Original Paper

Audiology & Neurotology

Audiol Neurotol 2005;10:220–233
DOI: 10.1159/000084843

Received: March 3, 2004
Accepted after revision: December 3, 2004
Published online: March 31, 2005

Permanent Threshold Shift Caused by Acute Cochlear Mitochondrial Dysfunction Is Primarily Mediated by Degeneration of the Lateral Wall of the Cochlea

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Key Words
3-Nitropropionic acid · Cochlea · Spiral prominence · Spiral ligament · Stria vascularis · Mitochondrial dysfunction · Permanent threshold shift · Rat

Abstract
Mitochondrial dysfunction in the cochlea is thought to be an important cause of sensorineural hearing loss. Recently, we have established a novel rat model with acute hearing impairment caused by exposure to the mitochondrial toxin 3-nitropropionic acid (3-NP) to analyze the mechanism of cochlear mitochondrial dysfunction. Both permanent and temporary threshold shifts were observed in this model depending on the amount of 3-NP used to induce hearing impairment. In this study, we demonstrate cochlear morphological changes in the permanent threshold shift model. Marked degeneration was detected in type 2 fibrocytes in the spiral prominence, type 4 fibrocytes in the spiral ligament, marginal cells and intermediate cells in the stria vascularis 3 h after 3-NP administration; these changes were progressive for at least 14 days. Less prominent degeneration was detected in type 1 and type 3 fibrocytes in the spiral ligament. These results indicate that permanent threshold shift caused by acute cochlear mitochondrial dysfunction is primarily mediated by cellular degeneration in the lateral wall of the cochlea, and suggest that therapy of cochlear hearing loss due to acute energy failure may be achieved through protection and regeneration of the cochlear lateral wall.

Introduction

Many mutations in mitochondrial DNA cause both nonsyndromic and syndromic sensorineural hearing loss [Hutchin and Cortopassi, 2000] that are primarily caused by cochlear dysfunction [Braverman et al., 1996; Sue et al., 1998; Yamasoba et al., 1996]. These facts suggest that cochlear cells are strongly dependent on mitochondrial function to maintain normal hearing. The most important cell physiological role of mitochondria is in oxidative

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phosphorylation to produce ATP, the primary source of cellular energy [Nicholls and Budd, 2000]. Mitochondria also function to integrate apoptotic pathways and to produce reactive oxygen species that cause cell damage [Green and Reed, 1998]. Both apoptosis and formation of reactive oxygen species have been reported to be involved in pathophysiological mechanisms of cochlear damage by ischemia, ototoxins, and noise [Huang et al., 2000; Yamane et al., 1995]. Thus, mitochondrial dysfunction is likely to play a critical role not only in hearing loss resulting from mitochondrial DNA mutations but also in other types of hearing loss.

To explore the effect of acute mitochondrial dysfunction in the cochlea, we established an animal model of acute cochlear energy failure by administering the mitochondrial toxin 3-nitropropionic acid (3-NP) into the rat round window niche [Hoya et al., 2004]. 3-NP is an irreversible inhibitor of succinate dehydrogenase, a complex II enzyme of the mitochondrial electron transport chain [Alston et al., 1977; Coles et al., 1979]. Systemic administration of 3-NP produces selective striatal lesion damage [Brouillet et al., 1995; Hamilton and Gould, 1987]. In our model, rats treated with 500 m*M* 3-NP exhibited a permanent threshold shift as measured by auditory brainstem response (ABR) while the same volume of 300 m*M* 3-NP caused only a temporary threshold shift [Hoya et al., 2004]. In the permanent threshold shift model, no response was detected by ABR using the maximal stimulus intensity for all tested frequencies (8, 12, 16, 20 kHz) 3 h after 3-NP administration, and this condition persisted for at least 28 days. In the temporary threshold shift model, the ABR thresholds deteriorated to the highest degree (approximately 80 dB SPL) at 1 day and recovered to normal levels at most frequencies in 14 days, although the ABR thresholds at 20 kHz remained significantly elevated (approximately 20 dB SPL) compared to the control even at 28 days [Hoya et al., 2004]. In the present study, we investigated structural and ultrastructural changes of the cochlea at various time points after 3-NP administration in the permanent threshold shift model.

Materials and Methods

Animals and Surgery

Experimental procedures reported in this study were approved by the Institutional Animal Care and Use Committee of the National Tokyo Medical Center. Female Sprague-Dawley rats weighing between 180 and 210 g (8–10 weeks old) were used. Before surgery, the animals were anesthetized with pentobarbital (30–40 mg/kg, i.p.) and an incision was made posterior to the left pinna near the external meatus after local administration of lidocaine (1%). The left otic bulla was opened to approach the round window niche. The end of PE 10 tubing (Becton Dickinson, N.J., USA) was drawn to a fine tip in a flame and gently inserted into the round window niche. 3-NP (Sigma, St. Louis, Mo., USA) was dissolved in saline at 500 m*M* and the pH was adjusted to 7.4 with NaOH. Saline alone was used as a control. The solution was administered for 2 min at a rate of 1.5 µl/min with a syringe pump. Following treatment, a tiny piece of gelatin was placed on the niche in order to keep the solution in the niche regardless of the head movement after awakening from anesthesia and the wound was closed. The right cochlea was surgically destroyed to avoid cross-hearing during ABR recording.

Histological Analysis

Histological analysis was performed at 3 h, 1 day, 7 days, and 14 days after 3-NP or saline administration (2 rats per time point). To confirm the level of hearing loss, ABR recording was performed before surgery, 3 h after 3-NP or saline administration and before transcardial perfusion for histological analysis at the end of the observation period (3 h, 1 day, 7 days, and 14 days after the administration) in each rat. Details of the method for ABR recording were described previously [Hoya et al., 2004]. Two untreated rats with normal hearing confirmed by ABR were also histologically analyzed as an additional control set. For fixation, rats treated with 3-NP as well as control rats were deeply anesthetized with pentobarbital and transcardially perfused with 0.01 *M* phosphate buffer (pH 7.4) containing 8.6% sucrose followed by fixative consisting of freshly depolymerized 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 *M* phosphate buffer (pH 7.4) containing 5% sucrose. After decapitation, left temporal bones were removed and immediately placed in the same fixative. Small openings were made at the round window, oval window and the apex of the cochlea. After immersion in the fixative overnight, the temporal bones were decalcified by placement in 0.1 *M* ethylene diamine tetra-acetic acid (pH 7.4) containing 5% sucrose that was stirred at 4°C for 6 days. The bones were then rinsed overnight in 0.1 *M* phosphate buffer containing 5% sucrose, postfixed in 1% osmium tetroxide in the same buffer for 150 min, dehydrated in a graded ethanol series, and embedded in Epon 812 resin. For light microscopy, semi-thin sections were cut in a horizontal plane parallel to the modiolus and stained with toluidine blue. The mean area of each spiral ganglion cell was measured in treated rats at each time point following the administration of 3-NP or control saline as well as in normal rats that did not receive any treatment. Measurements were based on 3 sections including the modiolus from each cochlea. First, images of spiral ganglia were taken with a light microscope (Eclipse E600; Nikon, Tokyo, Japan) equipped with a digital camera (PDMC-3; Nihon Poladigital, Tokyo, Japan). Second, the area of each individual spiral ganglion cell with a visible nucleus was measured in each section using digital image analysis software (MicroAnalyzer, Nihon Poladigital). For rats treated with control saline, the total number of spiral ganglion cells analyzed at each time point varied from 70 to 131 (mean: 97.5). For rats treated with 3-NP, the total number of spiral ganglion cells analyzed at each time point varied from 76 to 102 (mean: 89.8). In normal rats without any treatment, the total number of analyzed spiral ganglion cells was 101. For the statistical evaluation, the mean areas of spiral ganglion cells in rats treated with 3-NP and control saline were compared at each time point by the unpaired t test. Then, the mean areas of spiral ganglion cells in the normal rats and in rats treated with 3-NP were compared

by analysis of variance followed by the multiple comparison method.

For transmission electron microscopy, thin sections were cut at the apical side of the second turn, double-stained with lead citrate and uranyl acetate, and examined with a Hitachi H600 electron microscope. Because of the technical reason in cutting thin sections, ultrastructural observation was restricted to the organ of Corti, spiral prominence, stria vascularis, and a lower half of the spiral ligament which exhibited the most significant structural changes by light microscopy.

Results

General Structural Features of the Cochlea

The cochlear structures of both the experimental and control rats were examined at different time points following treatment. In these rats, no responses could be detected by ABR at 3 h after 3-NP administration using the maximal intensity at each tested frequency, and no recovery was observed thereafter. A typical tracing of the ABR result is shown in figure 1. Control rats did not exhibit a threshold shift following saline administration.

By light microscopy, there was a basal-to-apical gradient in the extent of cochlear damage in the rats treated with 3-NP, and the structural changes were most evident in the lateral wall in the middle and basal turns with lesser changes in the organ of Corti and modiolus. All structures in the apical turn were well preserved throughout the observation period. In the basal turn, inner and outer hair cells showed mild structural changes at 3 h after 3-NP administration, and these changes became severe by 7 days after administration. Supporting cells in the organ of Corti showed only mild degenerative changes throughout the observation period. The spiral ligament first exhibited mild structural changes at 3 h after 3-NP administration and the changes became severe at 7 days after administration. The stria vascularis exhibited mild structural changes at 7 days after 3-NP administration and became moderate at 14 days after administration. The structure of spiral ganglion cells remained normal during the observation period.

Although the histological changes were more severe and rapid in the basal portion of the cochlea, commonly recognized patterns of histological features were observed in both the middle and basal turns. Because of the relatively slow progression of histological changes in the apical side of the middle turn, we were able to analyze the process of morphological change in detail over time in this area. Thus, in the following sections, we describe the details of structural and ultrastructural changes of the cochlea at the apical side of the middle turn at various time points following 3-NP administration. There was little variation in the morphological findings between the 2 rats at each time point in both the experimental group and control group.

