Morphological Studies on the Vomeronasal Organ in the Golden Hamster

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Abstract. Morphological features of the vomeronasal organ in the golden hamster were examined by light and electron microscopy. This organ consisted of a 7 mm long tubular structure situated bilaterally in the anteroventral nasal septum and enclosed completely by a bony capsule. Posteriorly it ended blindly, whereas anteriorly it communicated with the nasal cavity through an orifice at its rostral end. The sensory epithelium lining the medial wall of the lumen of this organ showed a cell configuration similar to that of the olfactory epithelium except the basal cells. Free surface of the sensory cells in the sensory epithelium was covered with structures intermediate between cilia and microvilli. Capillaries often penetrated into the sensory epithelium, and the vomeronasal nerve observed under the sensory epithelium consisted of nonmyelinated nerve fibers. The respiratory epithelium lining the lateral wall of the lumen of this organ was typically ciliated and pseudostratified, devoid of goblet cells. Mucous and serous acini of Jacobson's glands were distributed on the dorsolateral and ventromedial sides of the lumen. The glands were PAS-positive, different from alcian blue-positive olfactory glands of Bowman. Myelinated and nonmyelinated nerve fibers were often distributed among the acini.

Although the vomeronasal organ has been studied by many investigators in various species after the first description by Jacobson in 1811 [8], its function is still unknown. Recently, still fragmentary, several electron microscopic observations of this organ [2, 7, 11-13, 15, 16, 18] became available, but these seem to be far from complete. The present paper intends to reveal the whole morphological aspects of this organ with light and electron microscope to make clear its real nature.

Materials and Methods

Specimens were taken for light and electron microscopy from 14 adult golden hamsters of both sexes. For the light microscopy, specimens were

fixed in Lillie's solution, decalcified in a mixture of 10% formic acid and 10% formalin, embedded in paraffin, sectioned serially at 7 μ m, and stained with hematoxylin-eosin (HE), and alcian blue-periodic acid Schiff reagent (PAS).

For the electron microscopy, specimens were double-fixed in modified Karnovsky's solution and 1% osmium tetroxide in 0.1 M cacodylate buffer at pH 7.3, and embedded in Epon 812 by the routine procedure. Ultra-thin sections were stained with uranyl acetate and lead citrate and examined with a JEM 100S electron microscope. Semi-thin sections were stained with toluidine blue for the light microscopy.

Results

Light Microscopy

The vomeronasal organ of the golden hamster consists of an about 7 mm long

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Fig. 1. A. Schematic drawing of a parasagittal section of the nasal cavity showing the location and opening of the vomeronasal organ. An arrow indicates the opening of this organ. (vo: vomeronasal organ).

B. Schematic drawings of transverse sections of the vomeronasal organ. Sections I to III correspond to the transverse sections at I to III in A. (Jg: Jacobson's glands, vn: vomeronasal nerve, vs: venous sinus).

tubular structure situated bilaterally at the base of the nasal septum and enclosed completely by a bony capsule. Posteriorly it ends blindly, whereas anteriorly it communicates with the nasal cavity through an orifice at its rostral end (Fig. 1). While the lateral wall of the lumen of this organ is lined with the respiratory (non-sensory) epithelium, the medial wall is lined with the sensory epithelium (Fig. 2).

The sensory epithelium (Fig. 3), similar to the olfactory epithelium, consists of sensory and supporting cells arrayed alternately. Oval nuclei of the supporting cells are arranged longitudinally in the upper third of the sensory epithelium con-

stituting a distinct zone. Round nuclei of the sensory cells are densely arranged in layers in the middle to basal regions of the epithelium. Different from the olfactory epithelium, the sensory epithelium is almost devoid of the basal cells. Capillaries are often seen to enter the epithelium.

Under the sensory epithelium is observed the vomeronasal nerve consisting of non-myelinated nerve fibers. This nerve leaves the organ, runs through the submucosa in the nasal septum and passes through an orifice of the cribriform plate to reach the accessory olfactory bulb.

The respiratory (non-sensory) epithelium (Fig. 4) lining the lateral wall of the lumen is ciliated and pseudostratified with a height of about $50\ \mu\text{m}$ and possesses oval nuclei in its middle to basal regions. Small basal cells are also observed at the bottom of the epithelium. Unlike the nasal respiratory epithelium, the vomeronasal respiratory epithelium lacks the goblet cells.

Jacobson's glands are situated on the dorsolateral and ventromedial side of the lumen and consist of mucous and serous acini. Their secretion is PAS-positive, different from that of olfactory glands of Bowman in that the secretion of the latter is alcian blue-positive. Their ducts open into the lumen in the transitional region from the sensory to the respiratory epithelium. Myelinated and nonmyelinated nerve fibers are often observed among the acini.

