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BRAIN RESEARCH

Research Report

Effects of heat stress on filamentous actin and prestin of outer hair cells in mice

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ABSTRACT

When the ear is exposed to traumatic loud noise, outer hair cells (OHCs) are damaged and thus permanent hearing loss occurs. Recently, prior conditioning with heat stress has been reported to protect OHCs from traumatic noise exposure by increasing the stiffness of the OHC soma and has also been reported to enhance distortion product otoacoustic emissions [DPOAEs; Murakoshi, M., Yoshida, N., Kitsunai, Y., Iida, K., Kumano, S., Suzuki, T., Kobayashi, T., Wada, H., 2006. Effects of heat stress on Young's modulus of outer hair cells in mice. Brain Res. 1107, 121–130]. In the present study, to further investigate the heat stress-induced protective mechanism of hearing and such stress-induced DPOAE enhancement mechanism, the amount of filamentous actin (F-actin), which is concerned with cell stiffness, and the amount of prestin, which is concerned with the generation of DPOAEs, were examined in OHCs, with and without heat stress. Heat stress was found to increase the amount of F-actin 6–24 h after heat stress. The greatest increase in the amount of F-actin was observed at the cuticular plate where F-actin anchors the roots of the stereocilia to the cell body. Based on this result, the part of the stereocilia most reinforced and protected by heat stress was concluded to be the roots of the stereocilia. In contrast with F-actin, heat stress did not affect the amount of prestin.

1. Introduction

Outer hair cells (OHCs), located on the basilar membrane as a part of the organ of Corti in the cochlea, can change their length in response to changes in membrane potential, which is known as electromotility (Ashmore, 1987; Brownell et al., 1985; Kachar et al., 1986; Santos-Sacchi and Dilger, 1988). The force generated by this electromotility of OHCs amplifies the vibration of the basilar membrane, i.e., cochlear amplification, which enables the high sensitivity, wide dynamic range and sharp frequency selectivity of hearing in mammals.

When the ear is exposed to intense and/or prolonged sound, the organ of Corti and hair cells, especially the OHCs, are damaged, resulting in a permanent threshold shift (PTS). Recently, it has been reported that OHCs can be protected from traumatic noise exposure by prior sublethal conditioning such as nontraumatic sound exposure, heat stress, ischemia and physical restraint (Canlon et al., 1988; Yoshida et al., 1999; Yoshida and

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Abbreviations: CLSM, confocal laser scanning microscopy; DPOAE, distortion product otoacoustic emissions; ECL, enhanced chemiluminescence; F-actin, filamentous actin; FITC, fluorescein isothiocyanate; HRP, horseradish peroxidase; HSP27, heat shock protein 27; OHC, outer hair cell; PTS, permanent threshold shift

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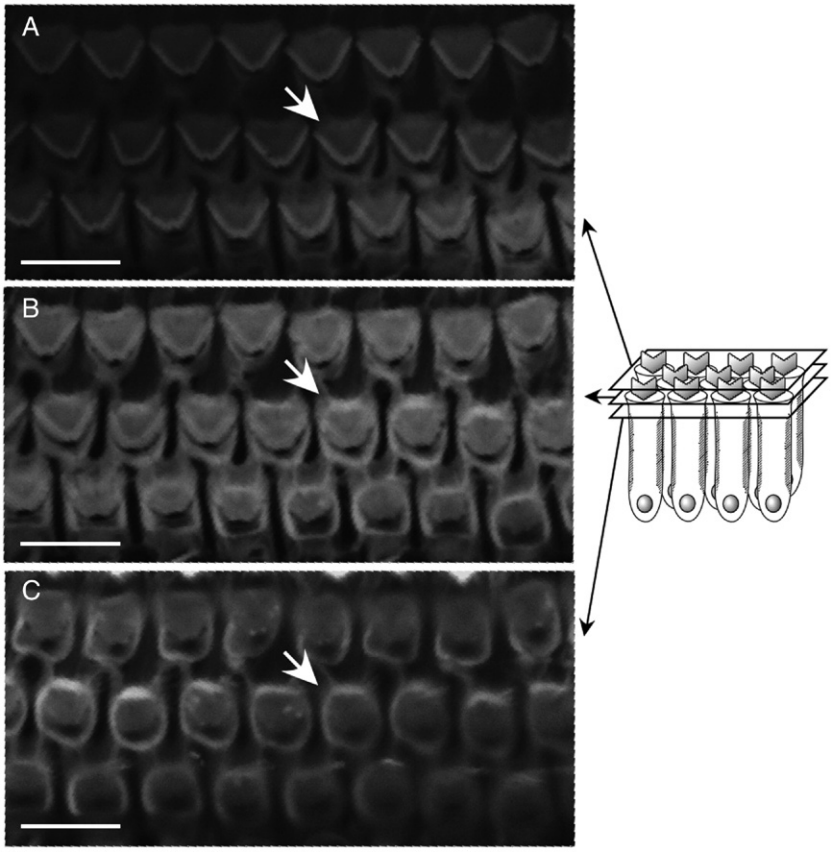

Fig. 1 – Fluorescence micrographs of the organ of Corti labeled with rhodamine–phalloidin in the normal mouse cochlea. The stereocilia, cuticular plate and lateral wall of the OHC were defined by referring to the OHC indicated by arrows. (A) Stereocilia (2 μm above the cuticular plate). (B) Cuticular plate. (C) Lateral wall (3 μm below the cuticular plate). Scale bars represent 10 μm.

Liberman, 2000; Wang and Liberman, 2002; Welch, 1992). Yoshida et al. (1999) reported that heat-stressed mice showed a reduction of PTS caused by subsequent intense sound exposure.

Although the mechanism of this protective effect remains unclear, one possibility is that modification of the OHC stiffness reduces the damage induced by traumatic exposure. Recently, heat stress has been found to increase the amount of filamentous actin (F-actin) in the OHC lateral wall with a consequent increase in wall stiffness (Murakoshi et al., 2006). Due to this increase in stiffness, intense noise causes less strain in OHCs, resulting in prevention of cell destruction.

However, to clarify the mechanism of heat stress-related hearing protection, investigation of only OHC lateral wall modification is insufficient because in many cases, noise-induced

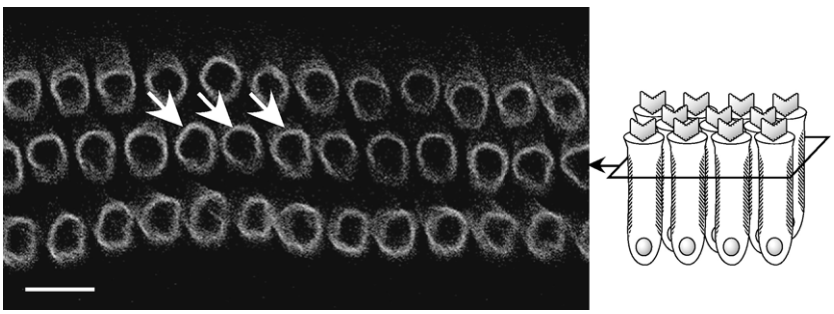

Fig. 2 – Fluorescence micrograph of the organ of Corti labeled with anti-prestin (C-16) primary antibody and FITC conjugated secondary antibody in the normal mouse cochlea. Because the surface of the organ of Corti is undulant, the z position of the cuticular plate differed from OHC to OHC. This micrograph was obtained at 2 μm below the cuticular plate of the OHCs which were indicated by arrows. Scale bar represents 10 μm.