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INTRACELLULAR STRUCTURE OF THE OUTER HAIR CELL OF THE ORGAN OF CORTI

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Abstract

The intracellular structure of the outer hair cells of the normal guinea pig organ of Corti was investigated three dimensionally by scanning electron microscope. Freeze fracturing technique followed by maceration with a 0.1% OsO_4 solution (osmic maceration method) was used. Among the cell organelles, the endoplasmic reticulum (ER) showed the most interesting features, such as subsurface cisternae and lamellar bodies. The subsurface cisterna which formed a stratiform network covered the inner surface of the cell membrane and the stratiform structure disappeared at the infranuclear region. Variously shaped mitochondria (spherical, cylindrical and branched) were found on the innermost layer of the subsurface cisterna. The lamellar body which was located beneath the cuticular plate consisted of dilated cisternae and tubular ER and was surrounded by mitochondria. The tubular ER of the lamellar body were continuous with the subsurface cisterna.

KEY WORDS: organ of Corti, outer hair cell, subsurface cisterna, lamellar body, scanning electron microscopy, A-O-D-O method, guinea pig

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Introduction

Although the intracellular structure of the sensory hair cells of the organ of Corti had been intensively studied using transmission electron microscope (TEM), it is fairly difficult with such micrographs to see continuity, interrelation or three dimensional structure of the organelles using that technique. This study was designed to reveal the intracellular structure of the outer hair cell of the organ of Corti under scanning electron microscopy (SEM).

Materials and Methods

Guinea pigs of 250-350g body weight were used. The specimens were prepared by the A-O-D-O method (Tanaka and Mitsushima, 1984). The organ of Corti was prefixed with 0.5% glutaraldehyde and 0.5% paraformaldehyde mixture buffered at pH 7.4 with phosphate buffer solution by cardiac perfusion under general anesthesia. After removal of the temporal bone, the round window and the oval window were perforated and the perilymphatic space was gently perfused with the same fixative for 2 minutes. Then the cochlea was post fixed at 4 C for 30 minutes in 1% OsO_4. After being rinsed in buffer, the specimens were immersed in 25% dimethyl sulfoxide (DMSO) for 30 minutes and 50% DMSO for 1 h. They were then frozen in liquid nitrogen and were fractured with a chisel. The fractured specimens were again rinsed in the buffer solution and were macerated at 23 C in 0.1% OsO_4 for 60-100 h (A-O-D-O method). The specimens were again fixed for 30-40 minutes in 1% OsO_4 solution and fixed with 2% tannic acid at 2 C for 2 h. Further post-fixation with 1% OsO_4 was done for 30 minutes. Following dehydration with graded ethanol, the specimens were treated with isoamyl acetate, critical point dried (liquid CO_2) and were coated with platinum. A high resolution field emission scanning electron microscope (HITACHI S-800) was used for observation.