Electron Microscopy

Sensory epithelium

The cytoplasm of the sensory cells protrudes slightly into the lumen and is covered with many microvilli running radially (Fig. 5). These microvilli are slender cylindrical processes about 2.8 to $3.6\ \mu\text{m}$ in length and 0.12 to $0.14\ \mu\text{m}$ in diameter. As many as 8 to 16 microvilli are observed per one section of the cell. About 20 micro-

tubules run longitudinally in a microvillus and tend to extend into the cytoplasm to congregate at one area just under the free surface (Fig. 6). This tendency is also recognized in the dog, cat as well as in several other species in our unpublished observations. Clusters of centrioles are observed around this area among mitochondria, a few vesicles and neurotubules running arbitrarily (Fig. 7). In the neighbourhood of centrioles are also observed some ciliary structure precursor bodies [10] and granular bodies [14] (Fig. 8) which seem to be their storage forms.

Below this area, numerous mitochondria aggregate densely and then are abruptly replaced by neurotubules. These neurotubules run parallel in large numbers proximally to reach the supranuclear cytoplasm. There, sometimes Golgi apparatus, rER, lysosome as well as mitochondria are well-developed, and sometimes sER is well-developed instead of them (Fig. 9). This sER consists of a tubular structure arrayed regularly (Fig. 10).

The cytoplasm of the supporting cells possesses a high electron density and is covered also with microvilli. These microvilli, different from those of the sensory cells, are mere processes of the unit membrane and their microtubules do not extend into the cytoplasm. The cytoplasm is rich in free ribosomes and mitochondria and also possesses rER and microtubule, but unlike in the sensory cell, these organelles are scattered throughout the cytoplasm.

The sensory and supporting cells are connected together by the junctional complex near the free surface of the epithelium, though no other junction is observed at any other part of the epithelium.

Respiratory (non-sensory) epithelium

The free surface of the respiratory epi-

thelium is covered with cilia and microvilli with a length of about 6 μm and 4 μm respectively (Fig. 11). Cilium has a diameter of about 0.3 μm and possesses a well-developed basal body at its foot (Fig. 12). Numerous mitochondria and free ribosomes are present in the cytoplasm, and mitochondria are inclined to cluster near the free surface and the basal lamina. Though rER and Golgi apparatus are also present, they are poorly developed. Cells in the epithelium are connected firmly by the junctional complex near the free surface and by other junctions in the middle to basal parts of the epithelium.

The basal cells are small and amorphous, observed to be small in number near the basal lamina and having cytological features almost similar to those in the usual epithelium.

Jacobson's glands

Mucous acini consist of acinar cells (Fig. 15) with a high electron density and containing many secretory granules. The nucleus is oval to spindle-shaped and situated in the basal part of the cell. The karyoplasm is dark and deposits of chromatin material are scattered at the periphery of the nucleus. Obviously well-developed rER is often observed to encircle secretory granules.

Serous acini consist of acinar cells (Fig. 16) with a low electron density. The nucleus is round and situated in the middle part of the cell. The karyoplasm is light and chromatin materials are disposed around the periphery of the nucleus. Mitochondria and sER are observed instead of rER in the cytoplasm.

Discussion

Although numerous functions have been ascribed to the vomeronasal organ, the real functions are still unknown. Some of func-

tions ascribed to this organ are as follows: acting as a receptor of the odour of the water [3]; acting as a receptor of pressure [1]; relating to the feeding behavior [4, 9]; relating to the reproductive behavior [17, 19] or "flehmen" [5].

The vomeronasal organ communicates with the oral or the nasal cavity according to species. Such a difference in the way of its opening may constitute an obstacle for finding out the functional mechanism common to every species. Further, since the opening is very small whether it communicates with the nasal or the oral cavity, only a small amount of odorants can be thought to enter this organ. For these reasons, it has to be hesitated to ascribe some positive role to its sensation of smell. Recently, relations of this organ with the reproductive behavior are discussed by many investigators and it becomes prevailing that this organ may be a receptor of some kind of pheromone. Even if it is so, the difference of its communication and the smallness of its opening may exert some influence on such a function.

