Hearing Research xxx (2018) 1–14

Contents lists available at ScienceDirect

Hearing Research

journal homepage: www.elsevier.com/locate/heares

Research Paper

The endocochlear potential as an indicator of reticular lamina integrity after noise exposure in mice

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ARTICLE INFO

Article history:
Received 21 November 2017
Received in revised form
23 January 2018
Accepted 30 January 2018
Available online xxx

Keywords:
Stria vascularis
Noise-induced hearing loss
Tight junction
Cochlea
Holes
Tears
Mouse

ABSTRACT

The endocochlear potential (EP) provides part of the electrochemical drive for sound-driven currents through cochlear hair cells. Intense noise exposure (110 dB SPL, 2 h) differentially affects the EP in three inbred mouse strains (C57BL/6 [B6], CBA/J [CBA], BALB/cJ [BALB]) (Ohlemiller and Gagnon, 2007, Hearing Research 224:34-50; Ohlemiller et al., 2011, JARO 12:45-58). At least for mice older than 3 mos, B6 mice are unaffected, CBA mice show temporary EP reduction, and BALB mice may show temporary or permanent EP reduction. EP reduction was well correlated with histological metrics for injury to stria vascularis and spiral ligament, and little evidence was found for holes or tears in the reticular lamina that might 'short out' the EP. Thus we suggested that the genes and processes that underlie the strain EP differences primarily impact cochlear lateral wall, not the organ of Corti. Our previous work did not test the range of noise exposure conditions over which strain differences apply. It therefore remained possible that the relation between exposure severity and acute EP reduction simply has a higher exposure threshold in B6 mice compared to CBA and BALB. We also did not test for age dependence. It is well established that young adult animals are especially vulnerable to noise-induced permanent threshold shifts (NIPTS). It is unknown, however, whether heightened vulnerability of the lateral wall contributes to this condition. The present study extends our previous work to multiple noise exposure levels and durations, and explicitly compares young adult (6–7 wks) and older mice (>4 mos). We find that the exposure level-versus-acute EP relation is dramatically strain-dependent, such that B6 mice widely diverge from both CBA and BALB. For all three strains, however, acute EP reduction is greater in young mice. Above 110 dB SPL, all mice exhibited rapid and severe EP reduction that is likely related to tearing of the reticular lamina. By contrast, EP-versus-noise duration examined at 104 dB suggested that different processes contribute to EP reduction in young and older mice. The average EP falls to a constant level after ~7.5 min in older mice, but progressively decreases with further exposure in young mice. Confocal microscopy of organ of Corti surface preparations stained for phalloidin and zonula occludens-1 (ZO-1) indicated this corresponds to rapid loss of outer hair cells (OHCs) and formation of both holes and tears in the reticular lamina of young mice. In addition, when animals exposed at 119 dB were allowed to recover for 1 mo, only young B6 mice showed collapse of the EP to ≤5 mV. Confocal analysis suggested novel persistent loss of tight junctions in the lateral organ of Corti. This may allow paracellular leakage that permanently reduces the EP. From our other findings, we propose that noise-related lateral wall pathology in young CBA and BALB mice promotes hair cell loss and opening of the reticular lamina. The heightened vulnerability of young adult animals to noise exposure may in part reflect special sensitivity of the organ of Corti to acute lateral wall dysfunction at younger ages. This feature appears genetically modifiable.

1. Introduction

Mammalian cochlear injury targets of noise exposure include the organ of Corti and the stria vascularis. Changes in either can

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https://doi.org/10.1016/j.heares.2018.01.015
0378-5955/

affect the endocochlear potential (EP), which provides much of the electrochemical driving force for sound-induced currents through hair cells (Wangemann, 2006). Depending on model and conditions, the EP may temporarily either increase or decrease. Acute EP increases have been attributed to changes in the organ of Corti such as prolonged hair cell depolarization or decreased conductance of transducer channels (Ohlemiller et al., 2016). Acute EP decreases appear attributable to injury to the stria or spiral ligament (Ohlemiller and Gagnon, 2007; Ohlemiller et al., 2011a) or to increased leakiness of the organ of Corti, which may 'short out' the EP (Ahmad et al., 2003). Within this framework, the EP may serve either as an indicator of events in the organ of Corti, or as the primary metric of interest, since lateral wall pathology that directly decreases the EP will elevate thresholds independent of any changes in the organ of Corti.

Work in mice has shown that the extent of noise injury to the stria and spiral ligament and resulting EP reduction are genetically modifiable. Following a 2-hr broadband exposure at 110 dB SPL, three inbred mouse strains (C57BL/6J [B6], CBA/J [CBA], BALB/cJ [BALB]) show different phenotypes, such that B6 mice are unaffected, CBA mice show temporary EP reduction, and BALB mice show temporary or permanent EP reduction (Ohlemiller and Gagnon, 2007; Ohlemiller et al., 2010, 2011a). The EP reduction is correlated with histological injury metrics to stria vascularis, spiral ligament, and Reissner's membrane. By contrast, little evidence was found for 'holes' left by rapid hair cell loss, or tears in the reticular lamina. Thus we suggested that the genes and processes that underlie these strain differences primarily impact cochlear lateral wall. One or more of the underlying genes lies on proximal chromosome 18 (*Nirep* QTL) (Ohlemiller et al., 2010, 2016).