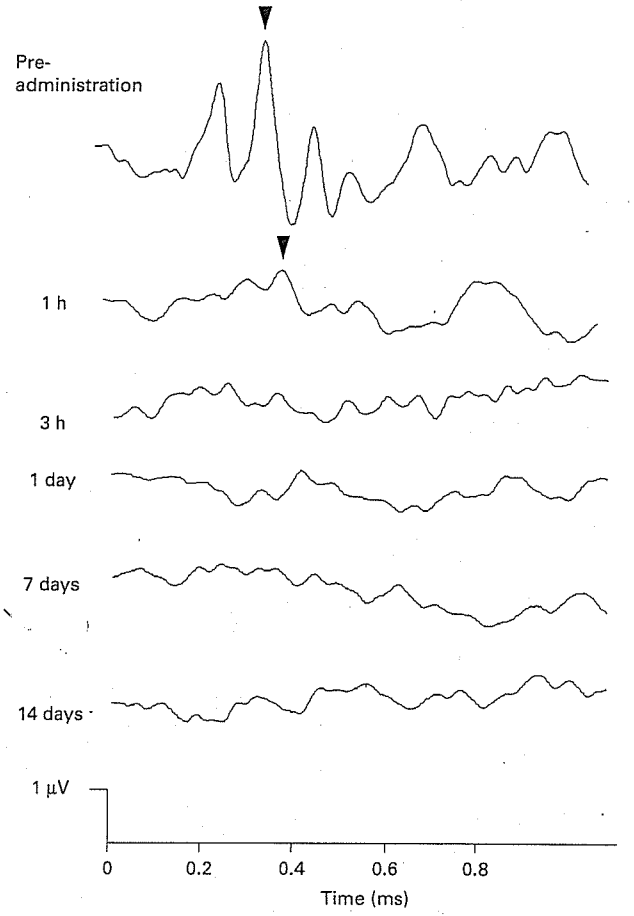


Fig. 1. A typical tracing of ABR results showing changes after administration of 3-NP. The stimuli consisted of pure tone bursts of 20 kHz and were delivered at 79 dB. Time after administration is indicated to the left of each tracing. Arrowheads indicate wave II. An additional recording 1 h after 3-NP administration is included to show the changes more clearly in this animal model.

Structural Changes in the Lateral Wall

Light microscopy revealed the enlarged extracellular spaces of the spiral prominence 3 h after 3-NP administration (fig. 2b). At 1 day after 3-NP administration, the extracellular spaces of the spiral ligament were also enlarged (fig. 2c), which became more apparent 7 days after

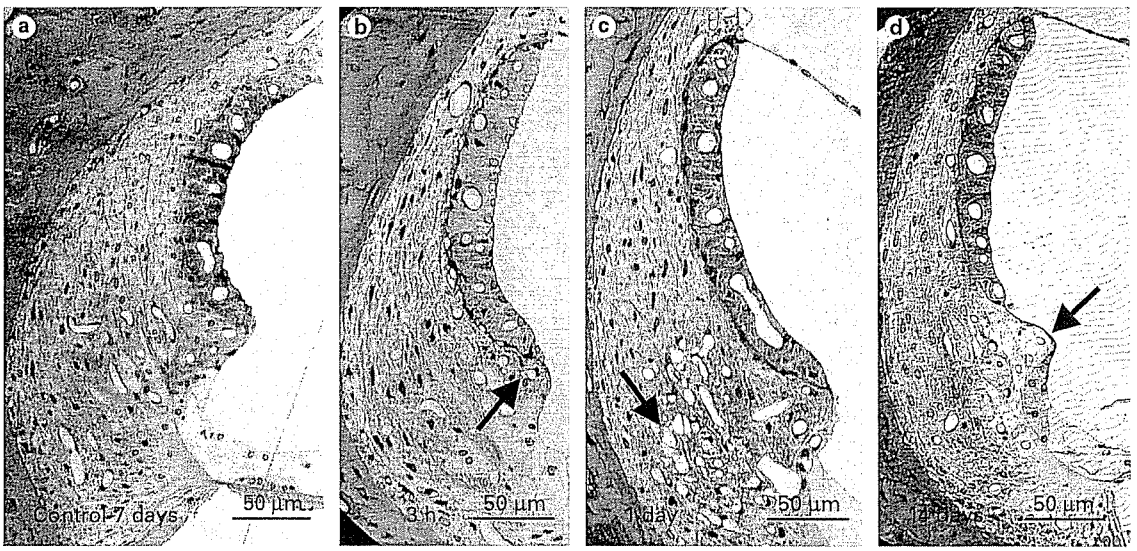


Fig. 2. Light microscopy of the cochlear lateral wall after the administration of control saline (**a**) or 3-NP (**b–d**). **a** Normal morphology at 7 days after saline administration. **b** At 3 h after 3-NP administration, the extracellular space in the spiral prominence was slightly enlarged (arrow). **c** At 1 day after 3-NP administration, the extracellular space in the spiral ligament was also remarkably enlarged (arrow). **d** At 14 days after 3-NP administration, fibrocyte loss was conspicuous in the spiral prominence (arrow).

the administration, and the majority of spiral prominence fibrocytes disappeared by 14 days following administration (fig. 2d). These histological changes were not observed in the control rats at any time point (fig. 2a). In the stria vascularis, no morphological changes were detected by light microscopy within the observation period after 3-NP administration in the experimental rats (fig. 2b–d) or saline treatment in the control rats (fig. 2a).

Fibrocytes of the spiral ligament are divided into 4 cell types based on structural features, immunostaining patterns and general location [Spicer and Schulte, 1996]. The classification is summarized in a scheme (fig. 3), and the following transmission electron microscopic findings are described according to this classification. Atrophy of type 2 fibrocytes and the enlargement of extracellular spaces in the spiral prominence were detected at 3 h after 3-NP administration (fig. 4b), which became more pronounced over time during the observation period. Nearly total loss of type 2 fibrocytes was noted at 14 days (fig. 4c). In contrast, spiral prominence capillaries and epithelia were maintained. In control rats, type 2 fibrocytes maintained normal morphology in that they contained numerous mitochondria and presented interdigitated cellular processes that were closely apposed by gap junctions (fig. 4a).

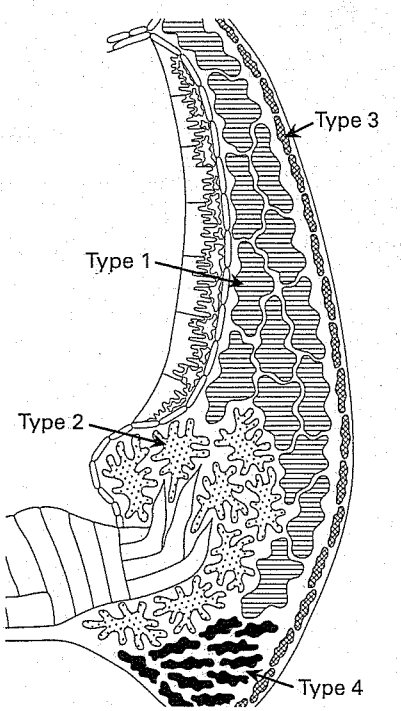
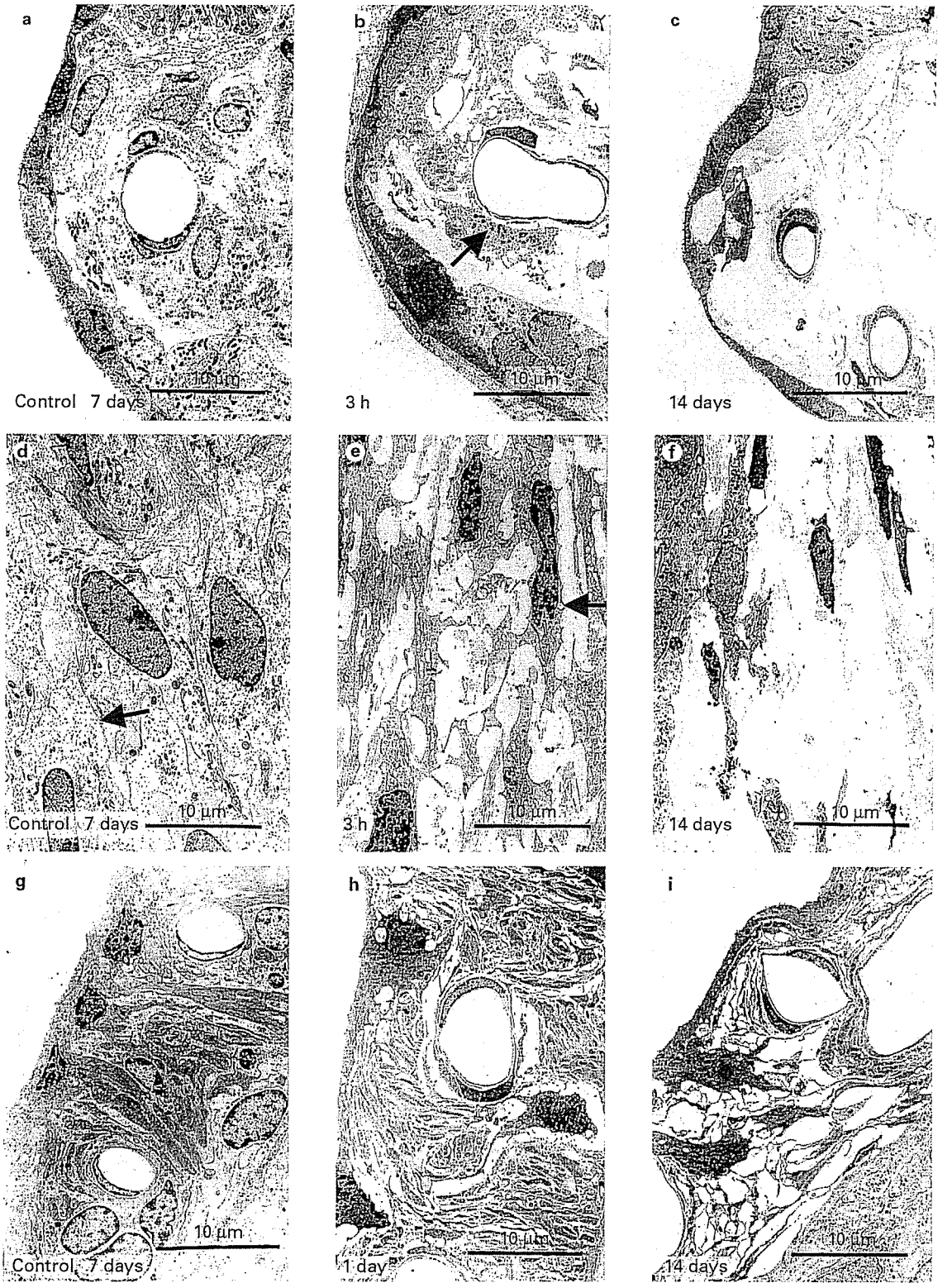


Fig. 3. Summary diagram of the lateral wall indicating the spatial relationship of the 4 types of fibrocytes in the spiral ligament.