From a morphological point of view, the resemblance of the vomeronasal sensory epithelium to the olfactory epithelium has appealed to many investigators. Both the sensory and the olfactory epithelia are derived from the olfactory placode embryologically and the sensory cell seems to be a receptor of some kind of odorant like the olfactory cell. Nevertheless, the free surface of the olfactory cell is covered with olfactory cilia, and the free surface of the sensory cell is covered with microvilli as described above. Furthermore, about 20 microtubules run longitudinally in a microvillus, similar to the olfactory cilium where 20 microtubules are arranged in so-called "9+2" configuration. Therefore, if the presence of microtubules alone is essential to the per-

ception of odorous molecules, the morphological difference between cilia and microvilli in this case may have no substantial significance to the olfactory sensation.

As for the secretion of the glands, that of Jacobson's glands is PAS-positive and that of Bowman's glands is alcian blue-positive. Therefore, the vomeronasal sensory and the olfactory epithelia are thought to be covered with neutral and acid mucopolysaccharide respectively. Thus, if the vomeronasal sensory epithelium is related to the olfaction, the difference in the nature of the secretion on the epithelial surface may cause some influences on the receptor when odorants touch the surface to give some kind of information to the receptor.

Considering the regeneration of the neuroepithelium at issue, since the basal cells are hardly observed in the sensory epithelium, Graziadei [6] asserts that the basal cells of the sensory epithelium are present in the transitional region from the sensory to the respiratory epithelium (Figs. 13, 14) and that the basal cells migrate from there to replace degenerated cells. He confirms his assertions by the autoradiographic observations. However, it is hard to explain how these cells could migrate to a distance to replace degenerated cells or what would cause them to do so. The mechanism of the regeneration of this epithelium is still open to controversy.

In short, many problems remain to be investigated on the vomeronasal sensory epithelium concerning its function or its mechanism of the regeneration. In order to solve these problems, it is inevitable to compare the fine structure of the vomeronasal organ in detail among many species, although the experimental morphological exploration in some selected species is also indispensable.

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要 約

ハムスター鋤鼻器官の形態学的研究: 谷口和之・望月 公子 (東京大学農学部家畜解剖学教室)——ハムスター鋤鼻器官の形態学的詳細について光顕および電顕的に検索を行なった。本器官は鼻中隔の前腹側に横たわり、骨におおわれた全長約 7 mm の一対の管状構造で、後方は盲端となって終るが、前方では小孔により鼻腔と交通していた。本器官の管腔内側壁をおおう感覚上皮は嗅上皮に類似した細胞配列を示したが基底細胞は見出されなかった。また感覚上皮の感覚細胞の自由縁は線毛と微絨毛の中間型を呈する構造物におおわれていた。感覚上皮にはしばしば毛細血管が侵入し、感覚上皮の下方に見られる鋤鼻神経は無髄線維より成っていた。本器官の管腔外側壁をおおう呼吸上皮は典型的な多列線毛上皮であったが、杯細胞は認められなかった。管腔の背外側および腹内側に多数分布するヤコブソン腺は粘液性と漿液性の2種の腺胞から構成されており、粘液腺主体であった。またヤコブソン腺は P.A.S. 陽性であり、嗅上皮のボウマン腺がアルシアン・ブルー陽性であるのと異っていた。ヤコブソン腺の腺胞の間にはしばしば有髄および無髄の神経線維が分布していた。

Explanation of Figures

- Fig. 2. Transverse section of the vomeronasal organ (se: sensory epithelium, re: respiratory epithelium, vs: venous sinus). $\times 70$.
 Fig. 3. Vomeronasal sensory epithelium. $\times 260$.
 Fig. 4. Vomeronasal respiratory epithelium. $\times 260$.
 Fig. 5. Upper part of the vomeronasal sensory epithelium. $\times 2,400$.
 Fig. 6. Free surface of a sensory and a supporting cell in the sensory epithelium (se: sensory cell, su: supporting cell). $\times 6,000$.
 Fig. 7. Distal end of the cytoplasm of a sensory cell. An arrow indicates the connection of microtubule of centriole with that of granular body. $\times 20,000$.
 Fig. 8. Granular body. $\times 32,000$.
 Fig. 9. Sensory cells of various types. $\times 2,000$.
 Fig. 10. Tubular sER in the supranuclear cytoplasm of a sensory cell. $\times 15,000$.
 Fig. 11. Vomeronasal respiratory epithelium. $\times 1,200$.
 Fig. 12. Cilia with well-developed basal bodies in the vomeronasal respiratory epithelium. $\times 10,000$.
 Fig. 13. Upper part of the transitional region from the sensory to the respiratory epithelium. $\times 3,000$.
 Fig. 14. Lower part of the transitional region from the sensory to the respiratory epithelium. $\times 2,000$.
 Fig. 15. Mucous acinar cell of Jacobson's glands. $\times 4,000$.
 Fig. 16. Serous acinar cells of Jacobson's glands. $\times 2,000$.