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Fine structure of the vomeronasal sensory epithelium of Korean goats(Capra hircus)

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Abstract

The vomeronasal sensory epithelium of goats native to Korea was examined morphologically by light and electron microscopy. The vomeronasal organ is a tubular structure situated bilaterally at the base of the nasal septum. The sensory epithelium lines the medial wall of the vomeronasal lumen and consists of receptor, supporting, and basal cells. The receptor and supporting cells have prominent microvilli on the luminal surface. In the basal region, four types of basal cells can be distinguished morphologically. Two of them are attached to the basal lamina. Morphological characteristics of Korean goat vomeronasal sensory epithelium are discussed in comparison to other mammalian vomeronasal epithelia.

Introduction

The vomeronasal organ(VNO) is a chemoreceptor organ, the receptor cells of which project axons to the accessory olfactory bulb. The VNO plays an important roles in perception of conspecific chemical signals(see reviews, Halpern 1987, Ichikawa 1996, Meredith 1983, Wysocki 1979), and has a tubular structure with its lumen surrounded by two types of epithelium: the vomeronasal sensory epithelium(VSE) and the vomeronasal non-sensory epithelium. The VSE is

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thought to function as a signal detector. Mammalian VNOs have been studied anatomically(Adams and Wiekamp 1984, Harrison 1987, Johnson et al. 1985, Mo 1989, Salazar et al. 1994, 1996). The gross anatomical characteristics of the VNO vary among mammalian species. The fine structure of the VSE of many mammalian species has been examined and has also been found to vary from species to species(Adams 1986, 1992, Bhatnagar et al. 1982, Cooper and Bhatnagar 1976, Krazing 1971, Loo and Kanagasuntheram 1972, Mendoza 1993, Mendoza et al. 1994, Oikawa et al. 1994, Taniguchi and Mikami 1985, Taniguchi and Mochizuki 1983, Taniguhi et al. 1992, Vaccarezza 1981, Yoshida et al. 1995).

The Korean goat inhabits the fields of the Korean peninsula and Cheju island. Behavioral characteristics of this goat have not been studied extensively. The Korean goats have been captured at regular intervals for research. Mo(1989) has reported on the gross anatomy of the Korean goat, but the fine structure of the Korean goat VNO has not been studied. Published reports on the fine stucture of wild mammalian VNO are very few; being limited to ones on the bat(Bhatnagar et al. 1982), slow loris (Loo and Kanagasuntheram 1972) and marmoset(Taniguchi et al. 1992) VNOs. Thus, the aim of the present study was to examine the fine structure of the VSE of the present study was to examine the fine structure of the VSE of the Korean goat and to compare it to that of the VSEs of other mammalian species.

Materials and methods

Five male goats(Capra hircus) (5-7 month old, body weight was about 20kg) native to Korean were captured from a field on Cheju island, Korea(One goat in August 1996, two in October 1996 and two in January 1997).

Under deep anesthesia with xylazine hydrochloride(Bayer, Seoul), the goats were sacrificed by transection of the carotid artery. For collection of the VNO, the cranial part of the nasal bone and maxilla was dissected out with electric bone saw. The VNO was exposed, dissected out with nasal septum, and then immersed in fixative(2.5% glutaraldehyde in 0.1 M phosphate buffer) at 4C for more than 1 day. The fixed VNO was washed in 0.1 M phosphate buffer. Coronal slices(0.5 - 1.0 mm thicks) were cut with a razor blade and postfixed with 1% osmium tetroxide in 0.1M phosphate buffer. The fixed slices were dehydrated and embedded in epoxy resin(Quetol 812). One - μm - thick sections stained with toluidine blue

were prepared for determination of tissue orientation and for light microscopic observation. The VSE was trimmed down to a block. Ultrathin sections with a silver gray interference color were cut and mounted on Formval-coated, one-hole copper grids. After staining with uranyl acetate and lead citrate, the VSE was observed with an electron microscope(JEOL JEM 1200 EXII).

Results

Light microscopic observation

In the anterior region of VNO, the wall of the lumen consists of only respiratory epithelium, In the middle region of VNO, the medial wall of lumen is lined with the sensory epithelium. The lateral wall of VNO is covered with respiratory epithlium. In the posterior region of VNO, the ventral wall of lumen consists of sensory epithelium and the dorsal wall consists of respiratory epithelium. The VSE is most widely distributed in the middle region of VNO rostrocaudally(Fig. 1). Thus, we observed the VSE in the middle. The VSE shows pseudostratified structure and consists of receptor, supporting and basal cells(Fig. 1C). The superficial layer is formed by the upper prolongations of receptor and supporting cells. The cell bodies of the supporting cells are densely distributed in an upper layer, while cell bodies of receptor cells are distributed mainly in the deeper layer(Fig. 1C). Basal cells are scattered in the basal region of the epithelium(Fig. 1C). No blood vessels were detected in the epithelium. The distance from the surface to the basal lamina was about 60-80㎛.