a
Control 7 days
10 µm
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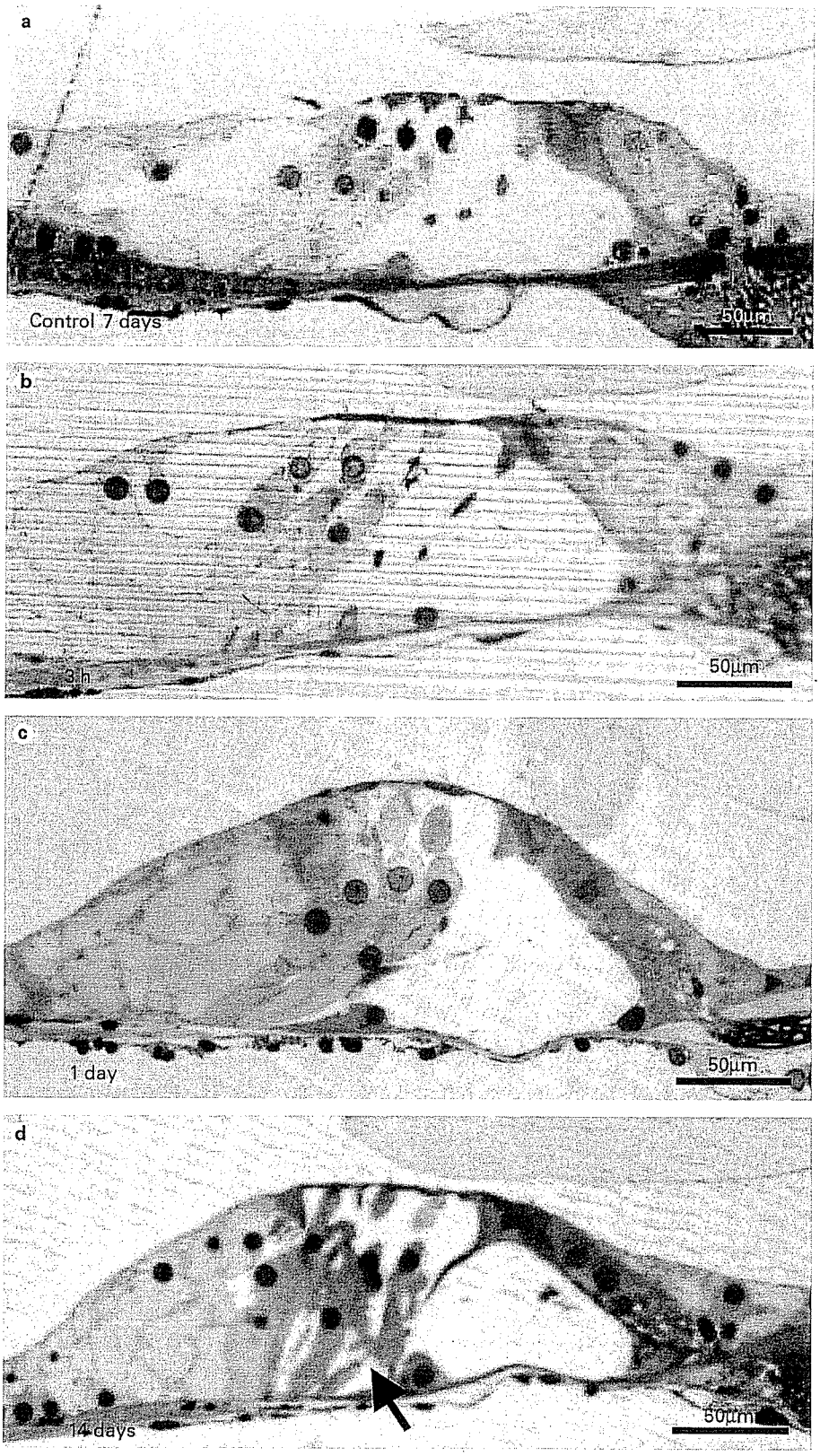


Fig. 5. Light microscopy of the organ of Corti after the administration of saline (**a**) or 3-NP (**b**, **c**, **d**). Normal morphology of the organ of Corti 7 days after saline administration (**a**), and at 3 h (**b**) and 1 day (**c**) after 3-NP administration. The organ of Corti exhibited slight shrinkage of the Deiters cells (arrow) but otherwise maintained normal structure 14 days after 3-NP administration (**d**).

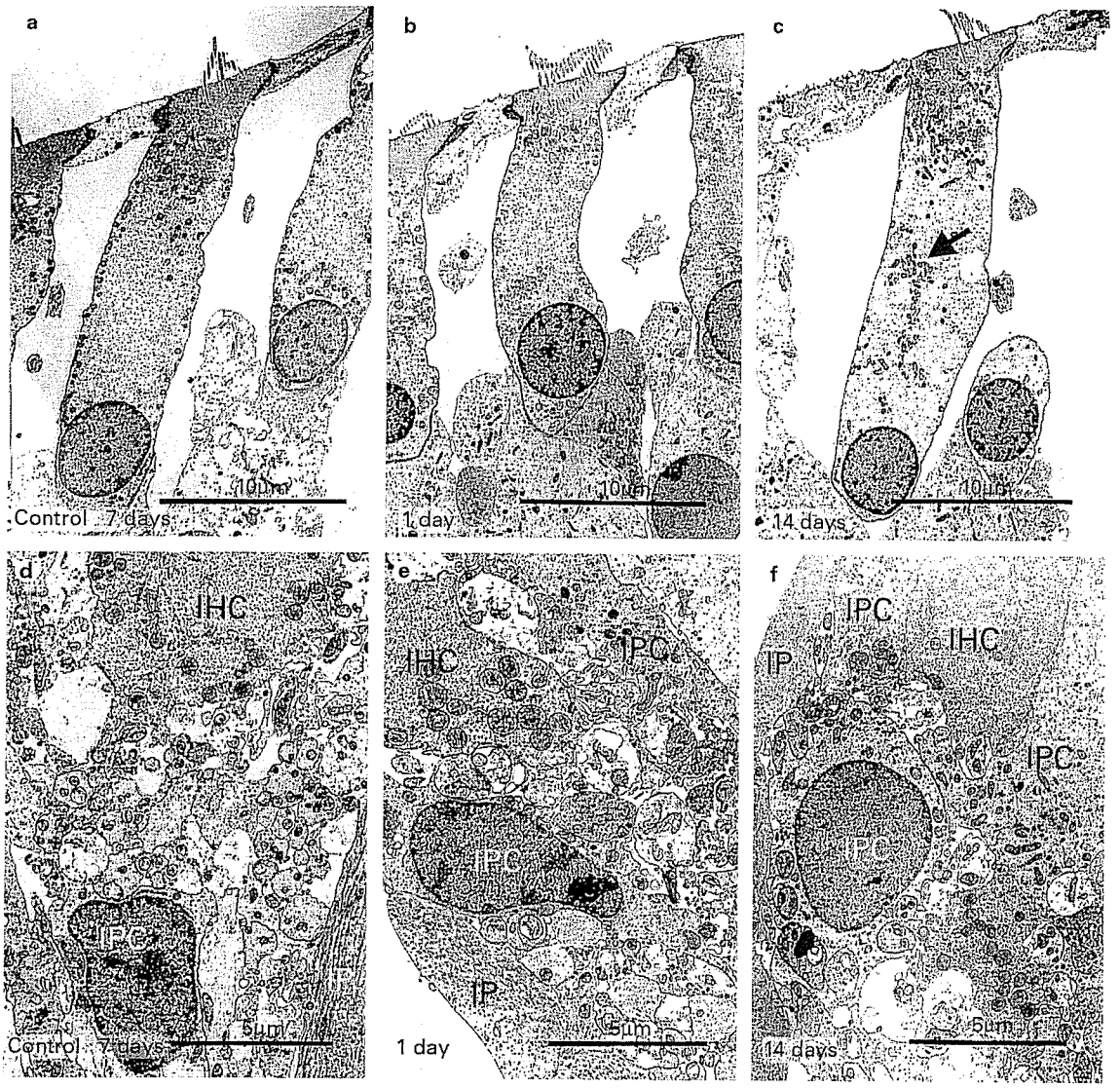


Fig. 6. Ultrastructural analysis of hair cells after the administration of saline (**a**, **d**) or 3-NP (**b**, **c**, **e**, **f**). **a** Normal structures in outer hair cells 7 days after saline administration. Mitochondria were distributed along the lateral wall of outer hair cells. **b** At 1 day after 3-NP administration, outer hair cells appeared structurally normal. **c** At 14 days after 3-NP administration, translocation of mitochondria into the center of cytoplasm (arrow) was noted. **d** Normal nerve endings 7 days after saline administration. Numerous nerve endings were seen at the bottom of the inner hair cell (IHC), and surrounded by the inner phalangeal cell (IPC) and the inner pillar cell (IP). At 1 day (**e**) and 14 days (**f**) after 3-NP administration, numerous nerve endings with the same structural features seen in the saline control were maintained at the bottom of the inner hair cell (IHC) and surrounded by the inner phalangeal cell (IPC) and the inner pillar cell (IP).

swelling 3 h after 3-NP administration (fig. 8b). The mitochondrial swelling became less apparent at 1 day following treatment. At 7 and 14 days, the structures appeared normal (fig. 8c). No ultrastructural changes were observed in the spiral ganglion cells of control rats (fig. 8a).

The structural changes observed in the cochlea following 3-NP administration are summarized in table 1.

