Hearing Research 327 (2015) 163–174

Contents lists available at ScienceDirect

Hearing Research

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

Research paper

Morphological correlates of hearing loss after cochlear implantation and electro-acoustic stimulation in a hearing-impaired Guinea pig model

Lina A.J. Reiss [a, *], Gemaine Stark [a], Anh T. Nguyen-Huynh [a], Kayce A. Spear [a], Hongzheng Zhang [a, b], Chiemi Tanaka [a, c], Hongzhe Li [a]

[a] Oregon Hearing Research Center, Department of Otolaryngology, Oregon Health & Science University, 3181 SW Sam Jackson Park Road, Portland, OR 97239, USA
[b] Department of Otolaryngology Head & Neck Surgery, Zhujiang Hospital of Southern Medical University, Guangzhou 510280, China
[c] Department of Communication Sciences and Disorders, John A. Burns School of Medicine, University of Hawai'i at Mānoa, 677 Ala Moana Blvd, Honolulu, HI 96816, USA

ARTICLE INFO

Article history:
Received 19 March 2015
Received in revised form
30 April 2015
Accepted 3 June 2015
Available online 16 June 2015

Keywords:
Hybrid
Cochlear implant
Electro-acoustic stimulation
Stria vascularis
Synapse
Hearing loss

ABSTRACT

Hybrid or electro-acoustic stimulation (EAS) cochlear implants (CIs) are designed to provide high-frequency electric hearing together with residual low-frequency acoustic hearing. However, 30–50% of EAS CI recipients lose residual hearing after implantation. The objective of this study was to determine the mechanisms of EAS-induced hearing loss in an animal model with high-frequency hearing loss.

Guinea pigs were exposed to 24 h of noise (12–24 kHz at 116 dB) to induce a high-frequency hearing loss. After recovery, two groups of animals were implanted (n = 6 per group), with one group receiving chronic acoustic and electric stimulation for 10 weeks, and the other group receiving no stimulation during this time frame. A third group (n = 6) was not implanted, but received chronic acoustic stimulation. Auditory brainstem responses were recorded biweekly to monitor changes in hearing. The organ of Corti was immunolabeled with phalloidin, anti-CtBP2, and anti-GluR2 to quantify hair cells, ribbons and post-synaptic receptors. The lateral wall was immunolabeled with phalloidin and lectin to quantify stria vascularis capillary diameters. Bimodal or trimodal diameter distributions were observed; the number and location of peaks were objectively determined using the Aikake Information Criterion and Expectation Maximization algorithm.

Noise exposure led to immediate hearing loss at 16–32 kHz for all groups. Cochlear implantation led to additional hearing loss at 4–8 kHz; this hearing loss was negatively and positively correlated with minimum and maximum peaks of the bimodal or trimodal distributions of stria vascularis capillary diameters, respectively. After chronic stimulation, no significant group changes in thresholds were seen; however, elevated thresholds at 1 kHz in implanted, stimulated animals were significantly correlated with decreased presynaptic ribbon and postsynaptic receptor counts. Inner and outer hair cell counts did not differ between groups and were not correlated with threshold shifts at any frequency.

As in the previous study in a normal-hearing model, stria vascularis capillary changes were associated with immediate hearing loss after implantation, *while little to no hair cell loss was observed even in cochlear regions with threshold shifts as large as 40–50 dB*. These findings again support a role of lateral wall blood flow changes, rather than hair cell loss, in hearing loss after surgical trauma, and implicate the

Abbreviations: ABR, Auditory brainstem response; ACE, Advanced Combination Encoder; AIC, Aikake Information Criterion; CAES, Chronic Acoustic Electric Stimulation; CAS, Chronic Acoustic Stimulation; CI, Cochlear implant; CIS, Chronic Interleaved Sampling; DAI, Direct Audio Input; EABR, Electrically-evoked auditory brainstem response; EAS, Electric and acoustic stimulation; IHC, Inner hair cell; IM, Intramuscular; NS, No stimulation; OHC, Outer hair cell; SV, Stria vascularis, stria vascular

* Corresponding author. Oregon Hearing Research Center, Department of Otolaryngology, Oregon Health and Science University, 3181 SW Sam Jackson Park Road, Portland, OR 97239, USA. Tel.: +1 503 494 2917; fax: +1 503 494 5656.
E-mail address: reiss@ohsu.edu (L.A.J. Reiss).

http://dx.doi.org/10.1016/j.heares.2015.06.007

endocochlear potential as a factor in implantation-induced hearing loss. Further, the analysis of the hair cell ribbons and post-synaptic receptors suggest that delayed hearing loss may be linked to synapse or peripheral nerve loss due to stimulation excitotoxicity or inflammation. Further research is needed to separate these potential mechanisms of delayed hearing loss.

1. Introduction

The goal of Hybrid or electro-acoustic stimulation (EAS) cochlear implants (CIs) is to provide high-frequency electric hearing while preserving residual low-frequency acoustic hearing for combined electric and acoustic stimulation in the same ear. The Hybrid or EAS CI is a shorter, thinner version of the traditional CI, and is implanted using soft surgery techniques which include careful, slow insertion of the electrode into the cochlea (Kiefer et al., 2002; Gantz and Turner, 2003). This type of CI is aimed for patients with a severe-profound high-frequency hearing loss, but good low-frequency hearing, who would not be candidates for a traditional full-length CI.