Electron microscopic observation

The goats were rounded up in three seasons, summer(August), autumn(October), and winter(January). No prominent differences in fine structure of the VSE were detected by electron microscopy among the goats rounded up in the different seasons.

Receptor cells

The rpcepter cells, bipolar in shape, extend a dendritic process to the luminal surface. At the luminal surface, apical cytoplasmic protrusion can be recognized(Fig. 2A). Numerous microvilli(20-40 in a single section) extended from the protrusion(Fig.

2A). These microvilli are 4-6㎛ in long and 60-100㎛ in diameter. Branching of microvilli was occasionally observed. In the luminal protrusions of receptor cells, a large number of tubular or vesicular structures were observed(Fig. 2A). In the adluminal portion of the receptor cell dendritic processes, clear vesicles, centrioles, ciliary precursor bodies and mitochondria were recognized(Fig. 2). The vesicles were numerous. Mitochondria, microtubules, and rough endoplasmic reticulum(ER) were observed in the dendritic processes. In the perikaryon, well-developed rough ER, Golgi apparatus, polyribosomes, mitochondria, and multivesicular bodies were observed(Fig. 3B). The nucleolus was clearly recognized in the nuclei. The axonal processes of receptor cells passed through the basal lamina(Fig.4). Bundles of axons were observed in the lamina propia(Fig.5).

Supporting cells

The supporting cells are also bipolar in shape. The upper cell process contain many free ribosomes, rough ER, and bundles of glial filaments(Figs. 3B and 6A). Cytoplasm of the supporting cell is electron-dense than that of receptor cells. At the luminal surface, many microvilli extend from the upper process(Fig. 1A). The number of microvilli on each supporting cell(10-20 microvilli in a single section) is much smaller than that on the receptor cell, and the microvilli on the supporting cells are longer than those of receptor cells. The supporting cell microvilli are more than 10㎛ long and 100-250㎛ in diameter. Glycocalyx-type materials are prominent on the microvilli of supporting cells(Fig. 1A). Occasionally, a thick microvillus or cytoplasmic process(sometimes free ribosomes were recognized in the process) is observed at the luminal surface. The deeper processes attached to the basal lamina. The perikaryal region is oval and contain mitochondria, rough ER, and ribosomes.

In the adluminal region, the junctional complexes are located between receptor cells and supporting cells(Fig. 1B). In the deep layer, tight junction was located between supporting cells.

Cells in the basal region

Four types of cells were observed in the basal region(Figs. 4-6). The first type is characterized by a flattened or oval nucleus and cell body and a narrow perikaryal cytoplasmic rim. Within the rim, polysomes, poorly developed ER, a few mitochondria, Golgi apparatus and bundles of thin filaments were observed(Fig.

6B). This type of cell was attached to the basal lamina. Desmosome-like structures were observed between cells of this type(Figs. 4 and 6B).

The second type of cell is characterized by an irregularly shaped electron-dense nucleus and cell body. The cell has a narrow electron-dense perikaryal cytoplasmic rim. In the perikarya, poorly developed rough ER, polyribosomes, and mitochondria were observed. Very frequently, bundles of thin filaments were observed in perikaryon(Fig. 6C). These cells were observed between the first type of cells and the receptor cell bodies.

The third type is characterized by an irregularly shape nucleus and cell body. The cell has a narrow perikaryal cytoplasmic rim. The cytoplasm and nucleoplasm in the third type of cells are more electron-lucent than those of the second type of cell. The nucleus of the third type of cells is more prominently heterochromatin in the third type of cell than those of the second type of cell. In the cytoplasm of cells of the third type, a small amount of smooth ER and a few free ribosomes were recognized. These cells were observed between the first type of cells and the receptor cells.

Only very few, cells of the fourth type were recognized(Fig. 5A). This type of cell is characterized by a flattened or oval nucleus and cell body and a narrow electron-dense perikaryal cytoplasmic rim. The nucleus is also electron-dense. In the perikarya of the cell of this type, poorly developed rough ER, polyribosomes, and mitochondria were observed. The type of cells were attached to the basal lamina.

Of the total number of these four types of cells, about 40% were of the first type, about 25% of the second type, about 25% of third type, and about 10% of fourth type.