Discussion

In the present study, we demonstrated that cellular degeneration in the lateral wall is the most remarkable change within the cochlea in response to acute mitochondrial dysfunction. The main physiological function of the cochlear lateral wall is ion transport and the generation of

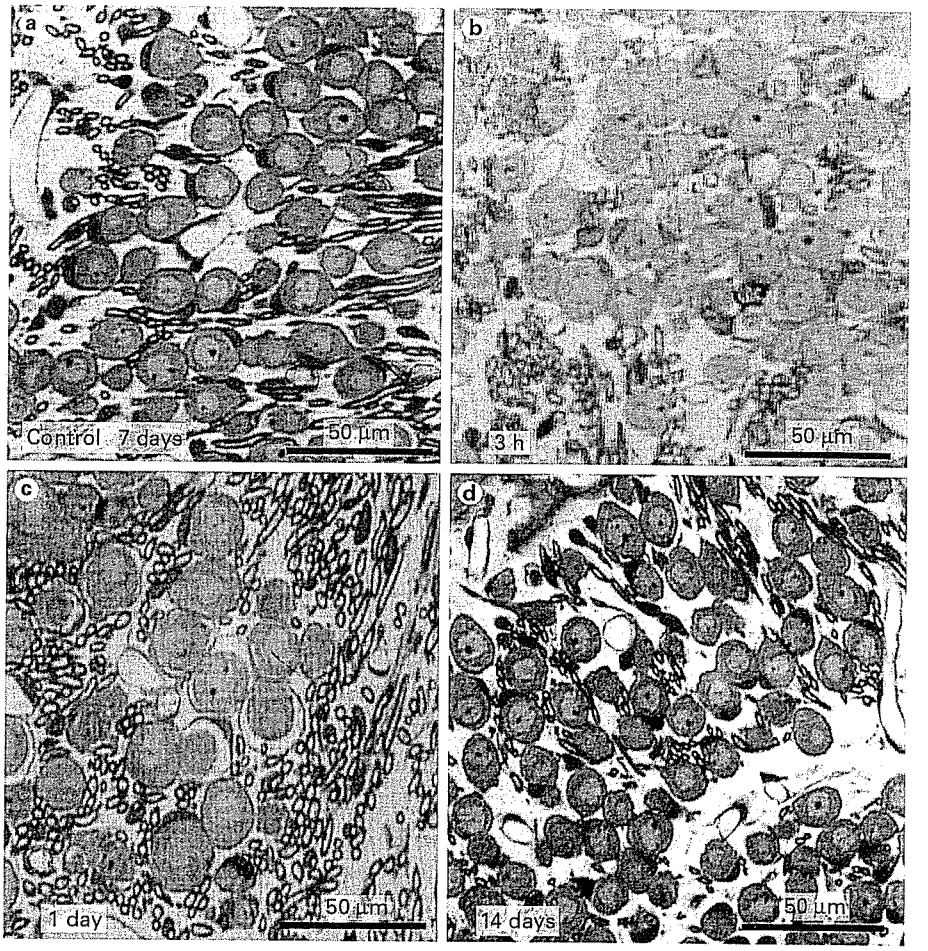


Fig. 7. Light microscopy of the spiral ganglion cells after administration of saline (**a**) or 3-NP (**b**, **c**, **d**). **a** Normal morphology in spiral ganglion cells 7 days after saline administration. At 3 h (**b**), 1 day (**c**), and 14 days (**d**) after 3-NP administration, spiral ganglion cells displayed normal structures.

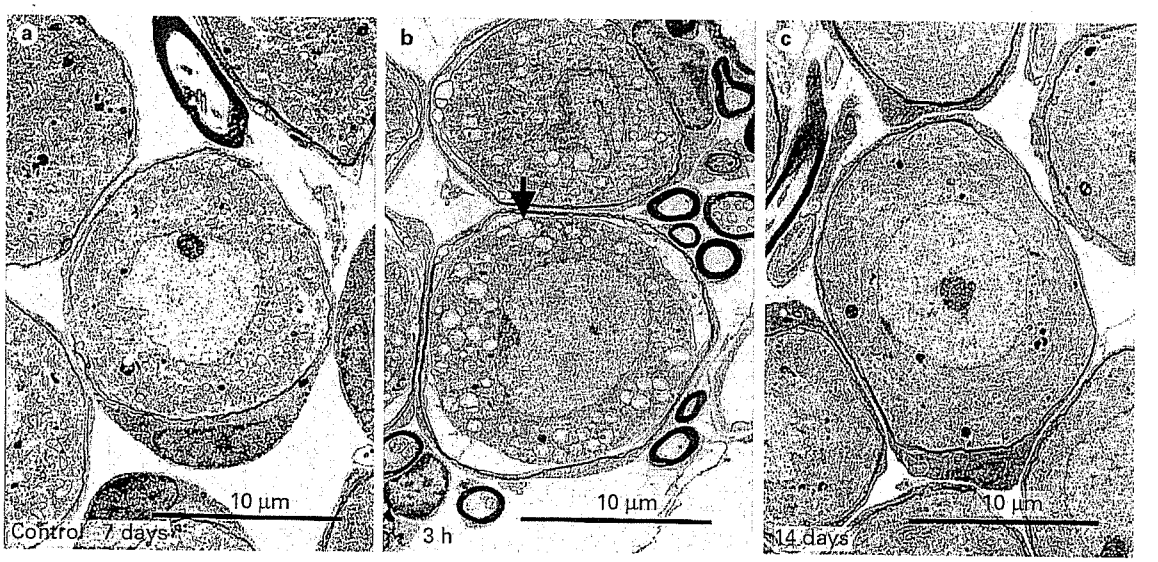


Fig. 8. Ultrastructural analysis of spiral ganglion cells after the administration of saline (**a**) or 3-NP (**b**, **c**). **a** Normal morphology in spiral ganglion cells 7 days after saline administration. **b** Mitochondrial vacuolation was prominent 3 h after 3-NP administration. **c** Mitochondria exhibited normal structures 14 days after 3-NP administration.

Table 1. Summary of structural changes in the cochlea at several time points after 3-NP administration

	3 h	1 day	7 days	14 days
Spiral ligament				
Type 2, type 4 fibrocytes	++	++	+++	+++
Type 1, type 3 fibrocytes	+	+	+	+
Spiral prominence				
Type 2 fibrocytes	++	++	+++	+++
Stria vascularis				
Marginal cell	++	++	++	+++
Intermediate cell	++	++	++	+++
Basal cell	N	N	N	N
Organ of Corti				
Outer hair cell	N	N	+	+
Inner hair cell	N	N	N	N
Deiters cell	N	N	N	+
Spiral ganglion				
Spiral ganglion cell	++	+	N	N

For the cells of the spiral ligament, spiral prominence and stria vascularis, the degree of cell degeneration is indicated. For the outer and inner hair cells, the occurrence of mitochondrial translocation is indicated. For the Deiters cell, the degree of shrinkage is indicated. For the spiral ganglion cell, the degree of mitochondrial swelling is indicated. None of these changes were observed in corresponding control rats. N = Normal morphology or absence of mitochondrial translocation; + = mild degeneration or mild mitochondrial swelling or presence of mitochondrial translocation; ++ = moderate degeneration or marked mitochondrial swelling; +++ = severe degeneration.

endocochlear potential [Salt, 2001; Wangemann, 1995; Wangemann and Schacht, 1996]. It has been postulated that the potassium recycling pathway toward the stria vascularis is critical for these functions although the exact mechanisms and pathway have not been definitely proved [Santos-Sacchi, 2000]. One candidate of such a ion transport system consists of extracellular flow through scala tympani and scala vestibuli, and transcellular flow through the organ of Corti, supporting cells, and cells of the lateral wall [Kikuchi et al., 1995; Spicer and Schulte, 1996]. According to this theory, type 2 and type 4 fibrocytes resorb potassium ions from the surrounding perilymph and from outer sulcus cells via the Na,K-ATPase. The potassium ions are then transported to type 1 fibrocytes, strial basal cells and intermediate cells through gap junctions, and are secreted into intrastrial space through the potassium channel. The secreted potassium ions are incorporated into marginal cells by Na,K-ATPase and the Na-K-Cl cotransporter, and are finally secreted into the endolymph through the potassium channel. In addition, a direct measurement of standing currents in the cochlea demonstrated ongoing ion movement, which most likely represented potassium ion movement, from scala tympani and scala vestibuli, through extracellular spaces in the spiral ligament, to the lateral surface of the stria vascularis [Zidanic and Brownell, 1990]. These potassium ion transport systems generate endocochlear potential and high potassium concentration in the endolymph, both of which are essential for the transduction of sound by hair cells [Santos-Sacchi, 2001].

Impairment of such a ion transport system has been assumed to result in hearing loss. For example, inhibition of the Na-K-Cl cotransporter with loop diuretics or mutations in the gene encoding the Na-K-Cl cotransporter reduces endocochlear potential, results in hearing loss and in an enlarged intrastrial space much like we found in the present study [Flagella et al., 1999; Quick and Duvall, 1970]. Another example that parallels our results is the Brn-4 gene-deficient mouse, which exhibits reduced endocochlear potential and hearing loss and shows severe ultrastructural alterations in spiral ligament fibrocytes, including cellular atrophy and a reduction in the number of mitochondria in these cells [Minowa et al., 1999].

In the present study, type 2 fibrocytes in the spiral prominence, type 4 fibrocytes in the spiral ligament, marginal cells and intermediate cells in the stria vascularis were all primarily affected. All these cell types are known to express Na,K-ATPase, and presumably constitute the potassium recycling pathway. Because of the alterations in the spiral ligament fibrocytes, we assume that intercellular communication through gap junctions was damaged, which may lead to the impairment of passive potassium transport through these cells. Marked atrophy or loss of type 2 and type 4 fibrocytes and less conspicuous alteration of type 1 and type 3 fibrocytes suggest impairment of active resorption of potassium ion and following diffusion through gap junctions. Marked atrophy of marginal and intermediate cells associated with loss of mitochondria in the marginal cells strongly indicates impaired potassium transport into the endolymph. The enlarged intrastrial space may be the result of impaired uptake of potassium ions by marginal cells, as was the case for inhibition of the Na-K-Cl cotransporter. Considering these morphological and functional correlations, elevated ABR thresholds observed in the present study may be the result of an impaired ion transport system in the lateral wall due to acute cochlear mitochondrial dysfunction.

In the organ of Corti, translocation of mitochondria in the outer hair cells and slight shrinkage of the Deiters cells were detected after administration of 3-NP in contrast to the maintained normal structures in inner hair cells and

their nerve endings. The major function of outer hair cell is to enhance the sensitivity to specific sound frequencies through their fast motility, and the Deiters cells structurally support the outer hair cells on the basilar membrane. Although it has been reported that this active amplification of sound by the outer hair cell is independent of ATP [Ashmore, 1987; Santos-Sacchi, 1989], and rather is operated by its membrane potential [Ashmore, 1987; Santos-Sacchi and Dilger, 1988], any changes in the mitochondrial function in the outer hair cell are likely to affect mechanisms controlling its membrane potential, which may result in outer hair cell dysfunction.

The changes in outer hair cells and Deiters cells were first detected at 7 days and 14 days after 3-NP administration, respectively, in contrast to the structural changes in the cochlear lateral wall, which were detected as early as 3 h after 3-NP administration. This time difference suggests that mitochondrial translocation in the outer hair cells and shrinkage of the Deiters cells may be secondary to the changes in the cochlear lateral wall rather than a direct effect of 3-NP on the mitochondria of these cells.

Prominent mitochondrial swelling was transiently detected in the spiral ganglion cells after 3-NP treatment. In contrast, the mean area of spiral ganglion cells in rats treated with 3-NP was not significantly different from that in rats treated with control saline at most time points. The mean area in 3-NP-treated rats was smaller than that in control rats only at 3 h after administration, but was not significantly different from that in normal, untreated rats, suggesting that the difference is not likely to be of pathological significance.

