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SCANNING ELECTRON MICROSCOPIC OBSERVATION OF THE CRISTA AMPULLARIS

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Abstract

The crista ampullaris of the guinea pig and the bull frog were investigated by scanning electron microscopy. The crista ampullaris were freeze fractured or sheared followed by maceration with 0.1% OsO_4 solution. Following this, three-dimensional intracellular structures were observed. The mitochondria of the sensory cells varied in shape from globular to long and slender. Golgi apparatus and endoplasmic reticulum of the sensory cells were also demonstrated clearly. Nerve elements, nerve endings and synaptic structures were also observed stereoscopically.

KEY WORDS: Crista ampullaris, Ultrastructure, Scanning electron microscopy, A-O-D-O method, Guinea pig, Bull frog, Nerve ending, Sensory cell, Supporting cell.

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Introduction

There have been many morphological studies on the crista ampullaris using transmission (TEM) and scanning electron microscopy(SEM). TEM study reveals intracellular fine structures, while SEM makes it possible to observe the surface structures three-dimensionally. Recent progress with the SEM and preparation technique made possible good access to intracellular structures. This study was designed to investigate the interior stereoscopic structure of the crista ampullaris using an osmium maceration technique.

Materials and Methods

Adult guinea pigs with normal Preyer's reflex and bull frogs were used for this study. Specimens were prepared by A-O-D-O method [4]. The guinea pigs were given cardiac perfusion with 0.5% glutaraldehyde and 0.5% paraformaldehyde mixture buffered with phosphate buffer solution (pH 7.4) under deep general anesthesia. Bull frogs were freshly decapitated. The cristae ampullaris were removed and fixed at 4°C for 30 min. in 1% OsO_4 solution. After rinsing, specimens were dipped into 25% dimethyl sulfoxide (DMSO) for 30 min. and 50% DMSO for 1 h. They were then sheared in the phosphate buffer solution or frozen in liquid nitrogen and fractured. The fractured or sheared specimens were again rinsed in the buffer solution, followed by maceration at 23°C in 0.1% OsO_4 solution for 60-100 h. Following this procedure, the cytoplasmic matrix was adequately removed. The specimens were post-fixed in 1% OsO_4 solution for 1 h. and conductive stained by 2% tannic acid for 2 h. and 1% OsO_4 solution for 1 h. Following dehydration with graded ethanol, the specimens were treated with isoamylacetate, critical point dried (CO_2) and were coated with platinum. High resolution field emission SEM (Hitachi S800 was used for all observation.

Results

When the crista ampullaris was prepared by the A-O-D-O method [4], a fine intracellular structure was clearly revealed three-dimensionally by SEM. At low magnification, flask-shaped type I cells and cylindrical type II cells, supporting cells and other structures were clearly observed (Fig 1). Under high magnification, intracellular structures such as mitochondria, endoplasmic reticulum, Golgi apparatus and internal structures of the nerve elements were also demonstrated. Inside the sensory cell, numerous mitochondria of 0.5-1 μm in diameter were clearly observed. They were generally round but occasionally long, slender and branched. In the cracked surface of the mitochondria, their cristae were clearly demonstrated. The cristae of the mitochondria were generally lamellar but occasionally tubular (Fig 2). The three-dimensional structure of the Golgi apparatus was also revealed. The Golgi cisterns piled tightly one upon the other and formed multiple lamellae. The surface of the outermost and innermost cisterns of the Golgi stack showed a net-like structure with many small pores (Fig 3). In addition, the Golgi cisterns were occasionally connected to the endoplasmic reticulum (Fig 4). The endoplasmic reticulum formed variable structures, such as tubular and multiple lamellae. Tubular endoplasmic reticulum was generally found in the supra-nuclear region and formed complicated net-like structures (Fig 5), while the multi-lamellar ones were generally observed in the infra-nuclear region. In the rough endoplasmic reticulum, the numerous ribosomes attached on its surface were demonstrated clearly (Fig 6).

