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Research Report

Late-phase recovery in the cochlear lateral wall following severe degeneration by acute energy failure☆

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ABSTRACT

We previously reported a model of acute cochlear energy failure using a mitochondrial toxin, 3-nitropropionic acid (3-NP), to study mechanisms of inner ear disorders such as inner ear ischemia. In this model, the main cause of hearing loss is apoptosis of fibrocytes in the cochlear lateral wall. Here, we analyzed the time course of structural and hearing level changes in the cochlea from the acute phase to the chronic phase up to 2 months after surgery. Hearing levels as determined by auditory brainstem response (ABR) thresholds exceeded the maximum acoustic output (>87 dBSPL) of the system at all frequencies 1 day after 3-NP treatment. Histology showed nearly complete loss of fibrocytes 2 weeks after 3-NP treatment. However, after 2 months, ABR showed significant recovery at low frequency (8 kHz) in four of five rats treated with 3-NP. ABR thresholds at 20 kHz occasionally showed some recovery. At 40 kHz, recovery of ABR thresholds was not observed. Histology of 3-NP-treated rats revealed partial recovery of the lateral wall and the regenerated fibrocytes in the spiral ligament expressed Na/K-ATPase in the cochlear basal turn 2 months after 3-NP treatment. These results indicate that ABR recovery is caused by regeneration of the cochlear lateral wall. Our findings demonstrate the recoverable capacity of the cochlear lateral wall that leads to functional recovery after severe damage.

1. Introduction

Fibrocytes found within the nonsensory regions of the cochlea play a critical role in regulating inner ear ion and fluid homeostasis (Delprat et al., 2005; Minowa et al., 1999). Along with other nonsensory cells, fibrocytes in the spiral ligament have the ability to repopulate themselves after noise or aminoglycoside exposure (Hirose and Liberman, 2003; Lang et al., 2003; Roberson and Rubel, 1994; Yamashita et al., 1999). However, mechanisms by which damaged cochlear fibrocytes repair themselves remains unknown. To study mechanisms of spiral ligament repair, some hearing loss models generated by noise

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or aminoglycoside have limitations because the primary region that is damaged in these models is the cochlear sensory epithelium while degeneration of the lateral wall fibrocytes is relatively mild.

We previously reported a rat model of acute inner ear energy failure that is generated by local administration of the mitochondrial toxin 3-nitropropionic acid (3-NP) into the round window niche (Hoya et al., 2004; Okamoto et al., 2005). This toxin 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 damage in the striatum of the cerebral basal ganglia (Brouillet et al., 1995; Hamilton and Gould, 1987). In our rat model of acute inner ear energy failure, local ATP deprivation in the inner ear results from inhibition of inner ear mitochondrial function. Therefore, this model replicates the etiology of inner ear energy failure caused by ATP deprivation due to inner ear ischemia. Local administration of a high concentration (500 mM) of 3-NP into the cochlea leads to an auditory brainstem response (ABR) threshold shift at all frequencies up to 1 month after treatment (Hoya et al., 2004). With administration of a lower concentration (300 mM) of 3-NP, a temporary threshold shift occurs at low frequencies, but threshold shifts at high frequencies persist after incomplete recovery up to 1 month after treatment. Marked degeneration of lateral wall fibrocytes caused by caspase-dependent apoptosis was observed upon histological examination, and we reported that the loss of fibrocytes and a portion of the ABR threshold shifts were treatable by mesenchymal stem cell transplantation into the inner ear (Kamiya et al., 2007) or by systemic administration of a pan-caspase inhibitor (Mizutari et al., 2008).

The 3-NP-induced acute inner ear energy failure model provides an opportunity to study the structure and function of lateral wall fibrocytes during repair of the spiral ligament because this model induces severe and selective damage in cochlear fibrocytes in the lower half turns of the cochlea (Okamoto et al., 2005). In our previous study using a rat model with severe damage in lateral wall fibrocytes, no recovery was observed in function or morphology at least 2 weeks after 3-NP treatment (Okamoto et al., 2005). In our current study, we monitored structural changes in this rat model for a longer time period in order to analyze the recoverable capacity of lateral wall fibrocytes.