The advantages of using EAS over using a traditional CI alone include superior speech recognition in the presence of background talkers (Turner et al., 2004; Dorman et al., 2008), superior musical melody and instrument recognition (Gfeller et al., 2006; Dorman et al., 2008), and improved ability to use localization cues to attend to a speaker in spatially separated noise (Gifford et al., 2013; Rader et al., 2013). Further, speech perception outcomes are superior when hearing is preserved *even when the CI is used alone without the acoustic hearing, i.e. without acoustic amplification*; this indicates the importance of minimizing the damage to neurosensory structures for effective electrical stimulation (Carlson et al., 2011; Fitzpatrick et al., 2014).

However, between 30 and 55% of EAS CI patients lose 30 dB or more of their residual low-frequency hearing within months after implantation (Gantz et al., 2009; Gstoettner et al., 2009; Santa Maria et al., 2013). The hearing loss does not typically occur right after surgery, but is slow and may take several months to manifest. While many EAS CI patients can function well on speech recognition in quiet with the electric CI component alone, they lose the additional benefits of EAS for speech recognition in noise, as well as for musical melody discrimination and sound localization (Gantz et al., 2009). Clearly, hearing preservation success rates need to be improved in order to allow full benefit from EAS.

The mechanism underlying this residual hearing loss is unknown. Retrospective analysis of subject risk factors in the Hybrid clinical trial indicate increased risk of implant-induced hearing loss with male gender, age, and an etiology of noise-induced hearing loss (Kopelovich et al., 2014). Studies in animal models are conflicting; some studies suggest that an inflammatory or immune response to electrode insertion trauma can lead to hair cell death (Eshraghi et al., 2013), while other studies have shown that this hearing loss is not explained by hair cell or spiral ganglion cell loss (Tanaka et al., 2014; O'Leary et al., 2013). Other proposed factors, such as fibrosis or bone growth, show weak correlations with hearing loss and are unlikely to play a major role in hearing loss after cochlear implantation (Tanaka et al., 2014; O'Leary et al., 2013).

In our previous study, we tested an alternative hypothesis, that electro-acoustic stimulation itself may cause hearing loss (Tanaka et al., 2014). Guinea pigs were implanted for up to 3 months, and hearing preservation and histology were compared in animals that were stimulated versus two sets of controls, implanted animals without stimulation and non-implanted animals. We were able to replicate both a high-frequency hearing loss immediately after surgery and a delayed low-frequency hearing loss in the stimulated group several weeks after implantation. The only histological changes associated with hearing loss were increased cross-sectional area and decreased blood vessel or capillary density of the stria vascularis (SV), the part of the cochlear lateral wall responsible for maintaining the high endocochlear potential or "battery" required for hair cells to transduce sound. This finding is consistent with a proposed role of the lateral wall in implantation trauma due to its vulnerable location in the path of the electrode insertion (Wright and Roland, 2013). However, this association was limited to the high-frequency hearing loss. The delayed low-frequency hearing loss was not explained by changes viewable under a light microscope, such as hair cell, spiral ganglion cell, or stria vascularis changes, or by fibrosis or ossification.

The previous findings suggest that the low-frequency hearing loss may instead be caused by more subtle changes, such as at the hair cell synapse, rather than hair cell death. Previous studies have shown glutamate excitotoxicity occurs after noise-induced hearing loss and can be mimicked by glutamate agonists (e.g. Pujol et al., 1985; Puel et al., 1994; Wang and Green, 2011). Further, such excitotoxicity can eventually lead to irreversible loss of the hair cell synapses and nerve fibers, even after "temporary" hearing loss (Kujawa and Liberman, 2009; Lin et al., 2011; Wang and Green, 2011), The clinical association of post-implantation hearing loss with an etiology of noise-induced hearing loss also suggests a possible excitotoxic mechanism that would only be visible in the hair cell synapses or nerve fibers (Kopelovich et al., 2014).

Another caveat is that normal-hearing animals were used in the previous study. It is possible that pre-existing hearing loss or sub-threshold damage may influence the effects of EAS on residual hearing. Thus, in this study, we extended the investigation of EAS effects on hearing to guinea pigs with high-frequency, noise-induced hearing loss similar to that in EAS CI patients, and used immunolabeling of both ribbon synapses and post-synaptic receptors to determine whether changes at the hair cell synapse could explain the delayed EAS-induced low-frequency hearing loss. Immunolabeling of SV vasculature was also used to further examine the relationship between SV capillary diameters and high-frequency hearing loss, and rule out a contribution of SV vasculature changes to delayed low-frequency hearing loss.

2. Materials and methods

2.1. Subjects

Sixteen male, 4-week old albino Dunkin-Hartley guinea pigs were purchased from Charles River Laboratories (Wilmington, MA). All animal protocols were approved by the Oregon Health & Science University Committee on the Use and Care of Animals and veterinary care was provided by the Department of Comparative Medicine (IACUC#IS00000672).

2.2. Research design

All animals were exposed to 24 h of octave-band noise (12–24 kHz) at 116 dB to induce a high-frequency hearing loss in