The three-dimensional structures of the fine nerve elements were also demonstrated. The type I cells have nerve chalice. Inside the nerve chalice, numerous mitochondria were observed. They were generally long and slender in the upper part of the nerve chalice (Fig 7), and were various shapes such as round or long and had occasional processes and branches in the lower part of the chalice (Fig 8). By the sheared preparation, the membrane of the nerve chalice was sheared and the mitochondrial figures revealed clearly (Fig 9). Inside the nerve chalice, there were fine fibrous structures forming a network around the mitochondria, considered to be endoplasmic reticulum. The nerve endings, both efferent and afferent, attaching to the type II cells were button shaped. In the efferent nerve endings, numerous synaptic vesicles 40-90 nm in diameter attached to the surface opposed to the sensory cell side and a synaptic cistern was also demonstrated inside the sensory cell (Fig 10). In this study, the fine structures of the synaptic bar was clearly demonstrated. The synaptic vesicle was linked to the synapti lod with fine stalks, giving an appearance of a bunch of grapes (Fig 11).

When the cytoplasmic membrane was sheared, a number of granules were observed inside the supporting cell. In the view of cracking surface, these granules looked like vacuoles, probably caused by maceration with 0.1% OsO_4 solution (Fig 12). These granules were 0.2-0.4 μm in diameter. Other intracellular organelles, such as mitochondria and Golgi apparatus also could be clearly demonstrated.

Discussion

Both TEM and SEM have been used for vestibular morphology [1,2,3]. However, three-dimensional intracellular structures were not well examined. Recent progress of SEM and the preparation technique made it possible to observe the intracellular fine structures three-dimensionally. The A-O-D-O method [4] made it possible to observe the intracellular structures by SEM. The shape of mitochondria varied greatly. They were round, long, slender and occasionally had processes and branches. And the mitochondrial cristae also varied such as tubular or lamellar. The Golgi apparatus was also demonstrated clearly and in this study, the connection of Golgi cisterns and endoplasmic reticulum was also observed. The endoplasmic reticulum also varied in shape. They were tubular, multi-lamellar and making a fine net-like structure. According to the nerve elements, nerve endings and synaptic structures were demonstrated three-dimensionally. In conclusion, A-O-D-O method [4] offers a great value in the study of vestibular morphology three-dimensionally.

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Discussion with Reviewers

D.J.Lim: One of the difficulties of the A-O-D-O technique is the preservation of the cell organelles. Did you encounter any such difficulty in the preservation of the

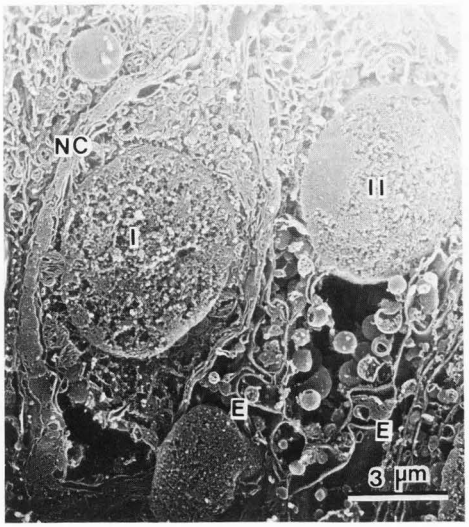

Fig 1. Fractured surface of the crista ampullaris (guinea pig)
I:type I cell, II:type II cell, NC:nerve chalice
E:nerve ending

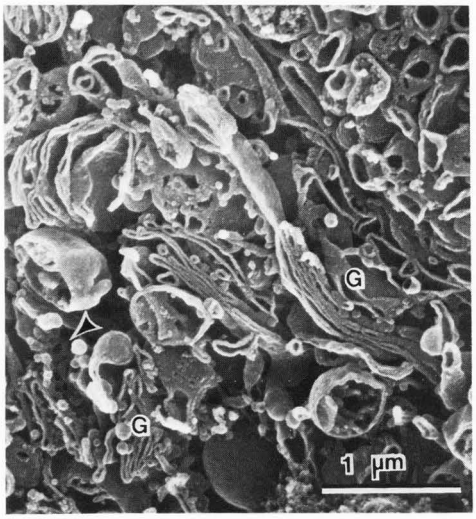

Fig 3. The Golgi cisterns pile up one upon the other to form multiple lamellae (G). The Golgi stack shows a net-like structure with many small pores (arrow head)