In the basal region, axonal processes of receptor cells were recognized(Fig. 4). Many desmosome-like structures were observed between cell processes(Fig. 6B, D). Occasionally electron-dense cytoplasmic processes which could not be traced to the perikarya were observed, but we could not determined whether the processes were ones of supporting cells or second or fourth types of cells in the basal region.

Legends

Fig. 1. (A) Binocular stereoscopic micrograph of slice of the vomeronasal organ. sc, septal cartilage; vc, vomeronasal cartilage; vno, vomeronasal orga. (B) Light micrograph of vomeronasal organ. Large arrows indicate the bound-

ary between the sensory epithelium(se) and nonsensory epithelium(ns). Small arrows indicate the veins. g, vomeronasal gland; lu, lumen of vomeronasal organ. (C) Light micrograph of sensory vomeronasal epithelium. Arrows indicate the level of the basal lamina. b, basal layer; lu, lumen of vomeronasal organ; mv, microvilli; r, receptor cell layer; s, supporting cell layer. Bars:1mm in (A), 0.5mm in(B), 20㎛ in(C)

Fig. 2. (A) Electron micrographs of luminal surface of receptor(R) and supporting (S) cells in the vomeronasal sensory epithelium. Arrows indicate microvilli of supporting cells. mv, microvilli of receptor cells; r, receptor cell; s, supporting cell. (B) Higher magnification view of junctional complex in(A). Arrows indicate the junctions. (C) Higher magnification view of centrioles in(A). Arrows indicate centrioles.
Bars:1㎛ in(A), 250 nm in(B) (C).

Fig. 3. Electron micrographs of supproting cell(A) and receptor cells(B). r, process of receptor cell; s, supporting cell; re, rough endoplasmic reticulum; n, nucleolus; pr, polyribosomes. Arrows indicate bundles of filaments.
Bars:1㎛

Fig. 4. Electron micrographs of cells in the basal region of sensory epithelium. Ⅰ, 1st type of cell; Ⅱ, 2nd type of cell; Ⅲ, 3rd type of cell; bl, basal lamina; p, processes of 2nd type of cell or supporting cell; Large arrows indicate axons of receptor cells. Small arrows indicate the desmosome like structure. Bar:1㎛

Fig. 5. (A) Electron micrographs of Micrographs of cells in the basal region of the sensory epithelium. Ⅲ, 3rd type of cell; Ⅳ, 4fth type of cell; bl, basal lamina. Bar:1㎛
(B) Non-myelinated axon bundles of receptor cells in the lamina propia. Bar:1㎛

Fig. 6. Electron micrographs showing the bundles of filaments in the perikaryion (arrows in A-C) and desmosome-like structure(arrowheads in B and D). s, supporting cell; bl, basal lamina; Ⅰ, 1st type of cell; Ⅱ, 2nd type of cell. Bars:500nm

