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Research paper

The effects of acoustic environment after traumatic noise exposure on hearing and outer hair cells

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ABSTRACT

Previous studies reported that exposure to non-traumatic level sounds after traumatic noise exposure reduced the degree of noise-induced hearing loss and hair cell stereocilia damage. The current study investigated the effects of a 3-day post-noise acoustic environment on the degree of noise-induced hearing loss and cochlear damage. Female chinchillas were exposed to traumatic continuous noise (4 kHz octave-band noise) at 107 dB SPL for 1 h and then placed in either an augmented acoustic environment (AAE) or deprived acoustic environment (DAE) for 3 days. The AAE group was exposed to a broad-band noise (4–20 kHz) at 80 dB SPL and the DAE animals were fit with conventional earplugs to minimize the level of acoustic stimulation. Auditory brainstem responses (ABRs) were recorded before and 3 days after the traumatic noise exposure. The AAE group showed a significantly lower average threshold shift at the frequencies of 4 and 8 kHz ($p < 0.01$). Correspondingly, significantly fewer missing and dying outer hair cells (OHCs) were observed in the AAE group than in the DAE group. Although the cochlear reduced and oxidized glutathione levels (GSH and GSSG, respectively) were essentially the same in two groups at day 3, significant correlations were found between GSSG levels and mean ABR threshold shift (1–16 kHz) in the AAE group; as well as GSSG and percentage of total OHC loss in the DAE group. The results suggest that post-noise acoustic environment influenced the degree of hearing loss and OHC deterioration after traumatic noise exposure.

1. Introduction

"Sound conditioning", also known as "toughening", is a well-established means to protect hearing from traumatic noise exposure and refers to the acquired resistance to noise-induced hearing loss (NIHL) by pre-exposure to a non-traumatic level noise (Canlon et al., 1988; Campo et al., 1991; Henselman et al., 1994). Interestingly, exposure to a non-traumatic level noise after traumatic noise exposure (i.e. augmented acoustic environment (AAE)) is also reported to reduce a degree of NIHL (Niu et al., 2004; Norena and Eggermont, 2005). Application of AAE in auditory research has a long history and was first employed in mice with genetic age-related sensorineural hearing loss (Turner and Willott, 1998; Willott and Turner, 1999). A series of studies suggested that AAE can ameliorate the severity and time course of hearing loss as well as OHC loss in C57BL/6J and DBA/2J mice (Turner and Willott, 1998; Willott and Turner, 1999; Willott and Bross, 2004; Willott et al., 2001, 2005, 2006a,b, 2008).

An AAE approach in NIHL reported that compared to a regular quiet sound environment, the ameliorative effect of short-term (24 h) AAE in guinea pigs is about 5–10 dB in auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE) threshold shifts (Niu et al., 2004). The effect was about 10–45 dB in ABR threshold shifts for long-term (>35 days) AAE in cats (Norena and Eggermont, 2005). The reduced DPOAE threshold shifts in the Niu et al. study (2004) by post-exposure AAE suggest the involvement of outer hair cells (OHCs) in the protective effect. However, these studies did not investigate the cochlear cellular correlates of the ameliorative effects. Additionally, Fukushima et al. (1990) concluded that sound stimulation after acoustic trauma is beneficial for hair cell stereocilia than resting the ear by sound deprivation. In this study, the researchers performed unilateral

Abbreviations: AAE, augmented acoustic environment; DAD, diode array detector; DAE, deprived acoustic environment; FITC, fluorescein isothiocyanate; GSH, reduced glutathione; GSSG, oxidized glutathione; HPLC, high-performance liquid chromatography; NIHL, noise-induced hearing loss; OHC, outer hair cells

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ossiculectomy, which yielded about 40–55 dB conductive loss, after traumatic noise exposure to create a deprived acoustic environment (DAE) and assigned hair cell stereocilia damage scores only in the apical boundary of the locus of damage, not in the entire cochlea. The investigators reported more hair cell stereocilia damage 1 week after traumatic noise exposure in this cochlear region in the sound deprived ear as compared with that of the non-deprived ear with intact middle ear structures. Given that the study examined only limited region of the cochlea and some recovery of hair cell stereocilia was observed at 12 week post-trauma in both deprived and non-deprived groups, Fukushima study provides a hint, but very weak evidence that auditory deprivation following traumatic noise exposure can influence auditory thresholds. Unlike AAE study in mice with genetic age-related hearing loss, no study investigated the auditory and cellular effects of acoustic environment following a traumatic noise exposure.

Numerous possible underlying mechanisms for the ameliorative effect of AAE have been speculated. Norena and Eggermont (2005) proposed that the partial conservation of hearing might be due to regeneration of auditory nerve fiber neurites and hair cell stereocilia, improvement of the microcirculation in the cochlea, or release of nerve growth factors. Niu et al. (2004) hypothesized that one possible mechanism may be related to increases in antioxidant capacity in the cochlea. This mechanism has been demonstrated to be part of the pre-exposure toughening phenomenon (Jacono et al., 1998). Metabolism of glutathione, a natural endogenous tripeptide antioxidant, is identified as a major line of defense for the protection of hair cells from oxidative stress caused by noise exposure (Evans and Halliwell, 1999; Henderson et al., 2006; Le Prell et al., 2007; Ohlemiller et al., 1999). Since the antioxidant enzymes in the cochlea are upregulated with pre-traumatic noise exposure sound conditioning, our hypothesis was that further upregulation of the antioxidant defense in the cochlea may be an underlying mechanism of the observed protective effect by AAE.

