서울醫大學術誌 제25권 제4호
The Seoul Journal of Medicine
Vol. 25, No. 4, December 1984
ISSN 0582-6802

Electron Microscopic Observation of Striated Organelle of Vestibular Hair Cells Treated with Tannic Acid

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INTRODUCTION

Striated organelle (Friedmann body, laminated cytoplasmic inclusion) was first observed in 1963 by Friedmann et al. in the degenerating utricular hair cells from patients with Menier's disease. They reported this Friedmann body, consisting of alternating thick and thin electron-opaque lines to form a characteristic pattern, near the surface or within the cytoplasm of degenerating cells.

The striated organelles have usually been associated with pathological conditions such as eighth nerve tumors (Hilding and House, 1964), Conn's syndrome (Friedmann et al., 1965), drug intoxication (Friedmann et al., 1966; Jahnke, 1969), or with old age (Rosenhall et al., 1974). Nevertheless, their consistent occurrence has also been noted in the inner ear hair cells of normal cat (Spoedlin, 1966), squirrel-monkey (Engstrom et al., 1972), chinchilla (Slepecky et al., 1980), and rat (Ross and Bourne, 1983). From these reports, striated organelle was proposed to be the normal constituent of hair cells (Slepecky et al., 1980).

Although several possibilities about the role of the striated organelle have been suggested (Slepecky, 1980; Jorgensen, 1982; Ross, 1982), no clear explanation has been achieved. Therefore, it is important to observe more morphological chacteristics of the structure which will become the basis of any functional investigation.

In order to obtain the enhanced visibility of the striated organelle, tannic acid which acts as a mordant between osmium treated structure and lead (Simionescu et al., 1976) was added to glutaraldehyde fixative.

MATERIALS AND METHODS

(All procedures described below were carried out at 0-4°C unless otherwise indicated.)

Young adult, Sprague-Dawley rats were sacrificed by decapitation. From the ventral orientation, the lower jaw and the soft tissues covering the nasopharynx were dissected away. After bullae were removed from the skull to expose the middle ear cavity, the obtained specimens were immersed in standard phosphate buffer (SPB) consisting of 100mM KCl, 5mM $MgCl_2$, and 6mM sodium phosphate at a final pH of 7.0 at 23°C. Perfusion was carried out through the opened oval window and round window by applying mild pressure with a rubber bulb of pasteur pipette. Maculae were dissected under a dissecting microscope and the otoconia were removed by flushing the buffer through with a pasteur pipette or a micropipette.

Triton Extraction

The Triton extraction method of demembranation was carried out with 1% Triton X-100 (a nonionic detergent, Sigma) in SPB at pH 7.0

† 접수일자 : 1984년 10월 25일

for 10 minutes (Mooseker, 1975; Flock, 1981) or 30 minutes at room temperature. Specimens were washed three times with SPB, pH 7.0 for 30 minutes and processed as indicated below.

Fixation

After a 10 minute wash in Sorensen's 0.1 M phosphate buffer at pH 7.0 with 0.1M sucrose, specimens were fixed with 1% glutaraldehyde containing 0.2% tannic acid (Begg et al., 1978) in Sorensen's buffer for 1 hour at room temperature. Specimens were washed three times, and then postfixed for 1 hour in 1% OsO_4 in Sorensen's 0.1M phosphate buffer, pH 6.0.

Electron Microscopic Procedure

The osmicated specimens were washed three times with distilled water for 30 minutes (Hirokawa et al., 1982), and stained en bloc for 2-3 hours in 0.5% uranyl acetate buffered to pH 5.0 with veronal acetate buffer. The specimens were then dehydrated, embedded, and sectioned. Thin sections were stained in uranyl acetate and lead citrate and examined with a Philips-400 electron microscope.