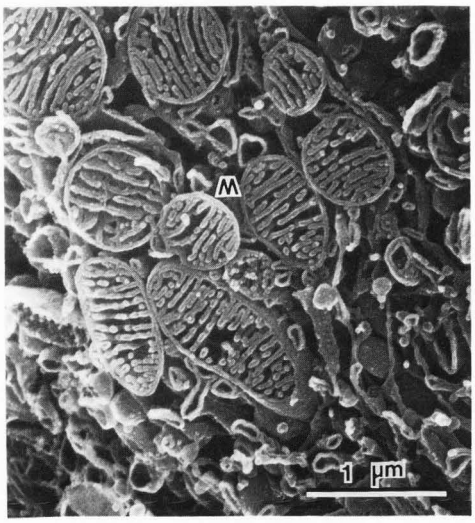

Fig 2. Inside the sensory cell, numerous mitochondria (M) and lamellar cristae are observed

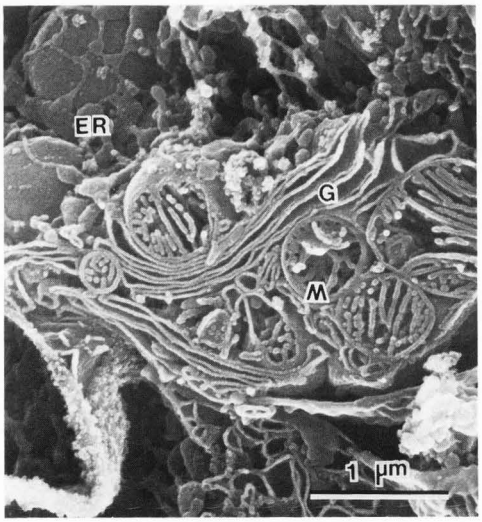

Fig 4. The Golgi cisterns (G) connect to the endoplasmic reticulum (ER).
M:mitochondria

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frog compared with the guinea pig?
Authors: We did not find that preservation of the frog specimen is more difficult than that of the guinea pig. Preservation technique is basically the same, except that cardiac perfusion is recommended in the guinea pig to get good preservation.

D.J.Lim: You say that you prepared the specimen by "sheared" preparation. Please elaborate on the technique involved and why you are using this technique.
R.Harrison and R.Mount: How is the shearing procedure carried out and do the results differ notably from the fractured preparation?
T.Inoue: What is the precise method of "sheared preparation"? This is obviously of

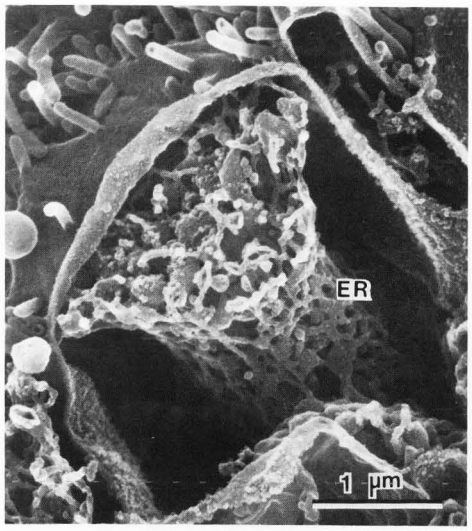

Fig 5. The supra-nuclear region of the sensory cell (guinea pig)
Smooth endoplasmic reticulum forms a fine net-like structure (ER)

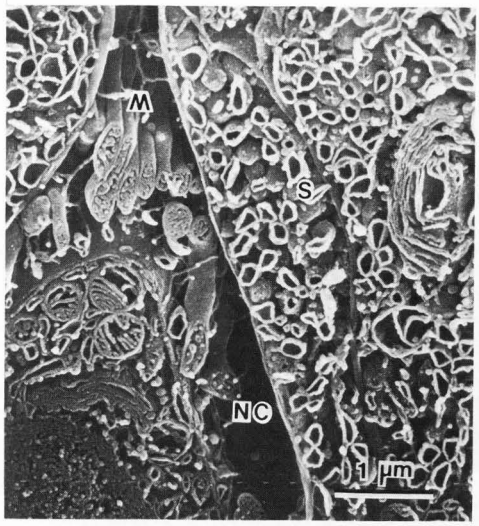

Fig 7. Upper part of the nerve chalice (guinea pig)
Long shaped mitochondria are generally seen (M).
NC:nerve chalice, S:supporting cell