2. Results

2.1. Threshold shifts induced by 500 mM 3-NP treatment

Rats treated with 3-NP exhibited elevated ABR thresholds, that exceeded the maximum acoustic output of the system at all frequencies 1 day after 3-NP administration (Fig. 1). Severe ABR threshold shifts were observed until 1 month after 3-NP treatment at all frequencies. However, 2 months after 3-NP treatment, ABR thresholds showed a significant recovery at low frequency (8 kHz) in four of five rats treated with 3-NP. The mean ABR threshold was 37.0±14.4 (S.E.M.) dBSPL. Moreover, the ABR threshold at 20 kHz showed some recovery in 2 of 5 rats treated with 3-NP. The mean threshold was 80.8±13.6 dBSPL. At 40 kHz, no ABR threshold recovery was observed up to 2 months after 3-NP treatment. No significant changes in the ABR threshold were observed in control rats treated with saline (data not shown).

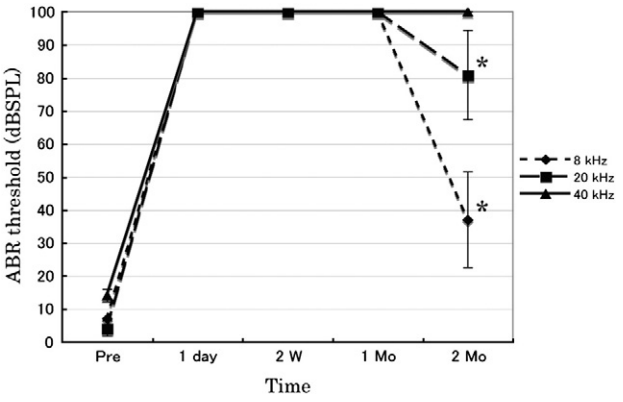

Fig. 1 – Time course of the ABR threshold shift. ABR threshold changes at 8, 20, and 40 kHz in rats treated with 500 mM 3-NP are shown. ABR thresholds at all frequencies are elevated to the scale-out level 1 day after 3-NP treatment and maintained until 1 month after 3-NP treatment. Thresholds at 8 and 20 kHz had partially recovered after 2 months. Asterisks indicate significant differences in ABR thresholds ($p<0.05$) at each frequency between 1 month and 2 months after 3-NP treatment. N=5 in each groups. Error bars indicate the standard error of the mean.

2.2. Histological changes in the cochlear lateral wall

We next evaluated histological and structural changes in cochlear structures in 3-NP-treated rats. Typically, fibrocytes of the spiral ligament are divided into five cell types based on structural features, immunostaining patterns, and general location (Spicer and Schulte, 1996). The classification is shown schematically in Fig. 2, and the following microscopic findings are described using this classification. Using light microscopy, we observed a basal-to-apical gradient in the extent of cochlear damage in rats treated with 3-NP, and all structures in the apical turn were well-preserved throughout the observation period (data not shown). There were also various structural changes in the upper side of the middle turn and the lower side of the basal turn in the cochlea. In the middle turn of the cochlea, type II fibrocytes in the spiral ligament exhibited severe degenerative changes 2 weeks after 3-NP treatment as compared to fibrocytes in untreated rats (Fig. 3). Loss of type II fibrocytes was apparent in the spiral prominence (Fig. 3B). The number of fibrocyte nuclei was also significantly decreased (Fig. 3G). Moreover, the stria vascularis demonstrated atrophic changes at this time point (Fig. 3B), and the thickness of the stria vascularis was also significantly decreased (Fig. 3H). Almost complete structural recovery of the fibrocytes in the lateral wall was observed 2 months after 3-NP treatment (Fig. 3C). The thickness of the stria vascularis also tended to increase at 2 months after 3-NP treatment (Fig. 3C) as compared to the thickness in untreated controls (Fig. 3H), but the difference between 2 weeks and 2 months was not statistically significant.

In the basal turn, there was a prominent loss and degeneration of all types of fibrocytes of the lateral wall except type V