RESULTS

The striated organelle was found in both type I and type II hair cells of macula utriculus. Fig. 1 is showing striated organelle in the specimen without tannic acid treatment. After the addition of tannic acid to glutaraldehyde, striated organelle and cuticular plate were shown more clearly although some cellular components including plasma membrane and mitochondria appeared extracted due to the Triton X-100 demembranation (Fig. 2~9).

Most of the striated organelles appeared very similar to the typical Friedmann body consisting of parallel thick and thin electron-opaque bands. The distance between the two adjacent thick (thin) electron-opaque bands was approximately 130-180nm. Each band was connected with the adjacent bands through filaments(Fig. 2 & 6). Rarely, the striated organelle was composed of only electrondense thick bands. The orientation of the striation was not in any particular direction regardless its location(Fig. 4 & 5). At times, more than one striated organelle seemed to merge to show curved appearence (Fig. 7).

The striated organelle was distributed inside the cuticular plate as well as outside including the area far below the base of cuticular plate (Fig. 8) Through filaments, the connection between the thin electron-opaque stripe and the electron-opaque area of the junctional complex seemed to exist (Fig. 2 & 3). The connection between the rootlet of the stereocilia and the striated structure was also observed (Fig. 9).

DISCUSSION

Utricular sensory epithelium, macula utriculus, consists of type I and type II hair cells along with supporting cells intervening between the hair cells. Each hair cell has one kinocilium which is morphologically similar to motile cilium and many(50-110) stereocilia which are modified microvilli. The filamentous area of the apical cytoplasm basal to the rootlets of the stereocilia is called cuticular plate. Because of the morphological resemblance of hair cells to intestinal epithelial cells (Mooseker et al., 1975), report of contractile proteins in the apical part of brush border cells (Bretscher and Weber, 1978) has increased the interest in the presence and organization of actin and actin-binding proteins in the hair cells.

In this study, we could obtain the well preserved, extensive striated organelle by using fixation method known to be optimal for the preservation of actin filaments (Maupin-Szamier et al., 1978): microdissection, glutaraldehyde fixation with tannic acid (Ishikawa, 1969; Simionescu, 1976), and postfixation with osmium

tetroxide (pH 6.0) in phosphate buffer at 0-4°C. After fixation in Karnovsky's fixative which has been conventionally used for fixation of inner ear tissue, the striations hardly appeared. Although striated organelle extended to the infracuticular area for various distances, it is mostly located in the apical region of the hair cell cytoplasm. Therefore, demonstrations of actin (Flock et al., 1977; Slepecky, 1982) and myosin (Macartney et al., 1980) in the apical part of hair cells suggest the existence of actin-myosin type interaction in the striated organelle. Also, observation of various cells such as intrafusal muscle fiber (Karlsson et al., 1968), smooth muscle cell (Ashton et al., 1975) and rhizoplast of the quadriflagellate green algae (Salisbury and Floyd, 1978) which have striated organelle correlated with contractile systems supports this idea.

The connection of the striated organelle with the rootlet of the stereocilia and electron-opaque material of the junctional complex implies the role of the striate organelle as an anchoring structure of the stereocilia rootlet to the cuticular plate. As suggested by Slepecky (1980), the striated organelle may have the function similar to that of the terminal web. It may arrange the stereocilia rootlet filament in the cuticular plate and fasten the filaments to the cell membrane by connecting it to the electron-dense material of the junctional complex. Another possibility from the above connection is the involvement of the striated organelle in conduction process by transmitting the signal from the apical part of the hair cell membrane to the lateral cell membrane (Ross, 1982) or to the synaptic structure as in the labyrinthine sensory cell of the ammocoete larva of the lamprey (Lowenstein and Osborne, 1964). The latter idea is supported by the location of striated organelle below the cuticular plate and close to the synapse.

In the inner hair cells of the chinchilla, the substructure of the striated organelle showed membraneous lamella (Slepecky, 1980). However, our study on the striated organelle of vestibular hair cell didn't show any morphological difference irrespective of section planes. This may reflect the difference in its functional role between the cochlea and vestibular sensory epithelium or between species.