Noise-related reticular lamina leaks that may reduce the EP appear to take two forms, both supported mostly by anecdotal evidence. At high noise levels ($\geq$124 dB in guinea pigs and $\geq$116 dB in mice) mechanical trauma may tear the reticular lamina (Fredelius, 1988; Fredelius et al., 1990; Henderson et al., 1994; Hirose and Liberman, 2003; Hirose et al., 2005; Spongr et al., 1998; Thorne et al., 1984; Wang et al., 2002; Zheng and Hu, 2012). At much lower noise levels (82 dB), studies in chinchillas support the notion of rapid hair cell death that leaves holes in the reticular lamina (Harding and Bohne, 2004). Both tears and holes would promote mixing of endolymph and perilymph, ostensibly exposing hair cells to toxically high K^+ levels, thereby magnifying hair cell and hearing loss. Such a process is supported by knockout models for tight junctional proteins such as vezatin, claudin-9, claudin-14, occludin, angulin-2 (Ildr1), and tricellulin (Bahloul et al., 2009; Ben-Yosef et al., 2003; Kitajiri et al., 2014; Morozko et al., 2014; Nakano et al., 2009; Nayak et al., 2013). Some of these models (claudin-9 and -14) show both reduced electrical resistance of tight junctions and hair cell loss, despite a normal EP. In mice, hair cell death that causes holes and EP reduction remains the exception to the rule, having only been reported in knockout models (Cohen-Salmon et al., 2002; Jin et al., 2016). On balance, observations of hair cell death from noise, ototoxins, or genetic causes support the predominance of mechanisms for quickly sealing off the reticular lamina so that electrical impedance and ionic integrity are maintained. The present work attempts to separate lateral wall and potential organ of Corti contributions to noise-related EP reduction.

Our previous work did not test the range of noise exposure conditions over which mouse strain differences apply. It thus remained possible that the relation between exposure level or duration and acute EP reduction simply has a higher exposure threshold in B6 mice compared to CBA and BALB. To address this, we measured EP changes in all three mouse strains in response to a wide range of 2-hr exposures (85–119 dB SPL). Our previous work also did not test for age dependence of results. It is well established that young adult animals are especially vulnerable to noise-induced permanent threshold shifts (NIPTS) (Ohlemiller et al., 2000, 2011b; Pujol, 1992; Saunders and Chen, 1982). It is unknown, however, whether heightened vulnerability of the lateral wall contributes to this early 'vulnerable period'. Alternatively, the organ of Corti of young mice may be more affected by acute lateral wall dysfunction than in older animals. In the present work we separately examined mice whose age fell near the peak of the vulnerable period for mice (6–7 wks), and outside this period (>4 mos) (Henry, 1982). Because exaggerated loss of outer hair cells (OHCs) is a known aspect of the vulnerable period (Ohlemiller et al., 2000), for some exposure conditions we examined surface preparations of the organ of Corti using antibodies for hair cell cuticular plates and tight junctions. Our observations confirm striking genetic dependence of cochlear noise-related lateral wall injury and EP reduction, such that B6 mice are more resistant than CBA or BALB under most exposure conditions. They further suggest that noise injury to the lateral wall in young CBA and BALB mice exacerbates injury to the organ of Corti in the form of rapid OHC loss and formation of both holes and tears in the reticular lamina. While recovery of the EP indicates that holes and tears are typically repaired, the EP in young B6 mice showed persistent collapse to $\leq$5 mV after 2-hr exposure at 119 dB SPL. Surface preparations in these mice suggest novel, permanent, disruption of tight junctions in the reticular lamina.

2. Methods

2.1. Animals

Procedures were approved by the Washington University Institutional Animal Care and Use Committee. Physiological recordings were conducted using C57BL/6J (n = 143), CBA/J (n = 200), and BALB/cJ (BALB, n = 208), all derived from breeders purchased from The Jackson Laboratory (JAX). Mice were either 6–7 weeks ('young') or 4–16 mos of age ('older') at the time of evaluation. The age of older BALBs was capped at 13 mos as our published data indicated that BALBs may show age-associated EP decline thereafter (Ohlemiller et al., 2006). All samples were randomly composed by gender, and no gender effects were detected in any feature reported here.

2.2. EP recording

All animals underwent a single EP measurement, obtained from the cochlear lower basal turn of the left ear. For EP recording, animals were anesthetized (60 mg/kg sodium pentobarbital, IP) and positioned ventrally in a custom headholder. Core temperature was maintained at 37.5 ± 1.0 °C using a thermostatically-controlled heating pad in conjunction with a rectal probe (Yellow Springs Instruments Model 73A). An incision was made along the midline of the neck and soft tissues were blunt dissected and displaced laterally to expose the trachea and left bulla. A tracheostomy was then made and the musculature over the bulla was cut posteriorly to expose the bone overlying the round window. Using a fine drill, a hole was made in the left cochlear capsule directly over scala media of the lower basal turn. Glass capillary pipettes (40–80 MΩ) filled with 0.15 M KCl were mounted on a hydraulic microdrive (Frederick Haer) and advanced until a stable positive potential was observed that did not change with increased electrode depth. The signal from the recording electrode was led to an AM Systems Model 1600 intracellular amplifier. A silver/silver chloride ball inserted into the neck muscles served as ground.

Please cite this article in press as: Ohlemiller, K.K., et al., The endocochlear potential as an indicator of reticular lamina integrity after noise exposure in mice, Hearing Research (2018), https://doi.org/10.1016/j.heares.2018.01.015