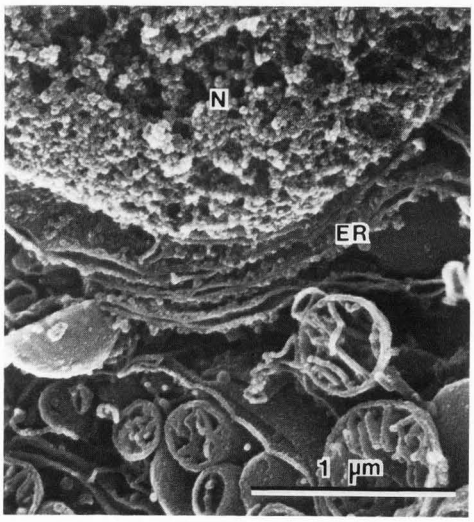

Fig 6. The infra-nuclear region of the sensory cell (guinea pig)
Rough endoplasmic reticulum (ER) form multiple lamellae. Each lamella is associated with numerous ribosomes. N:nucleus

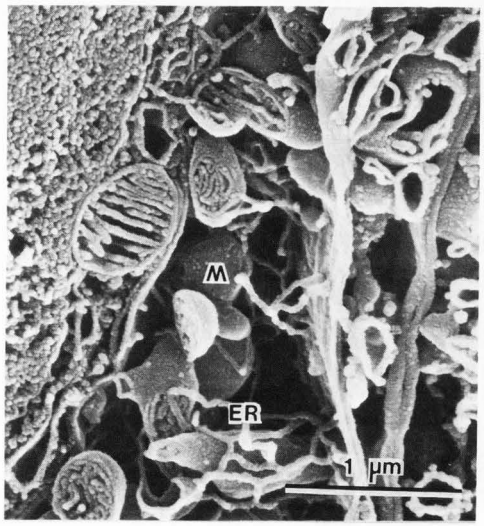

Fig 8. Lower part of the nerve chalice (guinea pig)
Mitochondria (M) show variable structures and tubular endoplasmic reticulum (ER) form a network around the mitochondria.

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importance to those who wish to use the method to examine such structures as you have shown.
Authors: After fixation, we used two fine forceps to hold the lateral parts of the crista in the phosphate buffer solution. Then, one of the forceps was gently moved away from the other to tear the specimen into two pieces. In the sheared specimen, the membrane of the cell body was peeled off (not fractured) and the outer surface of the cell body, nerve chalice, outer surface of the mitochondria etc were revealed more clearly than freeze fracturing.

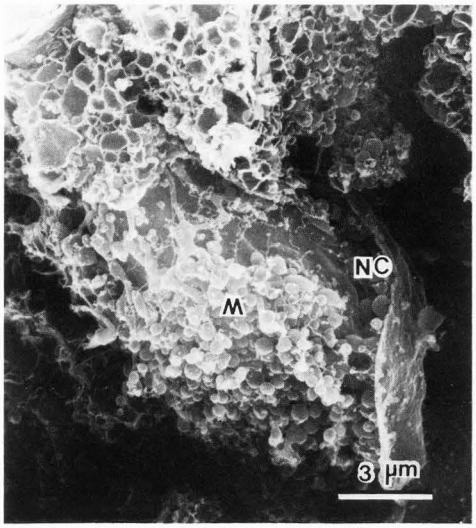

Fig 9. By the sheared preparation, the mitochondrial figures (M) inside the nerve chalice (NC) are revealed clearly

