rER, rough endoplasmic reticulum; Gc, Golgi complex; fG, fine dense granule; sER, smooth endoplasmic reticulum; Rb, lysosomal residual body; Mb, Myelin-like body.
Fig. 3. Transmission electron microscopy images of the submandibular gland of the big white-toothed shrew, *Crocidura lasiura* belonging to Crocidurinae. (A) Mucous acinar cells with well developed rough endoplasmic reticulum and numerous mucous acinar granules. Scale bar=2 μm. (B) Serous demilune cell with numerous serous acinar granules (Sg) composing of unusual substructure. Scale bar=2 μm. (C) Mucous acinar granules with a dense lateral border and several moderate dense bands producing a variety of pattern into the dense matrix. Scale bar=2 μm. (D-F) Serous aciner granules with geometric patterns having target like inclusions, dense inclusions or dense dots in the homogeneous pale matrix. Scale bar=0.5 μm. (G) Apical cytoplasm of granular duct cells with big fine dense granules. Scale bar=0.5 μm. (H) Myelin-like body having small granular vesicles and smooth endoplasmic reticulum in the cytoplasm of the granular duct cell. Scale bar=0.5 μm. (I) Myelin-like body having the homogenous dense core in lumen. Scale bar=0.5 μm. Mg, mucous granule; rER, rough endoplasmic reticulum; N, nucleus; Sg, serous granule; Gc, Golgi complex; M, mitochondria; fG, fine dense granule; L, lumen; Mb, Myelin-like body; sER, smooth endoplasmic reticulum.
serous acinar cells had the typical appearance of serous cells, and a well developed rough endoplasmic reticulum, few mitochondria and electron dense serous acinar granules (Fig. 2B). The mucous acinar granules had a dense lateral border and several dense bands producing a variety of patterns into the homogeneous and moderate dense matrix (Fig. 2C). Serous acinar granules were a round type and consisted of a moderate dense matrix composing of specks and a fine dense core often with moderate dense inclusions. As the granules mature, moderate dense inclusions had become a round type and had a homogeneously dark dense center (Fig. 2D). The granular duct cells had an irregular nucleus containing mostly euchromatin and many well developed mitochondria at the basal cytoplasm, as well as large numbers of fine dense granules of relatively large sizes at the apical cytoplasm (Fig. 2E). In the granular duct cells, the myelin-like body was composed of a layer of paired membranes and contained a smooth endoplasmic reticulum, and the lysosomal residual body was observed near the myelin-like body (Fig. 2F).

**Crocidura lasiura**

At the ultrastructural level, the mucous acinar cells (Fig. 3A) and serous acinar cells (Fig. 3B) had the typical appearance as those of *S. caecutiens* and *C. suaveolens*. The mucous acinar granules at the apical cytoplasm near the secretory canaliculi had a dense lateral border and several moderate dense bands producing a variety of patterns in the dense matrix (Fig. 3C). The serous acinar granules had a homogenous pale center surrounded by minute dense specks or had an unusual substructure in a pale matrix (Fig. 3D). Serous acinar granules showing a geometric pattern had a distinct limiting membrane and a pale matrix that was embedded with one or more different types of inclusions (Fig. 3B). Target like inclusions, a bundle or clump of dense inclusions or dense dots were observed in the homogeneous pale matrix (Fig. 3D and F). The granular duct cells were similar to those of *C. suaveolens* and had many large fine dense granules (Fig. 3E). The myelin-like body comprised of paired membranes with small vesicles and smooth endoplasmic reticulum was observed in the cytoplasm of the granular duct cells (Fig. 3H). The myelin-like body with a homogenous dense core also was observed in the lumen (Fig. 3I).

**DISCUSSION**

The mucous and serous acinar granules of the submandibular gland of *Sorex caecutiens* had a border of lucid corona, and those of *Crocidura suaveolens* and *C. lasiura* had a dense lateral border. This feature of acinar granules with a border of lucid corona distinguishes *S. caecutiens* from *C. suaveolens* and *C. lasiura*. Therefore a border of lucid corona of acinar granules is characteristic of *S. caecutiens*. Although Jeong et al. (2007) reported that the acinar granules of *S. caecutiens* did not have a limiting membrane on the border, the existence of a limiting membrane in the mucous and serous acinar granules of submandibular gland of *S. caecutiens* was confirmed. These results showing the existence of the limiting membrane in the acinar granules of the submandibular gland of *S. caecutiens* coincides with previous studies on the granules of the salivary gland in Soricidae species including Soricinae and Crocidurinae, revealing a limiting membrane and species-specific patterns (Schaffer, 1908; Raynaud, 1964; Mineda, 1981, 1985; Jeong & Jeong, 2005; Jeong et al., 2005a, 2005b).