Results

In the longitudinally fractured specimens,

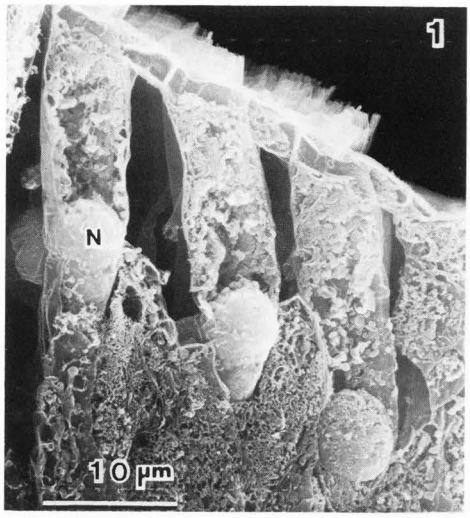
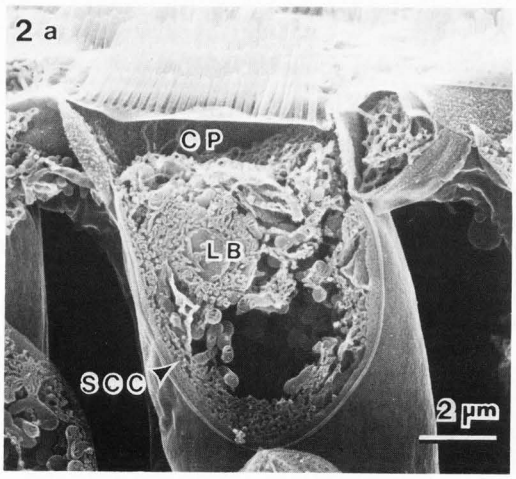
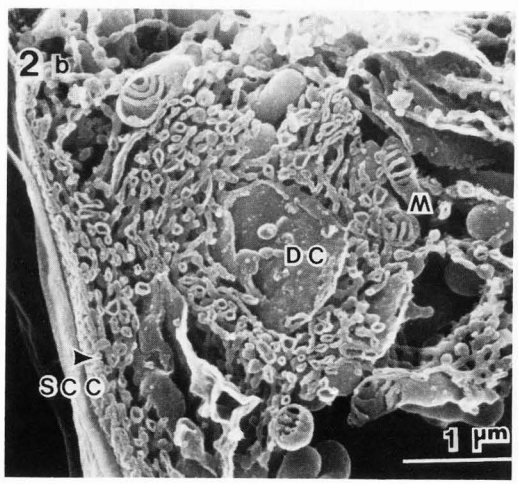
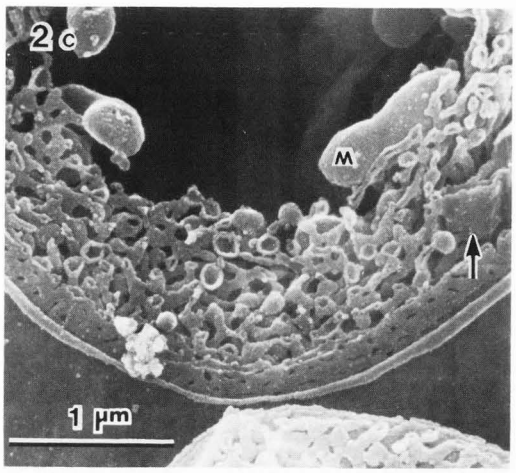


Fig. 1. Longitudinally fractured outer hair cells. The three dimensional structure of the cells is clearly demonstrated.
N: nucleus







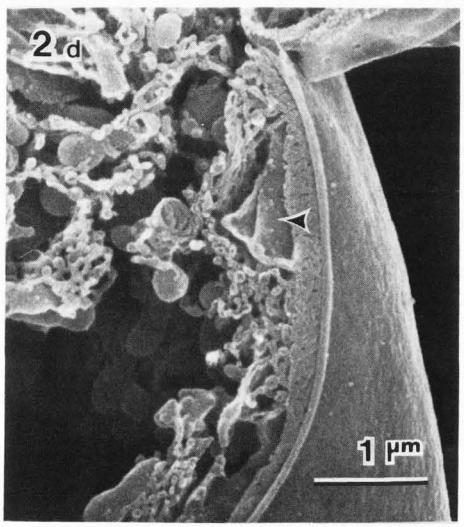
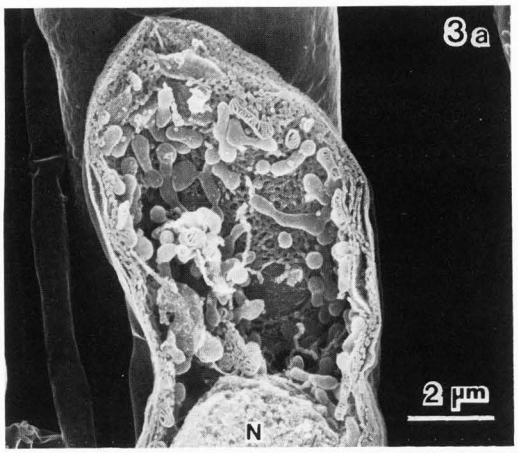