Figure 1

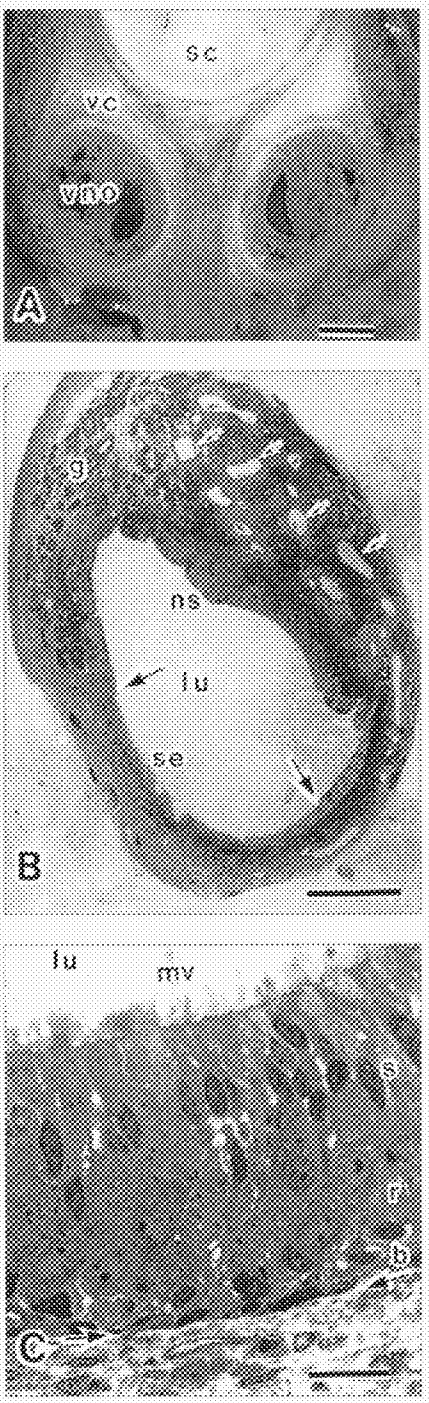


Figure 2

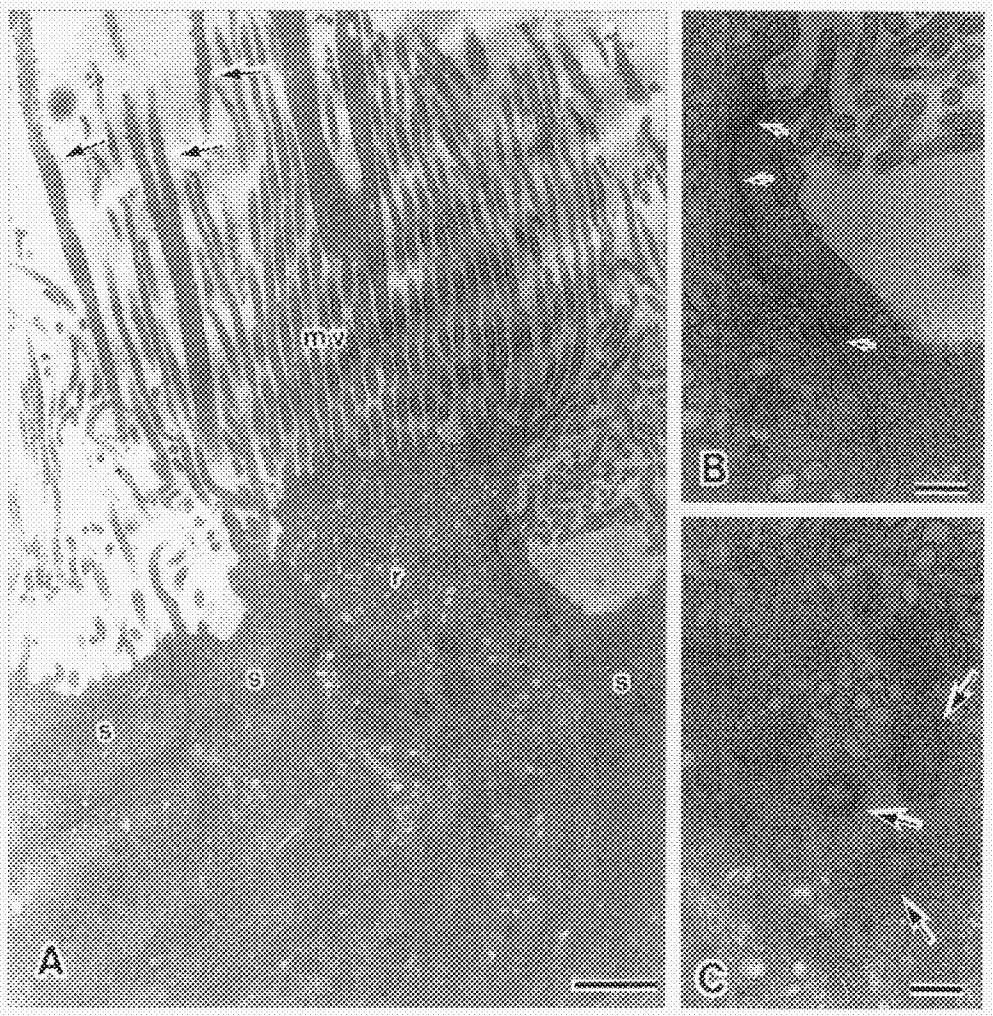


Figure 3

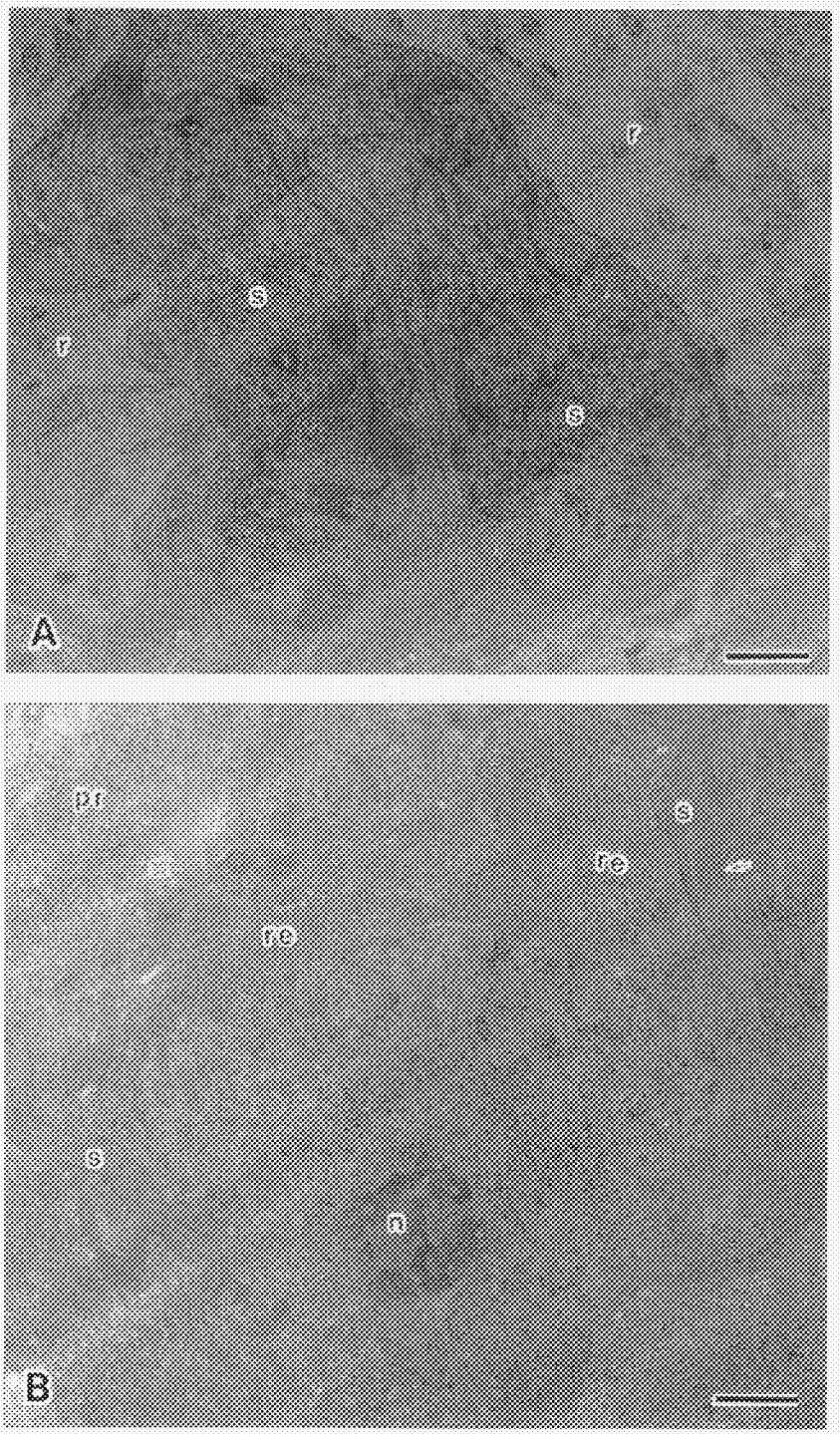


Figure 4

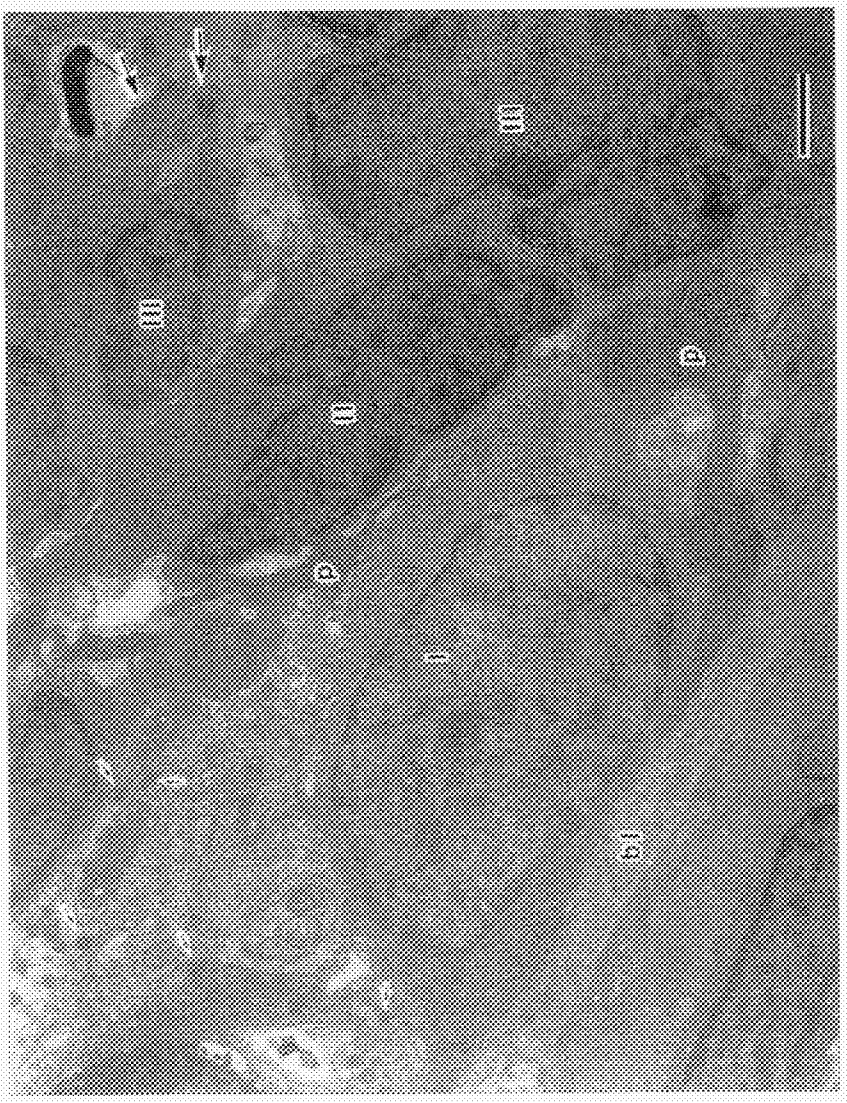