The newly reported substructure of the serous acinar granules of submandibular gland in *C. lasiura* differs considerably from the previous investigations (Jeong et al., 2005a). Jeong et al. (2005a) showed that serous cells have identical granules, a uniformly pale round center, a border of minute dense specks and no apparent substructure. In the present study, the matured and stored serous acinar granules of *C. lasiura* observed in the apical cytoplasm of the secretory cells of the submandibular gland had a homogenous pale center surrounded by minute dense specks or had an unusual substructure showing a geometric pattern according to the inclusions in the pale matrix of granules. This is the first report of an unusual substructure in *C. lasiura* showing a geometric pattern of serous acinar granules. The striking variation in secretory granule ultrastructure suggests species differences in the post-translational modification of a relatively small number of basic products (Tandler et al., 1990). These variations are not random but might be correlated with the genetic history (Tandler et al., 1986; Phillips et al., 1987a), diet (Phillips et al., 1987b; Tandler et al., 1990) and species isolation (Nagato et al., 1984). Therefore, interspecies ultrastructural differences between the homologous salivary gland secretory cells have potential evolutionary significance at the cellular level (Hand, 1980; Phillips and Tandler, 1987; Phillips et al., 1987a, 1987b; Tandler et al., 1990; Phillips et al, 1993; Tandler and Phillips, 1993; Tandler et al., 1994). Consequently, as based on the above data, the ultrastructure of the secretory granules in the submandibular salivary gland of the three shrew species inhabiting Korea might be used with a key classifying species belonging to Soricinae and Crocidurinae from other mammal species.

The myelin-like body is the most distinctive cytological feature of the granular duct cells in *S. caecutiens*, *C. suaveolens* and *C. lasiura*. The myelin-like body is a manifold form of membrane (Jeong et al., 2005a, 2005b) and has been observed as a whorled membranous body or whorled body (Tandler et al., 1998). A similar myelin-like body has been observed in variety of cells with known steroid-secreting ability (Bjersing, 1967; Black & Christensen, 1969; Hamilton
et al., 1969; Bourneva, 1973; Tandler et al., 1998). Tandler et al., (1998) reported that duct cells with a myelin-like body in the cytoplasm may be involved in the steroid metabolism. The myelin-like body has been reported to occur in the submandibular gland duct of five species shrews, the musk shrew, Suncus murinus, dsinezumi shrew, Crocidura dsinezumi, Korean spider shrew, C. caecutiens, lesser white-toothed shrew, C. suaveolens and big white-toothed shrew, C. lasiura (Mineda & Kasuga, 1985; van Lennep & Dryden, 1985; Ueyama et al., 1986; Tandler et al., 1998; Jeong et al., 2005a, 2005b, 2007). Moreover, the layers of the whorled bodies in S. murinus were unit membranes (Tandler, 1993; Tandler et al., 1998) and those of Crocidura were paired membranes (Mineda & Kasuga, 1985; Ueyama et al., 1986; Tandler et al., 1998). In the present study, the myelin-like body of S. caecutiens with layers of unit membranes was different from those of C. suaveolens and C. lasiura having paired membranes. Therefore, the layers composed of paired membranes of the myelin-like body might be one of the characteristics of Crocidura.

CONCLUSIONS

The existence of the limiting membrane in the acinar granules of the submandibular gland of S. caecutiens coincides with previous studies on the granules of the salivary gland in Soricidae species including Soricinae and Crocidurinae. The matured and stored serous acinar granules of C. lasiura observed in the apical cytoplasm of the secretory cells of the submandibular gland had a homogenous pale center surrounded by minute dense specks or had an unusual substructure. The myelin-like body of S. caecutiens with layers of unit membranes was different from those of C. suaveolens and C. lasiura. The ultrastructure of the secretory granules in the submandibular salivary gland of the three shrew species inhabiting Korea, suggesting that it might be used with a key classifying species belonging to Soricinae and Crocidurinae from other mammal species.

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