Further investigation using various techniques including electron microscopic immunocytochemistry, calcium-ATP induced contraction, and decoration of actin filaments with myosin subfragment S_1 may give us more information on the functional roles of striated structure.

SUMMARY

The striated organelle (laminated cytoplasmic inclusion, Friedmann body) of the utricular sensory hair cells of the rat was investigated under transmission electron microscope. Tannic acid-glutaraldehyde treatment following demembranation by Triton X-100 enhanced the visibility of the fine structure of the striated organelle. The following findings were observed:

1. Most of striated organelles observed resembled Friedmann body consisting of alternating thick and thin electron-opaque stripes connected through filaments.
2. Orientation of the striation of the striated organelle was not in any particular direction.
3. Striated organelle was connected with junctional complex and stereocilia rootlet through filaments.
4. More than one striated organelle located separately or merged could be seen in each hair cell.
5. Location of striated organelle was inside or outside the cuticular plate.

=국문초록=

탄닌산 처리후의 內耳感覺細胞內 橫紋小器官 微細構造의 觀察

서울大學校 醫科大學 解剖學敎室
李 秉 蘭
〈지도 : 成春暻 敎授〉

흰쥐 내이(inner ear)의 감각유모세포(utricular hair cell)내에 존재하는 횡문소기관(striated organelle)은 1963년 Friedmann의 보고 이후 병적인 현상이나 노화와 관련되어 생각되어 왔으나, 최근에 이르러서는 기능적역활을 가진 세포내 정상구조로 추정되고 있다. 저자들은 정상동물에서 이의 자세한 미세구조를 밝히기 위하여 glutaraldehyde 고정시에 탄닌산처리를 한 표본을 전자현미경으로 관찰하여 다음과 같은 소견을 얻었다.

1. 횡문소기관의 대부분은 전자밀도가 높은 굵은 띠(thick stripe)와 가는 띠(thin stripe)가 반복되어 구성하고 있었으며 두 띠간에는 세사(filament)들로 연결되어 있었다.

2. 횡문소기관은 세사를 통해 세포간결합복합체(junctional complex)나 부동모(stereocilia)의 소근(rootlet)과 연결되어 있는 것이 관찰되었다.

3. 횡문소기관의 배열방향은 일정치 않았으며 두 개의 소기관이 합치는 곳에서 횡문의 굴곡을 관찰할 수 있었다.

4. 횡문소기관은 소피판(cuticular plate)의 내부 혹은 아래쪽에 위치하고 있었다.

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LEGENDS FOR FIGURES

Fig. 1. Hair cell observed after ordinary glutaraldehyde-osmium fixation method. Stereocilia (SC) are seen protruding into the endolymphatic space (EL) and continued as rootlets (R) in the cuticular plate (CP) located in the apical cytoplasm. Arrows indicate thick and thin electron-opaque stripes of the striated organelle. ×42,900.

Fig. 2. Plasma membrane(M) is seen extracted due to Triton X-100 treatment. Filamentous structures are more clearely visible than those of non-treated tissue. The thin electron-opaque stripe (leftmost arrow) of the striated organelle in the infracuticular area is connected with the electron-opaque area of junctional complex(JC) through filaments. Adjacent supporting cell(S) shows a reticular membrane(RM). Tannic acid treatment. ×42,900.

Fig. 3. Striated organelles are seen in broad portion of the cuticular plate. Arrows indicate electron-opaque stripes intercommunicating through filaments (asterisk). Tannic acid treatment. ×63,250.

Fig. 4. Direction of the striation (arrows) of striated organelles is almost parallel to that of the apical surface of the cell. Tannic acid treatment. ×49,500.