Figure 5

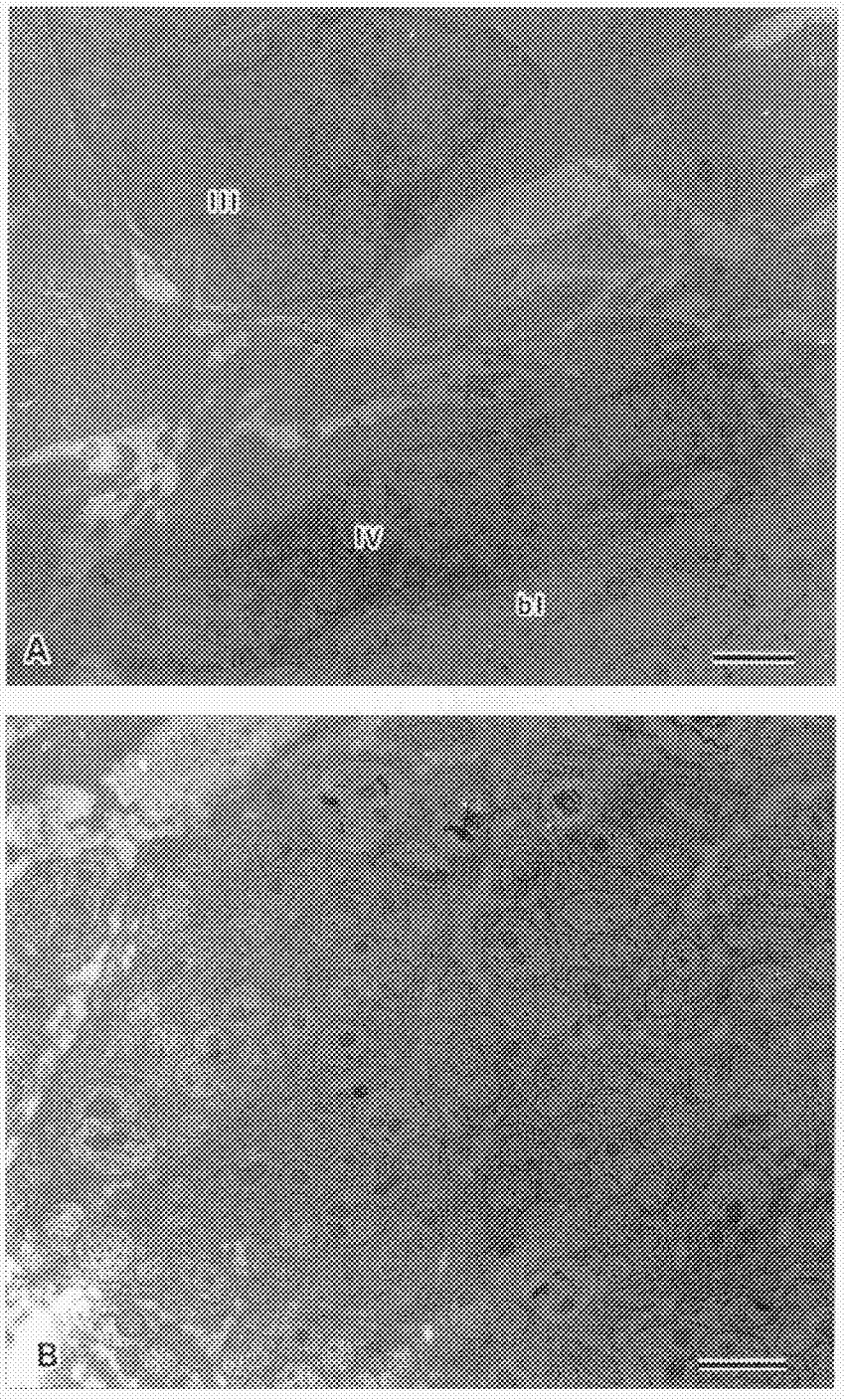
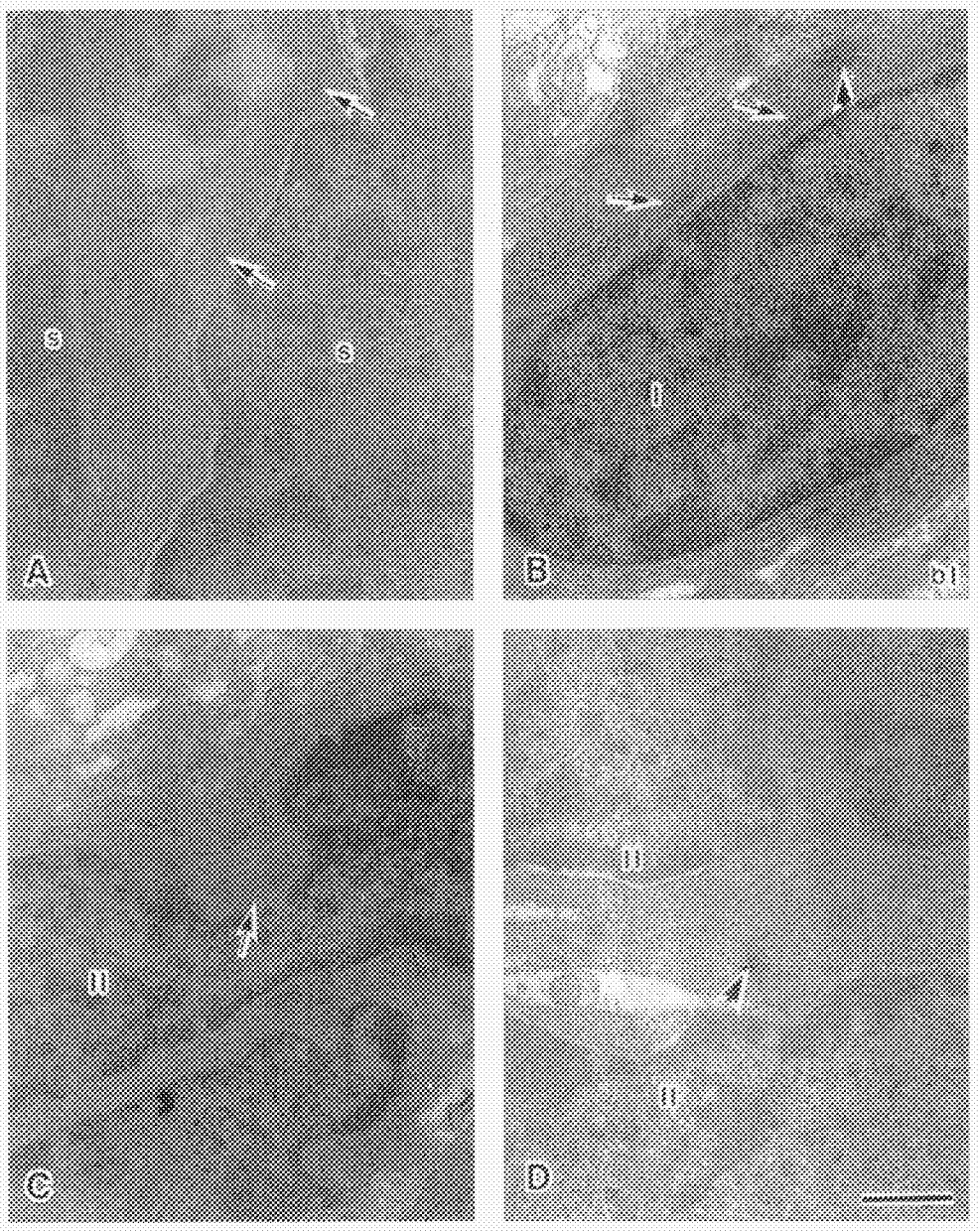


Figure 6



Discussion

Distribution of VSE

In the present observation of the semithin sections, the shape of the VNO lumen varied from ovoid to lunular. The VSE distributed from the medial wall in the middle region to the ventral wall in the posterior region. This result revealed the same distribution of sensory epithelium as that descrived in Mo's report(1989). Thus, the middle region of the VNO was examined electron microscopically in the present study.

