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Noise induced changes in the expression of p38/MAPK signaling proteins in the sensory epithelium of the inner ear

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ABSTRACT

Noise exposure is a major cause of hearing loss. Classical methods of studying protein involvement have provided a basis for understanding signaling pathways that mediate hearing loss and damage repair but do not lend themselves to studying large networks of proteins that are likely to increase or decrease during noise trauma. To address this issue, antibody microarrays were used to quantify the very early changes in protein expression in three distinct regions of the chinchilla cochlea 2 h after exposure to a 0.5–8 kHz band of noise for 2 h at 112 dB SPL. The noise exposure caused significant functional impairment 2 h post-exposure which only partially recovered. Distortion product otoacoustic emissions were abolished 2 h after the exposure, but at 4 weeks post-exposure, otoacoustic emissions were present, but still greatly depressed. Cochleograms obtained 4 weeks post-exposure demonstrated significant loss of outer hair cells in the basal 60% of the cochlea corresponding to frequencies in the noise spectrum. A comparative analysis of the very early (2 h post-exposure) noise-induced proteomic changes indicated that the sensory epithelium, lateral wall and modiolus differ in their biological response to noise. Bioinformatic analysis of the cochlear protein profile using "The Database for Annotation, Visualization and Integrated Discovery 2008" (DAVID — http://david.abcc.ncifcrf.gov) revealed the initiation of the cell death process in sensory epithelium and modiolus. An increase in Fas and phosphorylation of FAK and p38/MAPK in the sensory epithelium suggest that noise-induced stress signals at the cell membrane are transmitted to the nucleus by Fas and focal adhesion signaling through the p38/MAPK signaling pathway. Up-regulation of downstream nuclear proteins E2F3 and WSTF in immunoblots and microarrays along with their immunolocalization in the outer hair cells supported the pivotal role of p38/MAPK signaling in the mechanism underlying noise-induced hearing loss.

Abbreviations: NIHL, noise induced hearing loss; dB, decibel; SPL, sound pressure level; MAPK, mitogen activated protein kinase; WSTF, Williams syndrome transcription factor; IHC, inner hair cells; OHC, outer hair cells; SGN, spiral ganglion neurons; DPOAE, distortion product otoacoustic emission; DAVID, Database for Annotation, Visualization and Integrated Discovery; FAK, focal adhesion kinase; DEDAF, death effector domain-associated factor; Rb, retinoblastoma protein; PI, propidium iodide; PTS, permanent threshold shift; ATM, ataxia telangiectasia mutated.

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1. Introduction

Prolonged exposure to high intensity noise in occupational or recreational settings is a major hearing health care problem. Worldwide, noise exposure accounts for roughly 16% of cases of hearing loss in adults [1] and among combat personnel, the percentage rises to 50% [2]. Exposure to loud noise causes a number of pathological changes in the cochlea resulting in elevated hearing thresholds. Noise exposure can adversely affect all three regions of the cochlea (Fig. 1), the organ of Corti, the lateral wall and the spiral ganglion neurons (SGN) [3–7]. Much of the research on noise-induced hearing loss (NIHL) has focused on the sensory hair cells in the organ of Corti where auditory transduction occurs [8–11], but there is growing awareness that the SGN and lateral wall of the cochlea are adversely affected by noise [7,12]. The organ of Corti contains two types of sensory hair cells, outer hair cells (OHC) and inner hair cells (IHC). The OHCs, which are electromotile, act as a cochlear amplifier enhancing the sound-induced vibration of the basilar membrane [13]. The IHC, which make synaptic contact with 95% of SGN, play a major role in converting sound into neural activity and relaying this information through the auditory nerve fibers to the central auditory system. The hair cells, particularly OHCs, are considered to be the most susceptible to noise-induced damage.