Fig. 2. Upper part of the supranuclear region
a) Organelles aggregated beneath the cuticular plate. The cuticular plate was removed in the course of the specimen preparation.
b) The lamellar body consists of the dilated cisterna at the center and surrounded tubular ER with some layers. It is also seen that tubular ER of the lamellar body are continuous with the subsurface cisternae.
c) The whole layers of subsurface cisternae are demonstrated. The holes in the outermost layer are smaller than those in the other layers. In the deeper layers, a flattened cisterna (➔) is seen.
d) A dilated cisterna is seen among the subsurface cisternae (◄).
CP: cuticular plate, LB: lamellar body, DC: dilated cisterna, SSC: subsurface cisterna, M:mitochondria



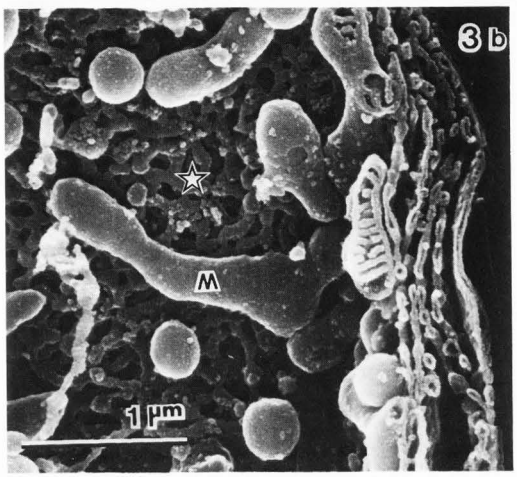


Fig. 3. Lower part of the supranuclear region.
a) Mitochondria on the subsurface cisternae are various in shape, round, long, processed or branched.
b) Various shaped mitochondria and the stratiform structure of the subsurface cisternae (☆) are demonstrated.
N: nucleus, M: mitochondria

the whole structure of the cylindrically-shaped outer hair cell was demonstrated. The nucleus was located at the basal part of the cell. Other organelles aggregated beneath the cuticular plate and the infranuclear part. They were also found close to the lateral plasma membrane (Fig. 1.).

In the upper part of the supranuclear region, a lamellar body was located just beneath the cuticular plate. The lamellar body which is also called Hensen's body consisted of dilated cisternae at the center, surrounded by a few layers of tubular ER. The entire structure of the lamellar body was spherical. The tubular ER which composed the lamellar body were continuous with the subsurface cisterna. The mitochondria around the lamellar body were spherical or cylindrical and were 0.3-0.4 μm in diameter (Figs. 2a,b). In the obliquely fractured specimen, the fine three dimensional structure of the subsurface cisternae could be demonstrated. The subsurface cisternae were composed of several layers of the tubular ER network. The size of the net varied among the layers. The net of the outermost layer was smaller than those in the other layers. In the deeper layer, cisternae, either flattened or dilated were seen (Fig. 2c and d).

In the lower part of the supranuclear region, the central portion of the cell was almost empty. This allowed close observation of the innermost layer of the subsurface cisternae. Among the network of tubular ER, flattened cisternae were rarely found in the lower part of the supranuclear region, whereas they were frequently observed in the upper part. The mitochondria on the innermost layer of the subsurface cisternae were spherical, cylindrical or sometimes branched (Fig. 3a). The thickness of the subsurface cisterna was approximately 0.4μm and formed 4 to 6 layers inside the cytoplasmic membrane (Fig. 3-b).

In the infranuclear region, the stratiform structure of the subsurface cisternae disappeared (Fig. 4).

