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Research Report

Effects of heat stress on Young's modulus of outer hair cells in mice

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Abbreviations:
ACh, acetylcholine
AFM, atomic force microscopy
BM, basilar membrane
CAP, compound action potential
CLSM, confocal laser scanning microscopy
DG, displacement generator
DPOAE, distortion product otoacoustic emission
F-actin, filamentous actin
FFT, fast Fourier transformation
HSP, heat shock protein
OHC, outer hair cell
PTS, permanent threshold shift
RL, reticular lamina

ABSTRACT

Intense sound exposure causes permanent hearing loss due to hair cell and cochlear damage. Prior conditioning with sublethal stressors, such as nontraumatic sound, heat stress and restraint protects the ear from acoustic injury. However, the mechanisms underlying conditioning-related cochlear protection remain unknown. In this paper, Young's modulus and the amount of filamentous actin (F-actin) of outer hair cells (OHCs) with/without heat stress were investigated by atomic force microscopy and confocal laser scanning microscopy, respectively. Conditioning with heat stress resulted in a statistically significant increase in Young's modulus of OHCs at 3–6 h after application, and such modulus then began to decrease by 12 h and returned to pre-conditioning level at 48 h after heat stress. The amount of F-actin began to increase by 3 h after heat stress and peaked at 12 h. It then began to decrease by 24 h and returned to the pre-conditioning level by 48–96 h after heat stress. These time courses are consistent with a previous report in which heat stress was shown to suppress permanent threshold shift (PTS). In addition, distortion product otoacoustic emissions (DPOAEs) were confirmed to be enhanced by heat stress. These results suggest that conditioning with heat stress structurally modifies OHCs so that they become stiffer due to an increase in the amount of F-actin. As a consequence, OHCs possibly experience less strain when they are exposed to loud noise, resulting in protection of mammalian hearing from traumatic noise exposure.

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1. Introduction

Outer hair cells (OHCs) of the mammalian cochlea are sensory cells which have a unique capability to alter their cell length in response to changes in membrane potential (Ashmore, 1987; Brownell et al., 1985; Kachar et al., 1986; Santos-Sacchi and Dilger, 1988). Due to this so-called electromotility, OHCs subject the basilar membrane (BM) to force, thereby leading to amplification of the BM vibration. This cochlear amplification is responsible for the high sensitivity, broad dynamic range, sharp frequency selectivity and nonlinear characteristics of mammalian hearing. Unfortunately, however, OHCs, especially the first row, are vulnerable to acoustic overstimulation. Histological findings have shown that in cases with a 40-dB permanent threshold shift (PTS) after overexposure to traumatic sound, the stereocilia of OHCs are damaged without any structural damage to the supporting cells and inner hair cells (Liberman, 1987; Liberman and Dodds, 1984). The loss of OHCs causes a hearing loss of approximately 40 dB as a result of damage to the cochlear amplifier (Miller, 1974).

The susceptibility of the auditory system to hearing loss from acoustic overstimulation has been shown to decrease with repeated exposures to nontraumatic sound, a phenomenon known as "conditioning" (Miller et al., 1963). This sound induced conditioning effect has been studied in a variety of animals, including guinea pigs (Canlon et al., 1988; Kujawa and Liberman, 1997; Skellett et al., 1998), rabbits (Franklin et al., 1991), rats (Pukkila et al., 1997), chinchillas (Campo et al., 1991), gerbils (Ryan et al., 1994), mice (Yoshida and Liberman, 2000) and humans (Miyakita et al., 1992). Besides nontraumatic sound exposure, it has also been elucidated that hearing loss caused by traumatic exposure can be decreased by previous conditioning with sublethal stress, such as heat stress and physical restraint (Wang and Liberman, 2002; Yoshida et al., 1999). However, study of the effects of such conditioning on the structure and/or function of the cochlear cells has been limited. Regarding the cochlear function, it has conventionally been evaluated by measuring compound action potential (CAP) or distortion product otoacoustic emission (DPOAE). Sound and restraint but not heat stress conditioning have been found to slightly enhance the CAP threshold and CAP input–output (I–O) function without statistical significance, and DPOAEs have also been found to be slightly enhanced in conditioned ears (Kujawa and Liberman, 1999; Wang and Liberman, 2002; Yoshida and Liberman, 2000; Yoshida et al., 1999). Morphologically, it has been shown that the vesicles in the basal pole of the OHC increased after sound conditioning (Canlon et al., 1993). Filamentous actin (F-actin) in OHCs, which is a primary component of their structural filaments, has been reported to increase as a result of sound exposure, while it decreased when exposure time and/or sound pressure were changed (Hu and Henderson, 1997). Another study reported that F-actin at the bent stereocilia of OHCs decreased by exposure to high-level noise (Avinash et al., 1993).