Oxidative stress is known to cause cell death though apoptosis and necrosis which have been identified as key cell death pathways in the noise-exposed chinchilla cochlea (Hu et al., 2002; Nicotera et al., 2003). Yang et al. (2004) investigated the expansion of cochlear lesions in two groups of chinchillas after exposure to different levels of a continuous noise (4 kHz octave-band noise at either 104 dB or 108 dB SPL for 1 h) at three time points (1, 4 or 30 days post-noise exposure) and found that the proportions of dying (apoptotic and necrotic) and missing OHCs were different after the high versus low noise exposure level. For example, the number of apoptotic OHCs was statistically greater than the number of necrotic OHCs at day 1 in 104-dB group, and at day 1 and 4 in 108-dB group. Based on this observation, we hypothesized that the proportion of OHC death may be influenced by the level of post-exposure sound environment. Thus, AAE and DAE may lead to the different proportions of the OHC death types.

In the current study, we examined the role that post-exposure acoustic environment plays in determining: severity of hearing loss, the degree and nature of cochlear damage, and possible biochemical pathways underlying the differences. Two different acoustic environments, AAE and DAE, were selected in order to examine the influence of post-exposure sound in two extreme acoustic environments. This study design enabled us to maximize the difference in acoustic levels between two groups to investigate whether sound after traumatic noise exposure matters for function and anatomy of the cochlea. Since we were interested in the underlying mechanisms, three variables were investigated after 3-day AAE or DAE treatment: (1) ABR threshold shifts; (2) percentage of dying and missing OHCs; (3) cochlear reduced glutathione (GSH) and oxidized glutathione (GSSG) levels as a measure of redox status. OHC morphology was investigated in the entire cochlea along with ABR thresholds at the day-3 time point, a point that has not been previously investigated. The chinchillas served as the experimental subject since this animal species has not been studied with AAE.

2. Materials and methods

2.1. Subject

A total of ten female adult chinchillas (522–751 g) with normal ABR thresholds were used in this study. They were obtained from Ryerson Chinchilla Ranch (Plymouth, OH) at various ages from 1 to 2 years. The animals were housed in a quiet colony. All animals were exposed to traumatic noise after baseline ABR threshold measurement and placed in one of the two different sound environments, AAE and DAE, for 3 days ($n = 5$ per group). Animals were sacrificed at the end of the treatment to examine cellular correlates of the effect of post-exposure sound treatment. All procedures involving use and care of the animals were reviewed and approved by the State University of New York at Buffalo Institutional Animal Care and Use Committee.

2.2. Traumatic noise exposure

Awake chinchillas were exposed to an octave-band noise with the center frequency at 4 kHz at the level of 107 dB SPL for 1 h. This noise parameter was selected to produce moderate damage to the cochlea based on a previous study (Yang et al., 2004). The noise was generated by TDT RPvdsEx software and RP 2.1 hardware (Tucker Davis Technologies, Alachua, FL), routed through an attenuator and SERVO 300 power amplifier (Samson, Hauppauge, NY), and presented through an acoustic horn attached to a speaker which was suspended directly above the animal cage. The noise level was calibrated at the level of the animals' head utilizing a calibrated Model 800B sound level meter (Larson Davis Inc., Depew, New York) and a 1/2" condenser microphone. Fig. 1 illustrates noise intensity levels (gray bar) in 1/3 octave band with various center frequencies measured at the level of animals' head in a noise exposure booth.

2.3. AAE and DAE treatments

Immediately after the traumatic noise exposure, the AAE group was exposed to controlled continuous noise, a broad-band noise (4–20 kHz) at 80 dB SPL, for 24 h per day for 3 days. The AAE noise was generated by TDT RPvdsEx software and RP 2 hardware, amplified by AMP 300 power amplifier (AudioSource Inc., Portland, OR), and presented through a speaker mounted on top of the ani-

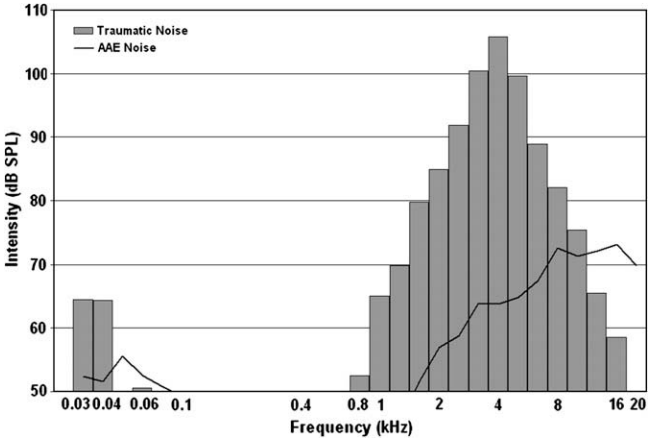

Fig. 1. Traumatic (gray bar) and AAE (black line) noise intensity levels (dB SPL) measured in 1/3 octave band with center frequency from 0.03 to 20 kHz.