Spiral ganglion cells are the primary afferent neurons that transmit signals from hair cells to the neurons in the brainstem, and dysfunction of the spiral ganglion cells causes hearing loss. For example, selective damage on the spiral ganglion neurons by application of ouabain in the gerbil cochlea resulted in profound hearing loss [Schmiedt et al., 2002]. Neural presbycusis is another example of such hearing loss, in which spiral ganglion neurons are predominantly affected in the aging cochlea [Schuknecht, 1964]. In the present study, dysfunction of cochlear ganglion cells is also likely to be involved in hearing impairment temporarily after 3-NP administration. However, it is unlikely that the dysfunction of these cells plays a role in persistent hearing loss because the mitochondria in these cells recovered their normal shape within 7 days following administration.

In contrast to the progressive degeneration observed in the lateral wall, mitochondrial swelling in these neurons was only temporary (at 3 h and at 1 day after 3-NP administration). At 7 days or 14 days after administration, the ultrastructural characteristics of the spiral ganglia were normal. The differences in the reaction to acute mitochondrial dysfunction may be explained by the distinct capacity to repair such damage in the spiral ganglion and the lateral wall. One of the candidate molecular mechanisms underlying such reparative capacity involves glial cell line-derived neurotrophic factor (GDNF) which has been reported to enhance survival of the spiral ganglion cells [Ylikoski et al., 1998]. GDNF family members and their receptors are expressed in the spiral ganglion cells of the mature rat cochlea [Stover et al., 2001] and GDNF is upregulated in the rat cochlea following exposure to noise [Nam et al., 2000]. It may be possible that GDNF was also upregulated following administration of 3-NP in the present study and played a role in repair of the damaged spiral ganglion cells. Further exploration of this hypothesis will be the subject of future studies.

To understand the basal-to-apical gradient in the cochlear damage observed in the present study, the pharmacokinetics of 3-NP applied to the round window membrane should be considered. Because of its relatively small size (molecular weight = 119), we assume that 3-NP applied to the round window membrane diffused in the perilymph within the scala tympani at the cochlear basal turn, and spread by local communication between two perilymphatic scalae, i.e. scala tympani and scala vestibuli, presumably across the extracellular spaces of the spiral ligament [Saijo and Kimura, 1984; Salt et al., 1991a, b]. Then, longitudinal gradients of 3-NP distribution along the cochlear scalae probably occurred, as is seen for ionic markers and gentamicin [Salt and Ma, 2001; Plontke et al., 2002]. Thus, exposure to 3-NP within the cochlea was expected to be higher at the basal region and lower at the apical region. This is in agreement with the basal-to-apical gradient of cochlear damage observed in this study.

Because there was a basal-to-apical gradient in the extent of cochlear damage and we mainly examined the apical side of the middle turn in the present study, we searched the literature for the effect of damage in the lateral wall of the cochlear basal turn on the middle turn. Previously, it has been proposed that damage in the lower cochlear turn has a depressant effect on the sound perception mechanism lying apical to this [Mattox and Simmons, 1977]. In an animal model of localized lesion in the cochlear lateral wall, it was also reported that endocochlear potential values were significantly depressed at all sites apical to the localized lesions in the cochlear lateral wall [Gao et al., 1998; Wu and Hoshino, 1999, 2001]. These results suggest that cochlear lateral wall damage in

the basal turn may affect endocochlear potential and ion composition in the apical turns when the damage is severe and their effect on the apical turns beyond the capacity of local maintenance of the endolymph ion composition [Salt, 2001]. In contrast, morphological changes were restricted to the region where the local lesion was produced in this animal model and normal structures were maintained in the apical sites [Gao et al., 1998; Miyashita et al., 1998; Wu and Hoshino, 1999, 2001]. This result indicates that, to a certain degree, damage in the lateral wall in the basal turn and associated changes in the endolymph ion composition do not affect the structure in the higher turns. Because the degree of lateral wall damage appeared structurally milder in the present study than in these reported studies, structural changes in the middle turn reported in the present study are likely to be the result of the direct and local effect of 3-NP rather than the effect of lateral wall damage in the basal turn.

In interpreting the apparent distinct susceptibilities to 3-NP of the lateral wall, organ of Corti, and spiral ganglion, the difference in exposure to 3-NP among these compartments should be considered. Basolateral membranes of the sensory and supporting cells in the organ of Corti and afferent nerve fibers of spiral ganglion cells are surrounded by cortilymph, which is continuous with perilymph. Fibrocytes in the spiral ligament are also surrounded by perilymph. Thus, the degree of 3-NP exposure in these structures at the same region of the cochlear turn was likely to be the same. In contrast, 3-NP exposure was likely to be low for the stria vascularis, where solute movement from the adjacent tissues or fluids is limited because of tight junctions between adjacent marginal cells and basal cells [Salt, 2001]. Furthermore, 3-NP exposure to the spiral ganglion cell bodies was also likely to be low because these cells are located in Rosenthal's canal, which has only limited interaction with the perilymph by way of the endoneurium of nerve fibers, habenula perforata and osseous spiral lamina. Taken together, these differences in the exposure to 3-NP did not coincide with faster and more severe morphological changes in the spiral ligament, stria vascularis, and spiral ganglion cell bodies than in the organ of Corti, and suggest that the susceptibility to 3-NP is higher in the lateral wall and spiral ganglion than in the organ of Corti.

Previously, the effects of acute energy failure on the cochlea have been studied using anoxia or chemical asphyxiants such as carbon monoxide, cyanide and nitric oxide [Brown et al., 1983; Kong et al., 1996; Nuttall and Lawrence, 1979; Pai et al., 1998; Rao and Fechter, 2000; Ruan et al., 1997; Tawackoli et al., 2001; Thalmann et al., 1977]. In these studies, electrophysiological methods were primarily used to investigate the pathophysiology of cochlear dysfunction, and only a small number of morphological studies limited to the organ of Corti and spiral ganglion cells have been conducted [Kong et al., 1996; Pai et al., 1998; Ruan et al., 1997]. No study demonstrated morphological changes in the cochlear spiral ligament.

In contrast to the models using anoxia or chemical asphyxiants, there have been a number of studies examining morphological changes of the cochlea exposed to ischemia-reperfusion which is likely to cause acute cochlear energy failure similar to that seen in the present model [Billett et al., 1989; Hakuba et al., 2003; Kimura and Perlman, 1958; Koga et al., 2003; Ohlemiller and Dugan, 1999; Perlman et al., 1959; Pujol et al., 1992; Puel et al., 1994; Tabuchi et al., 2002; Tsuji et al., 2002]. In most of these studies, however, the observation periods between the exposure and the morphological analysis were relatively short (usually several hours), and the cochlear structures most readily affected included afferent dendrites, hair cells, stria vascularis, pillar cells, Deiters and Hensen cells. In two animal models of the cochlear ischemia-reperfusion, cochlear morphology was examined after relatively long observation periods, 5 days and 7 days after exposure [Tsuji et al., 2002; Koga et al., 2003; Hakuba et al., 2003]. However, only the organ of Corti and spiral ganglion cells were examined in these studies.

Comparing the morphological features in the previous studies with those observed in the present study, degeneration of the stria vascularis and the relatively well-preserved organ of Corti were common features. However, morphological changes in the afferent dendrites frequently reported after exposure to ischemia-reperfusion were not detected in the present model. Morphological changes in the spiral ligament fibrocytes detected in the present model have not been reported in the previous studies. These differences were most likely due to the differences in the onset, duration and degree of cochlear energy failure between the present animal model and the previous models. Because a long observation period is possible following exposure to 3-NP using the present model, further study of the molecular mechanism underlying the functional and morphological changes observed in this model may lead to the discovery of a novel therapeutic strategy.

Conclusion

The present study showed that the degeneration of the cochlear lateral wall was the primary pathological change in the permanent threshold shift model due to acute mito-

chondrial dysfunction, and suggests that cochlear ion transport may be impaired in this model. Therefore, protection, repair, or regeneration targeted to the lateral wall may constitute a key therapeutic strategy for hearing loss due to acute energy failure such as cochlear ischemia.

Acknowledgements

We thank Mr. S. Nakagawa for his excellent technical assistance in light and electron microscopy. We also thank Dr. Seiji Bito for his help with the statistical analysis. This work was funded by a Health Science Research Grant from the Ministry of Health, Labor and Welfare of Japan (to T.M.).

References

Alston TA, Mela I, Bright HJ: 3-Nitropropionate, the toxic substance of Indigofera, is a suicide inactivator of succinate dehydrogenase. Proc Natl Acad Sci USA 1977;74:3767–3771.

Ashmore JF: A fast motile response in guinea-pig outer hair cells: The cellular basis of the cochlear amplifier. J Physiol 1987;388:323–347.

Billett TE, Thorne PR, Gavin JB: The nature and progression of injury in the organ of Corti during ischemia. Hear Res 1989;41:189–198.

Braverman I, Jaber L, Levi H, Adelman C, Arons KS, Fischel-Ghodsian N, Shohat M, Elidan J: Audiovestibular findings in patients with deafness caused by a mitochondrial susceptibility mutation and precipitated by an inherited nuclear mutation or aminoglycosides. Arch Otolaryngol Head Neck Surg 1996;122:1001–1004.

Brouillet E, Hantraye P, Ferrante RJ, Dolan R, Leroy-Willig A, Kowall NW, Beal MF: Chronic mitochondrial energy impairment produces selective striatal degeneration and abnormal choreiform movements in primates. Proc Natl Acad Sci USA 1995;92:7105–7109.

Brown MC, Nuttall AL, Masta RI, Lawrence M: Cochlear inner hair cells: Effects of transient asphyxia on intracellular potentials. Hear Res 1983;9:131–144.

Coles CJ, Edmondson DE, Singer TP: Inactivation of succinate dehydrogenase by 3-nitropropionate. J Biol Chem 1979;254:5161–5167.

Flagella M, Clarke LL, Miller ML, Erway LC, Giannella RA, Andringa A, Gawenis LR, Kramer J, Duffy JJ, Doetschman T, Lorenz JN, Yamoah EN, Cardell EL, Shull GE: Mice lacking the basolateral Na-K-2Cl cotransporter have impaired epithelial chloride secretion and are profoundly deaf. J Biol Chem 1999;274:26946–26955.

Gao J, Hoshino T, Iwasaki S, Wu R: Photochemically induced focal cochlear lesions in the guinea pig. 1. DAB staining and SEM study. Microsc Res Tech 1998;41:323–333.