Although these physiological and morphological studies suggest that OHCs are indeed structurally and/or functionally modified by conditioning with sublethal stress, the mechanisms underlying such structural and functional changes in OHCs and consequential conditioning-related cochlear protection remain unknown. To determine the effects of conditioning on the structure and function of OHCs in mice and to explore possible mechanisms of conditioning-related cochlear protection, Young's modulus, which indicates the elasticity of materials and is a factor determining stiffness, and the amount of F-actin, which is a primary component of cell structure, of OHCs were measured before and after conditioning by atomic force microscopy (AFM) and confocal laser scanning microscopy (CLSM), respectively. In addition, DPOAEs, which substantially rely on the electromotility of OHCs, were measured before and after conditioning. The conditioning was performed by subjecting animals to a high temperature (46.5 °C) for 15 min, i.e., heat stress, which has been proposed to be the most suitable model for exploring the mechanism of the conditioning effect on OHCs since heat stress dramatically suppresses PTS by about 25 dB in mice (Yoshida et al., 1999).

2. Results

2.1. *Changes in Young's modulus of OHCs*

The relationship between Young's modulus of the mouse OHCs in the apical turn and the cell length in each experimental group is shown in Fig. 1A. Open circles show results for the control group (n = 10), while filled circles, open diamonds, crosses, open squares and filled squares show those obtained for the anesthesia + heat groups with intervals of 3 h (n = 13), 6 h (n = 5), 12 h (n = 8), 24 h (n = 7) and 48 h (n = 12), respectively. Lengths of the apical-turn OHCs ranged from 15.5 μm to 23.0 μm and their Young's moduli ranged from 1.2 to 3.9 kPa. The regression line of the control group is given by $y = -0.025x + 2.5$ ($r = -0.11$), while those of the anesthesia + heat groups with intervals of 3 h, 6 h, 12 h, 24 h and 48 h are given by $y = -0.11\times + 4.9$ ($r = -0.23$), $y = 0.35x - 3.7$ ($r = 0.74$), $y = 0.069x + 1.3$ ($r = 0.061$), $y = 0.27x - 3.2$ ($r = 0.66$) and $y = -0.0088x + 2.6$ ($r = -0.026$), respectively. Statistical analysis indicated that there was no significant correlation between Young's modulus and the length of the OHC in the apical turn of the cochlea in each experimental group ($P > 0.05$ by Student's t-test). Young's moduli of OHCs in the anesthesia + heat groups with 3-h, 6-h and 12-h intervals tended to be larger than those of the control group and the anesthesia + heat groups with 24-h and 48-h intervals. As shown in Fig. 1B, the mean and standard deviation of Young's moduli of the apical-turn OHCs in the control group was 2.1 ± 0.5 kPa, while those in the anesthesia + heat groups with intervals of 3 h, 6 h, 12 h, 24 h and 48 h were 2.8 ± 0.8 kPa, 2.9 ± 0.6 kPa, 2.7 ± 1.0 kPa, 2.0 ± 0.3 kPa and 2.4 ± 0.6 kPa, respectively. Young's modulus of the mouse OHCs increased 1.3-fold by 3 h after heat stress and reached a peak at 6 h, which was 1.4 times as large as control level. It then began to decrease at 12 h after such stress, at which point it was still greater than that of the control group. Young's modulus returned to the pre-conditioning level by 24–48 h. Statistical analysis indicated significant differences between the control group and the anesthesia + heat groups with the two shortest intervals, i.e., 3-h and 6-h intervals, as shown by asterisks ($P < 0.05$ by Student's t-test).