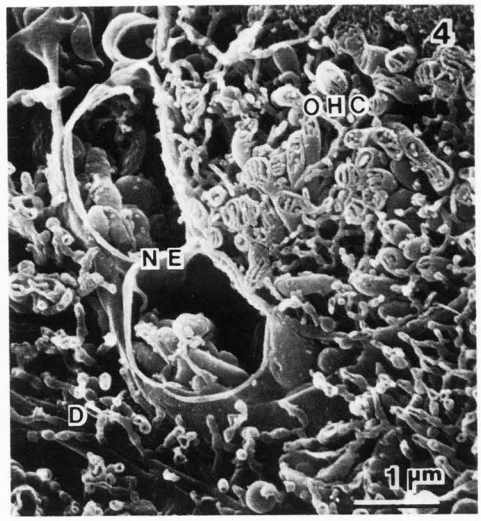


Fig. 4. The infranuclear part of the outer hair cell.
The stratiform structure of the ER network disappears in the infranuclear part.
OHC: outer hair cell, NE: nerve ending
D: Deiters' cell

Discussion

A number of studies on fine structure of the cochlear outer hair cells had been done using both SEM and TEM. The intracellular structure of the hair cell had been investigated mainly by TEM (Engström and Wersäll, 1953), while surface structure had been studied predominantly using SEM (Lim, 1969). An attempt had also been made to observe the intracellular structure of the

organ of Corti using SEM (Lim, 1971). However, close observation of the fine structure of the organelles could hardly be accomplished in the previous study. Yet, recent development of high resolution field emission SEM and preparation techniques, such as the A-O-D-O method, has permitted elucidation of the intracellular structure by SEM (Tanaka and Naguro,1981. Inoué, 1982. Harada et al,1985). The present study could further demonstrate fine intracellular structures, such as the subsurface cisterna, the lamellar body and mitochondria three dimensionally.

Both the subsurface cisternae and the lamellar body which are characteristic structures in the outer hair cell consisted of anastomosing tubular ER and dilated cisternae. Structural differences of the subsurface cisternae among different species have been reported. They are monolayered in human (Spoendlin, 1968) and the rat (Iurato, 1961), while multilayered in the guinea pig (Engstrom and Ades, 1973). Structural differences of the subsurface cisternae between each layer had been observed using TEM. The structure of the subsurface cisternae also varies between upper and lower parts of the supranuclear region. According to Saito (1983), in the upper portion of the supranuclear region of the outer hair cell, the outermost layer is a flattened cisterna with few fenestrae and the deeper layers are fenestrated cisternae with many disk-like areas. While in the lower supranuclear region, the outermost layer is tubular and the deeper layers are fenestrated cisternae. In the previous studies, ultra thin sections or freeze fracturing images were used for observation. Using these conventional TEM techniques, it is difficult to reveal the entire structure of subsurface cisternae, since these only allow observation of fairly limited areas of the subsurface cisternae. Compared to these methods, SEM allows three dimensional observation of wider regions. In the present study, the three dimensional structure of each layer of the subsurface cisternae and the lamellar body could be demonstrated. In our observation, subsurface cisternae did not literally appear cistern or sac shaped. They consisted of complicated networks of the tubular ER. Round spaces in the subsurface cisternae had been conventionally termed "fenestrae", but when observed stereoscopically they were more like loopholes in the network. Continuity between the tubular ER which forms a lamellar body and the tubular ER Which forms the subsurface cisternae was also observed in three dimensions. Therefore, the ER appears essentially a single system of tubules.

Several hypotheses had been proposed as to function of the subsurface cisternae, such as mechanical support of the cell body (Saito, 1983), maintenance of a potential gradient or insulation (Spoendlin, 1966), and transport of synaptic transmitter (Lubitz, 1981). Lim and Melnick (1971) suggested that the endoplasmic reticular system may have a function in regulating ions and therefore neural conduction. As another possibility, cisternae of this tubular system may be playing some role in pooling synaptic transmitter.

Acknowledgements

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References

Engström H,Wersäll J.(1953).Structure of the organ of Corti.1.Outer hair cells.Acta Otolaryngol.43,1-10.

Engström H,Ades HW.(1973).The ultrastructure of the organ of Corti.In:Friedmann I (ed) The ultrastructure of sensory organs.North Holland, American Elsevier,Amsterdam London New York,85-151.