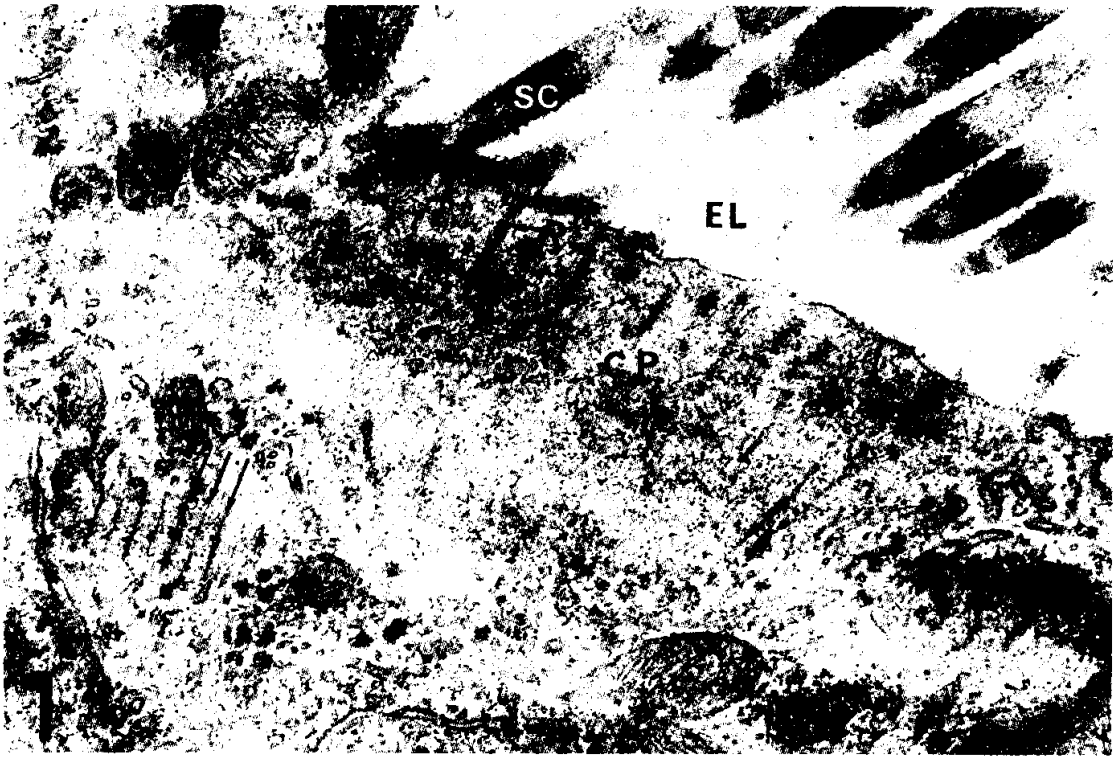
Fig. 5. Striation (arrows) is arranged in a different direction from that in Fig. 4. A mitochondrion(MT) is seen. Tannic acid treatment. ×49,500.

Fig. 6. A basal body(B) of kinocilium is located in the cuticle-free area of the apical cytoplasm. Striated organelles (arrows) are seen. Tannic acid treatment. ×30,000.

Fig. 7. A higher magnification of Fig. 6. Striation shows curved appearances (arrow heads) where twe striated organelles merge together. The connecting filaments (asterisk) are clearly demonstrable. Tannic acid treatment. ×106,500.

Fig. 8. Striated organelles (arrows) are located for various distances below the cuticular plate(CP). Tannic acid treatment. ×41,250.