Green DR, Reed JC: Mitochondria and apoptosis. Science 1998;281:1309–1312.

Hakuba N, Matsubara A, Hyodo J, Taniguchi M, Maetani T, Shimizu Y, Tsujiuchi Y, Shudou M, Gyo K: AMPA/kainate-type glutamate receptor antagonist reduces progressive inner hair cell loss after transient cochlear ischemia. Brain Res 2003;979:194–202.

Hamilton BF, Gould DH: Nature and distribution of brain lesions in rats intoxicated with 3-nitropropionic acid: A type of hypoxic (energy deficient) brain damage. Acta Neuropathol (Berl) 1987;72:286–297.

Hoya N, Okamoto Y, Kamiya K, Fujii M, Matsunaga T: A novel animal model of acute cochlear mitochondrial dysfunction. Neuroreport 2004;15:1597–1600.

Huang T, Cheng AG, Stupak H, Liu W, Kim A, Staecker H, Lefebvre PP, Malgrange B, Kopke R, Moonen G, Van De Water TR: Oxidative stress-induced apoptosis of cochlear sensory cells: Otoprotective strategies. Int J Dev Neurosci 2000;18:259–270.

Hutchin TP, Cortopassi GA: Mitochondrial defects and hearing loss. Cell Mol Life Sci 2000;57:1927–1937.

Kikuchi T, Kimura RS, Paul DL, Adams JC : Gap junction in the rat cochlea: immunohistochemical and ultrastructural analysis. Anat Embryol 1995;191:101–118.

Kimura R, Perlman HB: Arterial obstruction of the labyrinth. 1. Cochlear changes. Ann Otol Rhinol Laryngol 1958;67:5–23.

Koga K, Hakuba N, Watanabe F, Shudou M, Nakagawa T, Gyo K: Transient Cochlear ischemia causes delayed cell death in the organ of Corti: An experimental study in gerbils. J Comp Neurol 2003;456:105–111.

Kong W, Ren T, Nuttall AL: Electrophysiological and morphological evaluation of the acute ototoxicity of sodium nitroprusside. Hear Res 1996;99:22–30.

Mattox DE, Simmons FB: Natural history of sudden sensorineural hearing loss. Ann Otol Rhinol Laryngol 1977;86:463–480.

Minowa O, Ikeda K, Sugitani Y, Oshima T, Nakai S, Katori Y, Suzuki M, Furukawa M, Kawase T, Zheng Y, Ogura M, Asada Y, Watanabe K, Yamanaka H, Gotoh S, Nishi-Takeshima M, Sugimoto T, Kikuchi T, Takasaka T, Noda T: Altered cochlear fibrocytes in a mouse. Science 1999;285:1408–1411.

Miyashita H, Iwasaki S, Hoshino T: Photochemically induced focal cochlear lesions in the guinea pig. 2. A transmission electron microscope study. Microsc Res Tech 1998;41:334–340.

Nam Y, Stover T, Hartman SS, Altschuler RA: Upregulation of glial cell line-derived neurotrophic factor (GDNF) in the rat cochlea following noise. Hear Res 2000;146:1–6.

Nicholls DG, Budd SL: Mitochondria and neuronal survival. Physiol Rev 2000;80:315–360.

Nuttall AL, Lawrence M: Intracellular potential changes of Corti's organ with anoxia. Arch Otolaryngol 1979;105:574–578.

Ohlemiller KK, Dugan LL: Elevation of reactive oxygen species following ischemia – Reperfusion in mouse cochlea observed in vivo. Audiol Neurootol 1999;4:219–228.

Pai N, Zdanski CJ, Gregory CW, Prazma J, Carrasco V: Sodium nitroprusside/nitric oxide causes apoptosis in spiral ganglion cells. Otolaryngol Head Neck Surg 1998;119:323–330.

Perlman HB, Kimura R, Fernandez C: Experiments on temporary obstruction of the internal auditory artery. Laryngoscope 1959;69:591–613.

Plontke SKR, Wood AW, Salt AN: Analysis of gentamicin kinetics in fluids of the inner ear with round window administration. Otol Neurotol 2002;23:967–974.

Puel JL, Pujol R, Tribillac F, Ladrech S, Eybalin M: Excitatory amino acid antagonists protect cochlear auditory neurons from excitotoxicity. J Comp Neurol 1994;341:241–256.

Pujol R, Puel JL, Eybalin M: Implication of non-NMDA and NMDA receptors in cochlear ischemia. Neuroreport 1992;3:299–302.

Quick CA, Duvall AJ: Early changes in the cochlea duct from ethacrynic acid: An electron microscopic evaluation. Laryngoscope 1970;80:954–965.

Rao DB, Fechter LD: Increased noise severity limits potentiation of noise induced hearing loss by carbon monoxide. Hear Res 2000;150:205–214.

Ruan RS, Leong SK, Yeoh KH: Ototoxicity of sodium nitroprusside. Hear Res 1997;114:169–178.

Saijo S, Kimura RS: Distribution of HRP in the inner ear after injection into the middle ear cavity. Acta Otolaryngol 1984;97:593–610.

Salt AN: Dynamics of the inner ear fluids; in Jahn AF, Santos-Sacchi J (eds): Physiology of the Ear, ed 2. San Diego, Singular, Thompson Learning, 2001, pp 333–355.

Salt AN, Ma Y: Quantification of solute entry into cochlear perilymph through the round window membrane. Hear Res 2001;154:88–97.

Salt AN, Ohyama K, Thalmann R: Radial communication between the perilymphatic scalae of the cochlea: Estimation by tracer perfusion. Hear Res 1991a;56:29–36.

Salt AN, Ohyama K, Thalmann R: Radial communication between the perilymphatic scalae of the cochlea: Estimation by bolus injection into the sealed cochlea. Hear Res 1991b;56:37–43.
Santos-Sacchi J: Asymmetry in voltage-dependent movements of isolated outer hair cells from the organ of Corti. J Neurosci 1989;9:2954–2962.
Santos-Sacchi J: Cell coupling in Corti's organ. Brain Res Brain Res Rev 2000;32:167–171.
Santos-Sacchi J: Cochlear physiology; in Jahn AF, Santos-Sacchi J (eds): Physiology of the Ear, ed 2. San Diego, Singular, Thompson Learning, 2001, pp 357–391.
Santos-Sacchi J, Dilger JP: Whole cell currents and mechanical responses of isolated outer hair cells. Hear Res 1988;35:143–150.
Schmiedt RA, Okamura H, Lang H, Schulte BA: Ouabain application to the round window of the gerbil cochlea: A model of auditory neuropathy and apoptosis. J Assoc Res Otolaryngol 2002;3:223–233.
Schuknecht HF: Further observations of presbycusis. Arch Otolaryngol 1964;80:369–382.
Spicer SS, Schulte BA: The fine structure of spiral ligament cells relates to ion return to the stria and varies with place-frequency. Hear Res 1996;100:80–100.
Stover T, Nam Y, Gong TL, Lomax MI, Altschuler RA: Glial cell line-derived neurotrophic factor (GDNF) and its receptor complex are expressed in the auditory nerve of the mature rat cochlea. Hear Res 2001;155:143–151.
Sue CM, Lipsett LJ, Crimmins DS, Tsang CS, Boyages SC, Presgrave CM, Gibson WPR, Byrne E, Morris JGL: Cochlear origin of hearing loss in MELAS syndrome. Ann Neurol 1998;43:350–359.
Tabuchi K, Tsuji S, Fujihira K, Oikawa K, Hara A, Kusakari J: Outer hair cells functionally and structurally deteriorate during reperfusion. Hear Res 2002;173:153–163.
Tawackoli W, Chen G, Fechter LD: Disruption of cochlear potentials by chemical asphyxiants cyanide and carbon monoxide. Neurotoxicol Teratol 2001;23:157–165.
Thalmann R, Thalmann I, Ise I, Paloheimo S: Noxious effects upon cochlear metabolism. Laryngoscope 1977;87:699–721.
Tsuji S, Tabuchi K, Hara A, Kusakari J: Long-term observations on the reversibility of cochlear dysfunction after transient ischemia. Hear Res 2002;166:72–81.
Wangemann P: Comparison of ion transport mechanisms between vestibular dark cells and strial marginal cell. Hear Res 1995;90:149–157.
Wangemann P, Schacht J: Homeostatic mechanisms in the cochlea; in Dallos P, Popper AN, Fay RR (eds): The Cochlea. New York, Springer, 1996, pp 130–185.
Wu R, Hoshino T: Changes in off-lesion endocochlear potential following localized lesion in the lateral wall. Acta Otolaryngol 1999;119:550–554.
Wu R, Hoshino T: Long-term changes in off-lesion endocochlear potential after induction of localized lesions in the lateral wall. Ann Otol Rhinol Laryngol 2001;110:271–276.
Yamane H, Nakai Y, Takayama M, Iguchi H, Nakagawa T, Kojima A: Appearance of free radicals in the guinea pig inner ear after noise-induced acoustic trauma. Eur Arch Otorhinolaryngol 1995;252:504–508.
Yamasoba T, Oka Y, Tsukuda K, Nakamura M, Kaga K: Auditory findings in patients maternally inherited diabetes and deafness harboring a point mutation in the mitochondrial transfer RNA Leu (UUR) gene. Laryngoscope 1996;106:49–53.
Ylikoski J, Pirvola U, Virkkala J, Suvanto P, Liang XQ, Magal E, Altschuler R, Miller JM, Saarma M: Guinea pig auditory neurons are protected by glial cell line-derived growth factor from degeneration after noise trauma. Hear Res 1998;124:17–26.
Zidanic M, Brownell WE: Fine structure of the intracochlear potential field. 1. The silent current. Biophys J 1990;57:1253–1268.