Three modes of hair cell death have been reported in the inner ear — necrosis, apoptosis [9,14], and an atypical mode of cell death featuring loss of plasma membrane in the basal pole of the OHC [15]. The molecular mechanisms that regulate the balance of cell death and cell survival in the inner ear are not completely understood, but there is growing awareness that mitogen-activated protein kinases may be important. p38/MAPK (Mapk14), a stress-activated member of the family of mitogen-activated protein kinases, is an importing important signaling protein that links activity at the cell membrane to downstream signaling in the nucleus. Cellular processes in which p38/MAPK participates are numerous and include inflammation, cell cycle regulation and apoptosis [16]. p38/MAPK can be activated by a diverse spectrum of environmental factors and endogenous stimuli which include Fas-mediated pathways [17] and focal adhesion signaling [18]. Inhibitors of p38/MAPK have been shown to confer protection to the inner ear from stress induced by noise [19] and the ototoxic antibiotic, gentamycin [20].

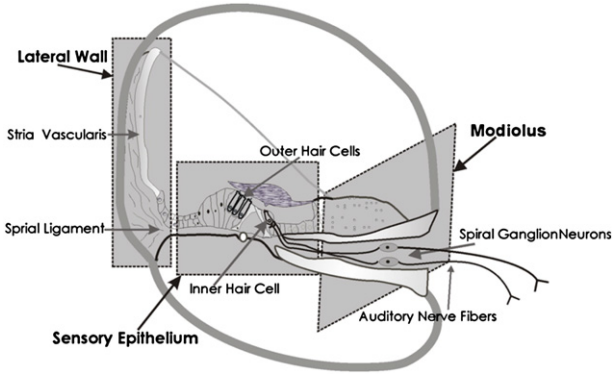

Fig. 1 – Schematic of the cochlea. The schematic illustrates the complex structure of the cochlea. The different cellular types included in the three discrete regions used for proteomic screening have been highlighted with dotted lines in this section of the cochlea.

Williams syndrome transcription factor (WSTF) and E2F3 are two of the many signaling proteins downstream of p38/MAPK. WSTF (Baz1b) is a nuclear signaling protein that modulates transcription through chromatin remodeling. Phosphorylation of serine-158 on WSTF is required for vitamin D-dependent transcription [21]. E2F3 is a transcription factor involved in cell cycle regulation and induction of apoptosis. E2F3 activity is repressed by binding of the retinoblastoma protein (Rb). Hyperphosphorylation of Rb by p38/MAPK and cell cycle dependent protein kinases causes the release of Rb and transcriptional activation of E2F3 target genes [22].

Although several groups have identified protein changes associated with NIHL using Western blots and immunolabeling [12,23,24], these methods do not lend themselves to studying large networks of proteins that are likely to increase or decrease during noise trauma. In order to provide a broad overview of the very early protein expression changes associated with NIHL, we used antibody microarrays to investigate proteomic responses [25,26] to acoustic overstimulation in three discrete regions of the chinchilla cochlea, viz. sensory epithelium, lateral wall and the modiolus containing the SGN. Importantly, an early two hour time-point was chosen to identify the initial proteomic responses that precede the noise-induced permanent shift in hearing threshold and loss of hair cells. Our results indicate that intense noise increased the levels of focal adhesion kinase phosphorylated on tyrosine-577, WSTF, diphosphorylated p38/MAPK and increased expression of E2F3. Collectively, these results suggests that the sensory epithelium responds to noise through p38/MAPK signaling involving regulation of focal adhesion junctions in stereocilia and in the apical aspects of hair cells.

2. Materials and methods

2.1. Animals

Long-tailed chinchillas (*Chinchilla lanigera*) weighing from 450 to 750 g were used for these experiments because there is an extensive literature dealing with the anatomical, physiological and behavioral consequences of NIHL in this species and because their hearing range is comparable to that of humans [27–29]. The animals were maintained in a temperature-controlled room with a 12-h light/dark cycle and allowed free access to food and water. The experimental protocol was reviewed and approved by the University at Buffalo Institutional Animal Care and Use Committee. The animals were handled and treated according to guidelines established by the National Institutes of Health and the Institutional Animal Care and Use Committee at the University at Buffalo.

2.2. Reagents

All reagents were purchased from Sigma Aldrich Chemical Company (St. Louis, MO) unless noted otherwise.