Fig. 9. A rootlet(R) of stereocilium(SC) seen connected with a electron-opaque stripe of a striated organelle (arrows) through filaments. Tannic acid treatment. ×88,750.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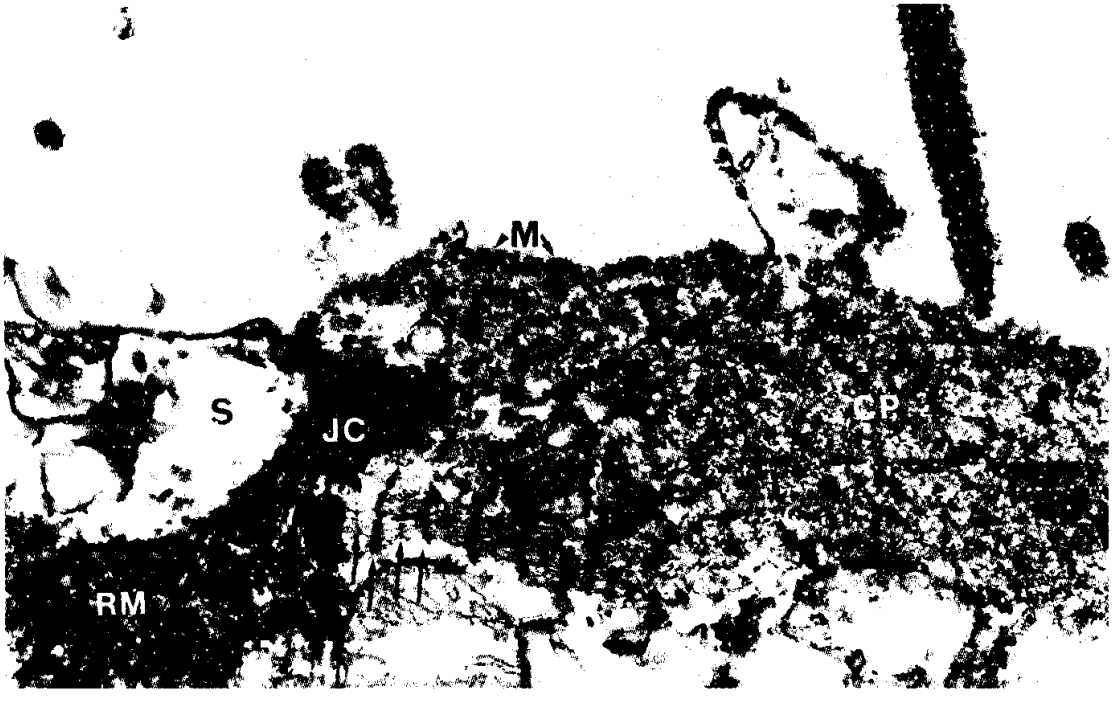