Microvilli

The Microvilli of receptor cells in VSE have been considered the primary location of occurrence of perception in the vomeronasal system. In the Korean goat, the microvilli of the receptor cells are shorter and more densely distributed than those of the supporting cells. Similar differences in microvilli between receptor cells and supporting cells have been reported for other mammals(Taniguchi and Mochizuki 1983, Taniguchi and Mikami 1985, Yoshida et al., 1995). The number of microvilli on the receptor cells in the Korean goat VSE is very large. The VSE receptor cells of horses, cattles, tree shrews, and slow lorises also have a large number of microvilli(Taniguchi 1985, Loo and Kanagasuntheram 1972). On the other hand, those of rats, mice and chinchillas have fewer number of microvilli than do those of the Korean goat(Taniguchi and Mochizuki 1983, Oikawa et al. 1994, Yoshida et al. 1995). The receptor cell microvilli in the Korean goat are longer than those in horses, rats and hamsters, shorter than those in sheeps, and mice, and similar in length to those in pigs and cattles(Taniguchi and Mochizuki 1983, Taniguchi and Mikami 1985, Adams 1992, Kratzing 1971, Vaccarezza 1981). In the presesent study, we did not determine whether the number or length of microvilli indicate the function of the vomeronasal epithelium, that is, ability of pheromonal perception. Morphological characteristics suggested that the microvilli of receptor cells is prominently developed in Korean goat.

The glycocalyx on the surface of the microvilli of the supporting cells is prominent in the Korean goat. The presence of a prominent glycocalyx on the supporting cell microvilli has also been reported in the other mammals : bats, pigs, and cattles(Adams 1986, 1992, Bhatnagar et al. 1982). The functional significance

of this glycocalyx has not yet been clarified.

Structure of adluminal dendritic region

A large number of clear vesicles were observed in the adluminal processes of the Korean goat receptor cells(Fig. 2A). The presence of such a large numbers of vesicles indicates that this is a highly active endocytotic region where odorant ligand-receptor protein complexes are internalized(Adams 1992).

Numerous centrioles and ciliary precursor bodies are observed in the adluminal processes of the Korean goat receptor cells(Fig. 2C). The presence of ciliary precursor bodies has been considered to indicate either blockage of ciliary development in a stage immediately after axonemal precursor formation, or incomplete centriole formation(Kolnberger and Altner 1971).

Basal cell

The basal region of the VSE of the Korean goat is composed of four types of cells. Multiple types of basal cells had not been previously identified in the mammalian VSE. Graziadei and Monti-Graziadei(1979) reported detecting two types of basal cells in the mouse olfactory epithelium, a basal cell proper and a globose basal cell. In the present study, the morphological characteristics of the first and fourth types of cells which were contact with the basal lamina were found to be similar to those of the basal cell proper in the olfactory epithelium, and those of the second and third types of cells to be similar to those of the globose basal cell in the olfactory epithelium. However, the functional meaning of the presence of two types of basal cells in the lofactory epithelium has not yet been clarified. Wang and Halpern(1982) reported the presence of undifferentiated precursor cells between basal cells and receptor cells in the regenerating VNO of a garter snake after transection of the vomeronasal nerve. It seems likely that the second and third types of cells in the basal region of the Korean goat VSE are undifferentiated precursor cells of receptor and / or supporting cells. However, further study is necessary to determine wether in fact they are.

Seasonal changes

Although as mentioned above the behavioral characteristics of Korean goat have

not been studied extensively, it is known that the reproductive season is autumn. In the present study, one of the goats was captured in August, another two in october, and the other two in January. Some studies have shown that in not only wild mammals but also domestic ones the vomeronasal function is enhanced in reproductive season(Hart, 1985). For example, male horsess show the special behaviors such as phremen in reproduction season. It is speculated that in the VSE the receptor cells develops in the reproductive season. In the present observation, no seasonal changes in the fine structure of VSE were recognized. For examination of seasonal changes of the Korean goat VSE, it is necessary to analyze the structure of the VSE quantitatively in each season such as in terms of the number of receptor cells and / or basal cells, and / or the number and length of microvilli on the receptor cells.

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