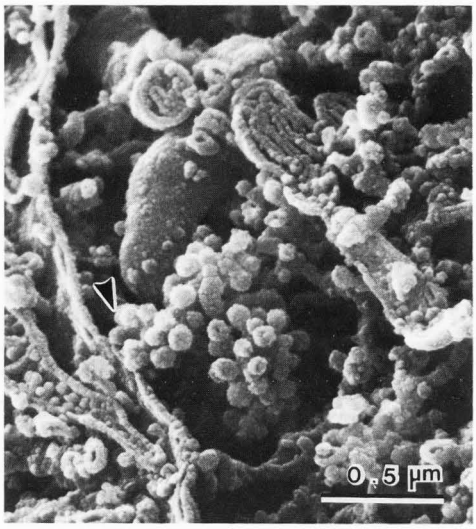

Fig 11. Synaptic bar (bull frog)
Each synaptic vesicle is linked to the synaptic rod with fine stalk (arrow head)

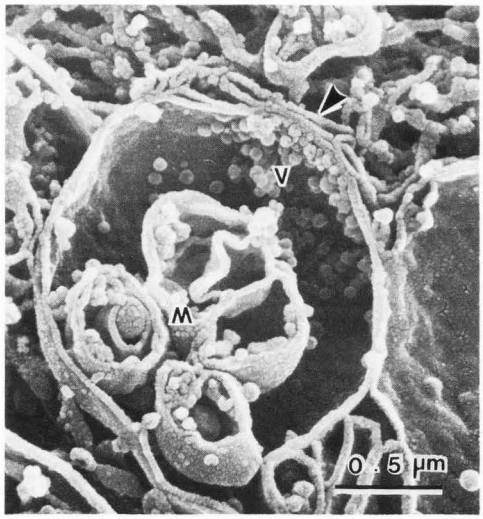

Fig 10. There are numerous vesicles attaching to the inner surface of the efferent nerve ending. Synaptic cisterns are also demonstrated (arrow head). M:mitochondria, V:synaptic vesicles

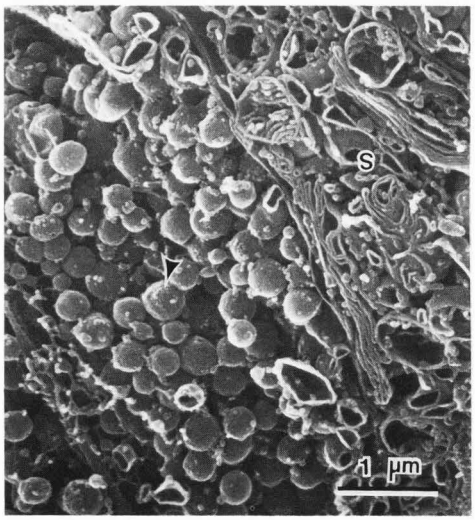

Fig 12. Supporting cell (guinea pig)
The cytoplasmic membrane is sheared and numerous granules inside the supporting cell are exposed (arrow head). S:sensory cell

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T.Inoue: How do you coat the specimen with metal?

D.J.Lim: What is the make of your platinum coater? What is the setting for the evaporation, and for how long do you evaporate? Do you have any idea of how thick the platinum coating is? The coating in Figs. 2, 3 and 4 appears to be much thinner than that in Fig 11. Is this because of differences in the magnification?

Authors: We use a cool sputter coating system (POLARON SEM coating system) equipped with a thickness monitor. Thickness of the coating was set either at 20 Å or at 40 Å. The thickness of coating in Fig 11 is 40 Å which had been

formerly used. Today, we prefer a coating thickness of 20 Å as are used in Figs. 2, 3 and 4, since 20 Å thickness generally results in better photograph.

D.J.Lim: Do you use a different buffer in the initial fixation of the tissue from guinea pigs and frogs?
Authors: We use phosphate buffer solution (pH 7.4) in fixation of both guinea pigs and bull frogs.

T.Inoue: In the original AODO method by Tanaka and Mitsushima (1984), specimens are macerated at 273K (20°C). However, the authors macerated the specimen at 23°C. Are there any merits for maceration of the specimens at a little higher temperature?
Authors: The effect of maceration is not really different between the temperature of 20°C and 23°C. However, the maceration process is faster at 23°C as compared to 20°C.

T.Inoue: Please add the accelerating voltage of the SEM during the observation and photography.
Authors: The accelerating voltage is 25 kV during both observation and photography.