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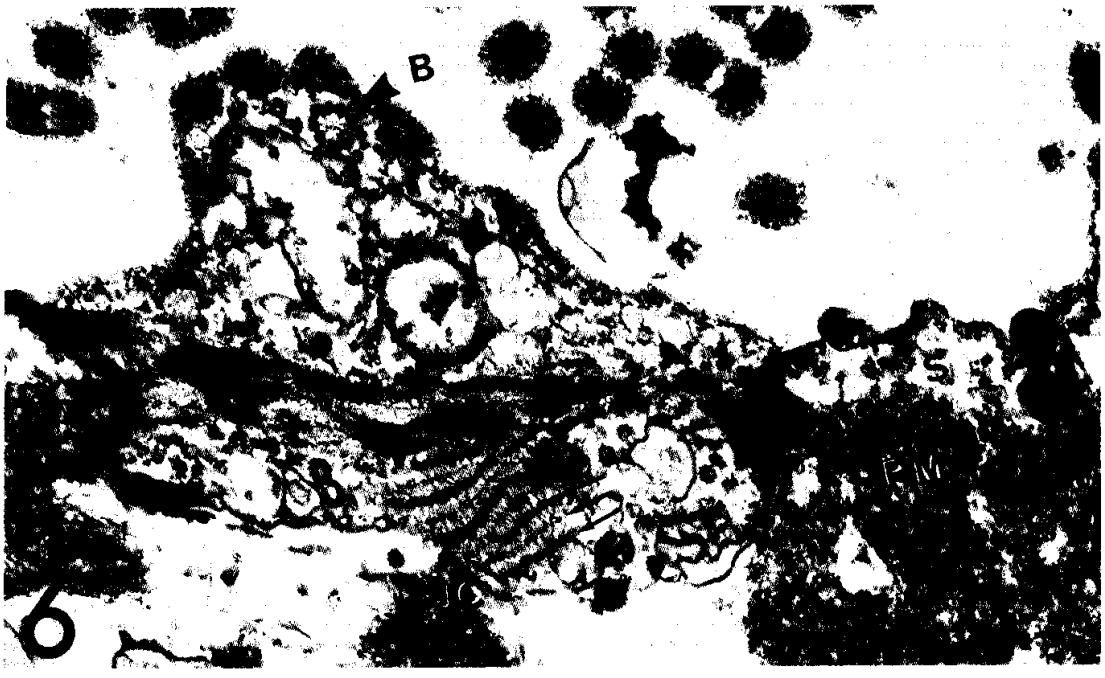
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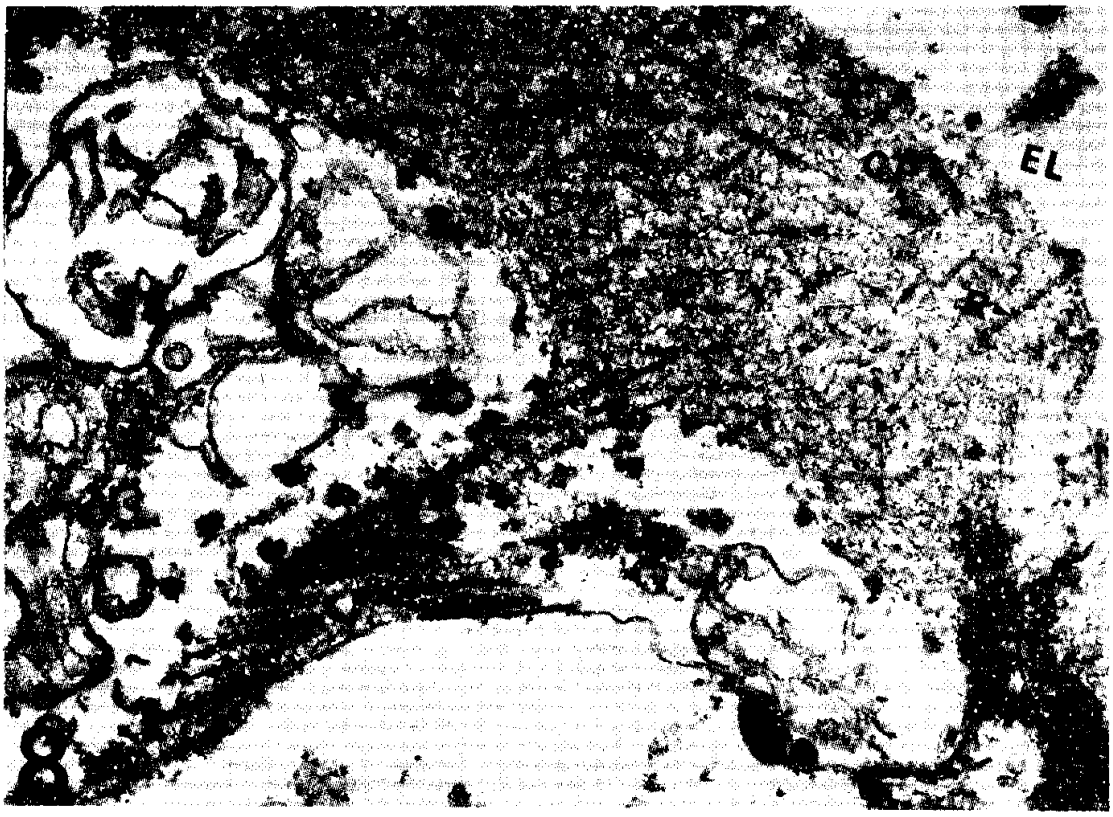
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