突発性難聴—最近の話題

神崎　仁*　松永達雄**

キーワード　突発性難聴　蝸牛外側壁障害　線維細胞　診断基準

はじめに

突発性難聴（以下突難と略す）の原因は依然として不明である．しかし，患者の死後の側頭骨病理所見，基礎的，臨床的研究によりウイルス説，循環障害説が二大原因となっている．

本稿ではこれらに加えて，近年注目されてきた蝸牛内・外側壁の線維細胞の機能とその障害による可逆性難聴の実験結果[1]を基に，新しい原因としての可能性を検討し，この所見を基に臨床における難聴の可逆性について考察した．また，そのほかの仮説，治療について述べ，厚生省研究班の診断基準（1973年）の修正を提案する．

Ⅰ．病因

内耳という骨組織に囲まれた蝸牛内で何が起こったのかについては，生前には障害を与えることで蝸牛の生検ができないこともあり，生前突難に罹患した患者が何十年か後に死亡した際の側頭骨病理から推測するしかない状態である．そのために種々の動物モデルが検討されてきた．

側頭骨の病理所見から，突難の二大原因としてウイルス性内耳炎と循環障害説があり，それぞれに動物モデルの作成が試みられている．

1．ウイルス説

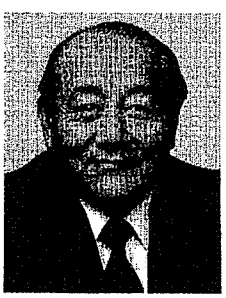
側頭骨の病理所見からは，ウイルス感染の所見と類似するためウイルス説が出された．しかし，これらの所見は突難罹患後何十年か後のもので，突難発症時の所見が相当修飾されており，はたして発症時の病態を推測できるかについては疑問があった．

ヘルペスウイルスを動物の内耳に接種して内耳炎を起こさせ，急性の不可逆性の難聴を起こすことはできる[2]．このモデルに対してステロイドと抗ヘルペスウイルス薬（アシクロビル）の併用をすると，聴覚の回復と組織障害の抑制に有効であったと報告された[3]．しかし，突難の患者にこの併用療法をステロイド単独療法と比較して行った臨床試験では，治療効果に差が認められなかった[4]．

2．循環障害説

難聴の発症が突発性であることから，原因として虚血が考えられる．そのために従来より，動物に実験的に循環障害と類似した病態を起こさせて検討した研究は多い．

蝸牛内の聴覚に関連した電気生理学的活動は，無酸素，低酸素に対して脆弱性がきわめて高い．このことを利用して，動物で人工呼吸器の一時的停止，あるいは一酸化炭素，一酸化窒素，シアン化物などの全身負荷により，酸素利用障害を起こして可逆性難聴を起こすことがで



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きる．しかし，この実験系では持続する難聴を生じるレベルの酸素利用障害を負荷すると，個体として生存できなくなるため，慢性実験ができないという欠点があった．

ほかには，蝸牛を栄養する内耳動脈を，選択的に一定時間圧迫することなどによって遮断する方法がある．この方法では全身的に影響を与えない利点の反面，手術侵襲が大きく，術後長期に動物を生存させて経過をみることができないとともに，難聴を緩徐に回復させることができなかった．近年，スナネズミを用いて椎骨脳底動脈の一過性の遮断で内耳虚血による難聴を起こし，長期経過を観察できるモデルが開発されたが，同様に難聴を緩徐に回復させることはできていない[5]．これらのモデルの解析から，障害は虚血の条件によっても異なるが，内有毛細胞とシナプスを形成する求心系神経終末，内・外有毛細胞，血管条，ラセン神経節，コルチ器の支持細胞に障害が生じることが明らかにされた．臨床例および実験的に，利尿薬による血管条障害によって可逆性難聴が生ずることから，可逆性難聴の病態として血管条性難聴が疑われている．

3. 最近の知見

1）蝸牛内・外側壁障害説（図1）

最近，新たな蝸牛内の障害部位によって，可逆性難聴が生ずることが明らかになり，突難のモデルとして今後研究に有用な方法となることが期待される．

Hoyaらは，ミトコンドリア電子伝達酵素の阻害薬である3-nitropropionic acid（3-NP）をラットに中耳経由で正円窓から内耳に投与することにより，低侵襲で酸素利用障害による高度の難聴を誘導することができることを示した[6]．この障害は一過性虚血と類似した病態によると考えられるので，広義には循環障害説に入るものと考える．この難聴は数週間以上の長期観察が可能であった．そのうえ，3-NP量によって緩やかに改善する可逆性の難聴と不可逆性の難聴の

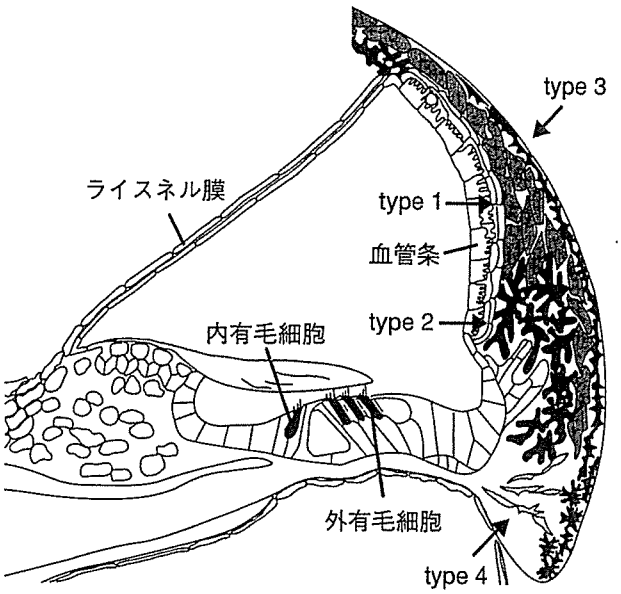

図1　蝸牛管の構造と蝸牛外側壁の線維細胞の分類
（Hirose K, Liberman MC : Lateral wall histopathology and endocochear potential in the noise-damaged mouse cochlea. *J Assoc Res Otolaryngol* 2003 ; 4 : 339―352 より引用改変）

両者が作成できた．これらの障害部位は主として蝸牛外側壁の線維細胞のtype 2とtype 4であった[6,7]．この部位は内リンパ液のイオン組成の形成と維持を行っている．特に，Kイオンのリサイクルに関係した部位として重要である．これらの線維細胞ではアポトーシスによる細胞死が生じていることに加えて，その周囲では再生が起こっており，この過程は聴力の回復と並行していた．これらの障害と回復の過程でのさまざまな遺伝子発現の網羅的解析が現在進行中である．

従来，可逆性難聴では蝸牛外側壁の血管条障害が考えられていた．しかし，Minowaらによる DFN3 非症候性難聴のマウスモデルの研究によって，蝸牛外側壁の線維細胞のみの障害でも蝸牛内電位の低下が起こり，難聴が生じることが明らかにされた[8]．蝸牛外側壁の線維細胞は4つないし5つのタイプに分類されており，蝸牛外側壁障害で実際に障害を受ける細胞の多様性，個体の回復力の差を考えると，臨床例の聴力改善速度の差，改善率の差など予後予測の精

度の低い理由も説明できると思われる．

2）ストレス反応仮説

ハーバード大学のグループは，突難が発症し加療中に心筋梗塞で死亡した側頭骨の病理所見をすでに報告していたが，今回改めて再検討し新たな仮説を示した[9]．その所見は支持細胞，有毛細胞にあったが，この説明として，ストレスに誘発され変化を受けることにより支持細胞内のnuclear factor kappa Bが病的に活性化されたためとした．支持細胞の障害はKイオンのリサイクルの障害を起こし，線維細胞障害と類似の病態を起こすと推測される．

Ⅱ．感音難聴の可逆性について

聴力検査の250，500，1000，2000，4000Hzの5周波数平均が40～90dB（grade 2とgrade 3）の症例について聴力改善率を検討した．[聴力改善率(%)＝患側治療開始時聴力－患側固定時聴力/患側治療開始時聴力－健側聴力×100]

●突難発症後1週以内に治療を開始した136例の検討では，発症後日数と固定時聴力との間に治癒率，聴力改善率（平均79.2±30.2%）の差はなかった．このことから，難聴の改善に自然治癒が関わっている可能性があると推測した．

●突難発症後1週以内の例では，grade 2（5周波数平均40～60dB）とgrade 3（60～90dB）に分けて検討しても聴力改善率に差がなかった．しかし，発症後2週以内までの例を含めるとgrade 2とgrade 3には差がみられた．

●突難発症後1週以内に治療開始した129例について検討した結果，聴力改善の速度には症例ごとに差があった．治療開始後1週以内に改善率が50%以上になる例の予後は良かった．しかし，回復速度が遅くても最終的には改善率が高い症例もみられた．これらのことからも1週以内では自然治癒が関与し，その後の改善の差には自然治癒も関与するが障害の程度，障害部位の差，回復力の差に影響されると推測した．

これらの所見は，線維細胞の可逆性障害としても説明できると考えられる．初診時聴力で予後の推測が必ずしもできないのは，現時点の聴力検査では難聴の程度が同じであると，異なる病態であってもその判別ができないからではないかと考えられる．

Ⅲ．診断基準

1973年に当時の厚生省の特定疾患突発性難聴研究班が作成した「突発性難聴診断の手引き」が一般に広く使われている．この手引きによる診断のポイントは，①突然発症する，②原因不明である，③高度の感音難聴である，の3点である．本来，この手引きは研究班の班員の研究のために作成されたものであり，曖昧な例を取り込まないようにとの配慮から，特に③がかなり厳しい基準となっていた．しかし，これが一人歩きしているうちに基準が正しく理解されないで使われるようになってきた．問題の1つ目は，①，②の基準を満たすが③に該当しない軽度，中等度の感音難聴も含まれるようになってきたことである．「高度」と断っていたのは，1つには高度でないと難聴が自覚されないこと，自覚されないといつ発症したかが曖昧になり「突然発症した」のかどうかが明らかでなくなるという理由があった．

2つ目は，鑑別診断の進歩の過程で，急性低音障害型感音難聴（以下低音障害型と略す）という500Hz以下の障害を示す例の扱いが問題になってきたことである．このタイプの難聴は軽度であり，診断の手引きの③に該当しない．さらに全周波数が中等度以上障害される例に比べ比較的回復しやすい．そのため，これらの症例を①，②の条件に該当するからとして突難に含めると，この割合が多い報告では当然治癒率が良くなる．このことからも聴力改善の判定は聴

力の重症度を考慮して行う必要があり，重症度分類が作成された．

3つ目の問題は，突難の診断を巡る医事紛争が出てきたことである．低音障害型ではしばしば難聴が自覚されずに耳閉塞感として訴えられるため耳管機能障害として扱われ，聴力検査が行われないことがある．治りが悪いと感じた患者が転医し，そこでは聴力検査が行われ低音障害型であったが，この後医がこれを突難と診断したために紛争になった例があった．患者には突難は早期治療が必要であるとの情報がインプットされていたため診断，治療の遅れとして前医が訴えられたのである．低音障害型には蝸牛型内リンパ水腫，メニエール病が含まれているために突難からは除外する必要がある．さらに，低音障害型は重症度分類の grade 1（5周波数平均 40dB 以下）に含まれるので，③の基準からも当然 grade 1 を除外するか別に扱い，臨床上の混乱を避ける必要があると考え，研究班の診断の手引きの修正を提案する．