Harada Y,Tagashira N,Takumida M,Suzuki M.(1985). Three-dimensional structure of the vestibular sensory epithelia.Acta Otolaryngol.99,411-418.

Inoué T.(1982).The continuity of endoplasmic reticulum in rat spermatids observed by scanning electron microscopy.J Electron Microsc.31,261-263.

Iurato S.(1961).Submicroscopic structure of the membranous labyrinth-II.The epithelium of Corti's organ.Z Zellforsch.53,259-298.

Lim DJ.(1969).Three dimensional observation of the inner ear with the scanning electron microscope. Acta Otolaryngol.Suppl.255.

Lim DJ.(1971).Scanning electron microscopic observation on non-mechanically cryofractured biological tissue.Scanning Electron Microsc. 1971:257-264.

Lim DJ,Melnick W.(1971).Acoustic damage of the cochlea.Arch Otolaryngol.94,294-305.

Lubitz DKJE von.(1981).Subsurface tubular system in the outer sensory cells of the rat cochlea.Cell Tissue Res.220,787-795.

Saito K.(1983).Fine structure of the sensory epithelium of guinea-pig organ of Corti: Subsurface cisternae and lamellar bodies in the outer hair cells.Cell Tissue Res.229,467-481.

Spoendlin H.(1966).The organization of the cochlear receptor.Adv Oto-Rhino-Laryngol.13, 1-227.

Spoendlin H.(1968).Ultrastructure and peripheral innervation pattern of the receptor in relation to the first coding of the acoustic message.In:De Reuck AVS,Knight J(eds) Hearing in vertebrates(A Ciba Symposium).Churchill,London, 89-119.

Tanaka K,Naguro T.(1981).High resolution scanning electron microscopy of cell organelles by a new specimen preparation method.Biomedical Res.2,Suppl.63-70.

Tanaka K, Mitsushima A. (1984). A preparation method for observing intracellular structures by scanning electron microscopy. J Microsc. 133, 213-222.

Discussion with Reviewers

J.T.Corwin: How were the cuticular plates removed from these hair cells during their preparation?
Authors: The elements which compose the cuticular plate, such as actin filament are digested in dilute osmium solution. That is the reason why all cuticular plates were removed.

J.T.Corwin: Have you observed any connections between the subsurface cisternae and the nuclear envelope?
Authors: No, we haven't.

J.T.Corwin: In the right half of figure 2a the cytoplasmic side of the apical (ciliated)surface of a hair cell is visible. In that partially transparent view the stereocilia appear to project above openings in the surface membrane, did your preparation method remove all the cytoskeletal elements that are normally associated with stereocilia as that image suggests?
Authors: The answer for this question is closely related to that of the first question. As is shown in the figure, all the cytoskeletal elements are digested and removed during the preparation process.

J.T.Corwin: Did you observe any specializations of subsurface cisternae in the vicinity of synapses?
Authors: No, we didn't.

J.T.Corwin: Would you care to advance a hypothesis that might explain the change in stratification that you observed in the subsurface cisternae in the infranuclear regions of these cells?
Authors: We have no idea of a functional hypothesis as to the change in stratification.

D.J.Lim: In your Fig. 1, I observed that the nuclei of the outer hair cells were not fractured. Is this because the nucleus was below the fracture line, or somehow the tissue was warm during the fracturing? A related question is, what in your estimation is the optimum temperature for a clean fracture of the organ of Corti?
Authors: As you pointed out, it is because the nucleus was below the fracture line. We fracture specimens in liquid nitrogen. Therefore the temperature of -196°C which is the boiling point of nitrogen is the only temperature we used.

D.J.Lim: You state that 60-100 hours is the time required for osmium digestion or maceration. Would you elaborate on what is the ideal time window to produce the best results? Please explain what if the specimens are under- or over-digested.
Authors: We think 100 hours is the best time at 23°C. The time of maceration should be changed according to the temperature of the maceration. When the specimens are under-digested, only fractured surface can be seen. While the specimens are over-digested, the intracellular structure would be destroyed, thus resulting in many artifacts.