Ⅳ．治療

原因不明なので，推定される原因（主に，ウイルス，循環障害）に対して治療薬が複数，いわゆるカクテルとして用いられてきた．このような成績を検討すると，どのような治療を行っても治癒率は大体同じようであったことからも，自然治癒が関わっていることが示唆された．また，厚労省の研究班が行った単剤治験の結果でも，明らかに治療効果のある薬剤はなかった[10]．

しかし，ステロイドについては例数が少ないが二重盲検試験で有効との報告がある．内耳の自己免疫性感音難聴の存在も認識されてきているが，臨床的に使用できる検査法はない．ステロイドで回復した難聴がステロイドを中止するとしばらくして難聴が再発する症例が見出され，ステロイド依存性感音難聴として知られるようになってきた[11]．この病態も明らかではないが，明らかにステロイドの投与で聴力が改善することが臨床的に証明できるので，ステロイドが禁忌でなければ，投与することは意味があると考えている．

近年，感音難聴に対して中耳腔より内耳窓経由でステロイドを内耳に浸透させる方法が試みられている．この方法をさらに発展させることにより，内耳液中へ治療薬あるいは細胞［胚性幹細胞（ES 細胞），あるいは線維細胞を含む間葉系細胞に分化する間葉系幹細胞］を移植させる方法が開発されれば，今後の突難への新しい治療法が出現する可能性がある．

おわりに

突難の原因の1つとして新たに提唱された虚血による蝸牛内・外側壁の線維細胞障害説について述べた．難聴の改善には自然治癒があること，同じ重症度でも改善率に差があることから，聴力改善には細胞レベルでの障害部位や障害機構の細部の差，回復力の個体差があることが推測された．このため，聴力改善の予後推測の精度が低いと考えた．40dB 以下の症例（grade 1）は突難以外の種々の疾患を含むこと，予後が比較的良いことから，突難の診断基準から除外することを提案した．治療については自然治癒があることから，エビデンスのある特効薬と認められた治療薬は現在のところ見当たらない．

文　献

1) 松永達雄：内耳エネルギー不全の病態解析に基づいた突発性難聴の新規治療法開発．厚生科学研究費補助金「感覚器障害研究事業」平成16年度総括報告書，3—17.
2) Stokroos RJ, Albers FW, Schirm J : The etiology of idiopathic sudden sensorineural hearing loss. Experimental herpes simplex virus infection of the inner ear. *Am J Otol* 1998 ; 19 : 447—452.
3) Stokroos RJ, Albers FW, Schirm J : Therapy of idiopathic sudden sensorineural hearing loss : antiviral treatment of

experimental herpes simplex virus infection of the inner ear. *Ann Otol Rhinol Laryngol* 1999 ; 108 : 423—428.
4) Westerlaken BO, Stokroos RJ, Dhooge IJ, *et al* : Treatment of idiopathic sudden sensorineural hearing loss with antiviral therapy : a prospective, randomized, double-blind clinical trial. *Ann Otol Rhinol Laryngol* 2003 ; 112 : 993—1000.
5) Koga K, Hakuba N, Watanabe F, *et al* : Transient cochlear ischemia causes delayed cell death in the organ of Corti : an experimental study in gerbils. *J Comp Neurol* 2003 ; 456 : 105—111.
6) Hoya N, Okamoto Y, Kamiya K, *et al* : A novel animal model of acute cochlear mitochondrial dysfunction. *Neuroreport* 2004 ; 15 : 1597—1600.
7) Okamoto Y, Hoya N, Kamiya K, *et al* : Permanent threshold shift caused by acute cochlear mitochondrial dysfunction is primarily mediated by degeneration of the lateral wall of the cochlea. *Audiol Neurootol* 2005 ; 10 : 220—233.
8) Minowa O, Ikeda K, Sugitani Y, *et al* : Altered cochlear fibrocytes in a mouse model of DFN3 nonsyndromic deafness. *Science* 1999 ; 285 : 1408—1411.
9) Merchant SN, Adams JC, Nadol JB Jr : Pathology and pathophysiology of idiopathic sudden sensorineural hearing loss. *Otol Neurotol* 2005 ; 26 : 151—160.
10) Kanzaki J, Inoue Y, Ogawa K, *et al* : Effect of single-drug treatment on idiopathic sudden sensorineural hearing loss. *Auris Nasus Larynx* 2003 ; 30 : 123—127.
11) Kanzaki J, O-uchi T, Tsuchihashi N : Steroid-responsive sensorineural hearing loss : combination therapy with prednisolone and Saire-to. *ORL J Otorhinolaryngol Relat Spec* 1993 ; 55 : 24—29.

Audiology Japan 49, 782～788, 2006

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突発性難聴の可逆性について

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要旨：突発性難聴の原因，メカニズムはなお不明であり，予後予測の精度も必ずしも高くない。本稿では，重症度分類のGrade 2と3の症例に限定して，治癒率，改善率を調べ，治癒率に影響する因子を検討し，本疾患における報告システムの必要性を提案した。治癒率を主に発症後7日以内と発症後8～14日以内の2群に分類して検討した。発症後7日以内に受診した例の治癒率は52.9%（文献報告37～60%）であった。Grade 2と3の症例では有意差はなかった。発症後8～14日以内になると36.6%であった(文献報告20～25%)。7日目の改善率が50%以上になった場合，聴力固定時の改善率が75%以上になる率が高く，これらの例には自然治癒例が含まれている可能性が示唆された。

以上から症例全体の治癒率は，①発症後7日以内の症例数，②同じく8～14日の症例数，③Grade 3 a（めまいあり）の症例数，④治療開始7日目の改善率，⑤自然治癒率に依存していると考えられた。

－キーワード－
突発性難聴，難聴の可逆性，自然治癒，治癒率，重症度分類

はじめに

突発性難聴（以下突難と略す）の特徴は原因不明で，突然きこえなくなることであるが，さらに感音難聴にも拘らず，一部の例は完全に，一部は部分的に聴力が改善することである。難聴の原因，メカニズムは依然として不明であり，予後の予測の精度も必ずしも高くない。本稿では自験例を厚生労働省の急性高度難聴研究班（以下研究班と略す）が作成した重症度分類のGrade 2と3の症例に限定して，治癒率，改善率を調べ，治癒率に影響する因子を検討し，突難の報告システムの必要性を提案した。またこれらの成績から自然治癒の可能性を検討し，さらに可逆性難聴の動物モデルの病態から突難の可逆性を類推し，加えて聴力改善の予後予測の精度が低い理由などについて考察を加えた。

対象と方法

対象症例はすべて研究班の重症度分類でGrade 2（5分法で40dB以上60dB未満）とGrade 3（60dB以上90dB未満）の症例に限った。

1）発症後7日以内に治療した症例

A. 治癒率，改善率

対象：難聴発症後7日以内に治療を開始し，初診時聴力がGrade 2とGrade 3の症例136例。

発症1日目に治療を開始した例を第1日群とし，以下同様に第2日群から第7日群とした。治癒の判

表1　発症後　病日と治癒率(N＝136)

	第1病日	第2病日	第3病日	第4病日	第5病日	第6病日	第7病日	平均
治癒率(%)	37	50	47	62	59	56	57	52.9%
症例数	8	34	32	29	17	9	7	136
改善率(%)	78.9±28.9	77.7±33.0	77.7±27.3	80.9±31.6	81.9±37.3	78.1±28.0	80.4±16.6	79.2±30.2

定は厚生労働省突発性難聴調査研究班の聴力回復の判定基準（1974）を用いた。また，各群の聴力改善率（治療開始時と固定時聴力の差）/（治療開始時患側と健側聴力の差）を求めた。

重症度分類は厚生労働省急性高度感音難聴研究班のものを用いた。

各群の治療開始時聴力レベル，めまい症例の割合はカイ二乗検定で各群間に有意差はなかった。

B. Grade 2 と Grade 3 症例の治癒率の比較

対象：症例はAと別のシリーズである。発症後7日以内の症例で初診時聴力は Grade 2 と Grade 3 の215例。

C. めまいの有無：Bのシリーズで Grade 2（71例）と Grade 3（144例）症例の治癒率の比較

対象：発症後7日以内に治療した Grade 2 a（13例）と Grade 2 b（58例）症例，Grade 3 a（32例）と Grade 3 b（112例）症例。

D. 改善速度

対象：A，Bと別のシリーズで発症後7日以内に治療した症例（129例）。治療後7日目の改善率を用いて改善速度を検討した。

改善率が1週間以内に50%以上になる例，25～50%，25%以下に留まる例の聴力固定時の改善率から回復速度を検討した。

2）発症後8～14日に治療した最近の Grade 2（13例）と Grade 3（17例）症例の計30例の治癒率

3）発症後14日以内に治療した1），2）とは別のシリーズで Grade 2（69例）と Grade 3（40例）の計109例の治癒率を検討した

1）～3）において治療法はステロイド，ウロキナーゼ，ATP などの単剤または併用療法をおこなった。いずれの群でも特定の薬物を用いることはなかった。

結　果

1）発症後7日以内に治療した症例

(A) 治癒率，改善率

治癒例は136例中72例（52.9%）であった。

治癒，著明回復，回復，不変の割合は分散分析にて各群間に有意差を認めなかった。改善率は全症例の平均値が79.2±30.2%（M±SD）で各群間に優位差を認めなかった（表1）。

(B) Grade 2 と Grade 3 の症例の治癒率

治癒率を比較すると，Grade 2 では59%，Grade 3 では54%で有意差はなかった。

(C) Grade 2 と Grade 3 の症例のめまいの有無での治癒率の比較

Grade 2 a は13例で治癒率は46.2%，Grade 2 b は58例で治癒率は62.1%で統計学的に有意差はなかった。Grade 3 a の治癒率は32例中37.5%，Grade 3 b の治癒率は112例中58.9%で有意差を認めた。

(D) 治療開始後7日目の改善率

7日目の改善率が50%以上になる例では聴力固定時の改善率が75%以上なる率が高い(93%)。一方，1週目では50%以下，25%以下でも固定時改善率が75%以上になる例がそれぞれ44%，25%みられた(表2)。

治療開始後7日以内の改善率より3群に分類した。

A群：50%以上の改善率を示し聴力固定時75%以上の改善率を示した群（急速回復群）：129例中65例（50%）

B群：50%以下の改善率を示すが，聴力固定時75%以上の改善率を示した群（緩徐回復群）：129例中20例（16%）

C群：50%以下の改善率を示し，聴力固定時75%以下の改善率を示した群（回復不良群）：129例中44例（34%）

2）発症後8～14日に治療した症例の治癒率