Hearing Research 249 (2009) 1–14

Contents lists available at ScienceDirect

Hearing Research

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

Research papers

Absence of strial melanin coincides with age-associated marginal cell loss and endocochlear potential decline

Kevin K. Ohlemiller [a,b,c,*], Mary E. Rybak Rice [c], Jaclynn M. Lett [a,b], Patricia M. Gagnon [a,b]

[a] Fay and Carl Simons Center for the Biology of Hearing and Deafness/Central Institute for the Deaf at Washington University, USA
[b] Department of Otolaryngology, Washington University Medical School, 660 S. Euclid, St. Louis, MO 63110, USA
[c] Program in Audiology and Communication Sciences, Washington University, St. Louis, MO, USA

ARTICLE INFO

Article history:
Received 29 October 2008
Received in revised form 15 December 2008
Accepted 16 December 2008
Available online 25 December 2008

Keywords:
Cochlea
Aging
Stria vascularis
Hair cells
Spiral ganglion
Spiral ligament
Fibrocytes
Presbycusis
C57BL/6
Mouse

ABSTRACT

Cochlear stria vascularis contains melanin-producing intermediate cells that play a critical role in the production of the endocochlear potential (EP) and in maintaining the high levels of K^+ that normally exist in scala media. The melanin produced by intermediate cells can be exported to the intrastrial space, where it may be taken up by strial marginal cells and basal cells. Because melanin can act as an antioxidant and metal chelator, evidence for its role in protecting the stria and organ of Corti against noise, ototoxins, and aging has long been sought. While some evidence supports a protective role of melanin against noise and ototoxins, no evidence yet presented has demonstrated a clear role for melanin in maintaining the EP during aging. We tested this by comparing basal turn EPs and a host of cochlear cellular metrics in aging C57BL/6 (B6) mice and C57BL/6-*Tyr*$^{c\text{-}2J}$ mice. The latter mice carry a naturally occurring inactivating mutation of the tyrosinase locus, and produce no strial melanin. Because these two strains are coisogenic, and because pigmented B6 mice show essentially no age-related EP decline, they provide an ideal test of importance of melanin in the aging stria. Pigmented and albino B6 mice showed identical rates of hearing loss and sensory cell loss. However, after two years of age, basal turn EPs significantly diverged, with 42% of albinos showing EPs below 100 mV versus only 18% of pigmented mice. The clearest anatomical correlate of this EP difference was significantly reduced strial thickness in the albinos that was highly correlated with loss of marginal cells. Combined with findings in human temporal bones, plus recent work in BALB/c mice and gerbils, the present findings point to a common etiology in *strial* presbycusis whereby EP reduction is principally linked to marginal cell loss or dysfunction. For any individual, genetic background, environmental influences, and stochastic events may work together to determine whether marginal cell density or function falls below some critical level, and thus whether EP decline occurs.

1. Introduction

The cells of cochlear stria vascularis work together to produce the high K^+ concentration in endolymph and the positive endocochlear potential (EP) that provide much of the electrochemical gradient driving receptor currents through hair cells. Strial degeneration and accompanying EP reduction are a common cause of age-related hearing loss that Schuknecht termed *metabolic* or *strial* presbycusis (Ohlemiller, 2004; Ohlemiller and Frisina, 2008; Schuknecht and Gacek, 1993; Schuknecht et al., 1974). In characterizing *strial* presbycusis, Schuknecht was limited by the availability and quality of temporal bone specimens, and was forced to infer EP reduction from audiograms and the appearance of the stria. However, three important principles that emerged from his observations appear to be correct. First, he suggested that the primary event in this condition was dysfunction or loss of strial marginal cells. This notion has found recent support in animal models, including the Mongolian gerbil (Schmiedt, 1993; Spicer and Schulte, 2005a), and BALB/cJ mouse (Ohlemiller, 2006; Ohlemiller et al., 2006). This need not imply that all *strial* presbycusis has the same etiology. Age-related strial degeneration and EP reduction secondary to microvascular pathology has long been argued (Gratton and Schulte, 1995; Gratton et al., 1996; Johnsson and Hawkins, 1972; Thomopoulos et al., 1997), and has recently found a potential example in NOD.NON-*H2*^{nbl} mice (Ohlemiller et al., 2008). Second, Schuknecht proposed that the stria possesses redundant functional capacity, so that nearly half of its total volume could be lost before the EP and hearing thresholds are affected (Pauler et al., 1988). This idea also has found support in animal

* Corresponding author. Address: Department of Otolaryngology, Washington University Medical School, 660 S. Euclid, St. Louis, MO 63110, USA. Tel.: +1 314 747 7179; fax: +1 314 747 7230.
E-mail address: kohlemiller@wustl.edu (K.K. Ohlemiller).

0378-5955/$ - see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.heares.2008.12.005

models (Schulte and Schmiedt, 1992). Third, among the major forms of presbycusis (*sensory*, *neural*, *strial*), strial presbycusis was proposed to be the most strongly influenced by genetics. This assertion has been supported by familial patterns in the incidence of apparent *strial* presbycusis in humans (Gates et al., 1999). Among animal models, this latter principle can be best explored in mice and rats, since these exist as many highly inbred strains. Only recently, however, has evidence for divergent EP aging patterns across strains begun to accumulate. Only some inbred mouse strains of those tested show clear age-related EP decline by the end of their life expectancy (Ohlemiller et al., 2006), which fact supports a substantial genetic component. Notably, however, all animal models presently known to undergo age-associated EP reduction feature an environmental or stochastic component, such that at most half of subjects exhibit an abnormally low EP at advanced ages. Examples of this include the gerbil (Gratton and Schulte, 1995; Gratton et al., 1996; Schmiedt, 1993; Schulte and Schmiedt, 1992), Fischer 344/NHsd rat (Bielefeld et al., 2008), and *Tyrp1*$^{B\text{-}lt}$ (Cable et al., 1993), BALB/cJ (Ohlemiller, 2006), NOD.NON-*H2*^{nbl} (Ohlemiller et al., 2008), and CBA/CaJ mouse models (Ohlemiller and Gagnon, 2007a). In only one of these models (*Tyrp1*$^{B\text{-}lt}$) is a specific locus implicated, and it is unlikely that the same locus promotes EP reduction in all cases. Thus the probabilistic character of EP reduction in all these models may reflect the impact of multiple kinds of influences upon a small set of limiting resources.

One feature common to the Fischer 344/NHsd rat, BALB/cJ (BALB) mouse, and NOD.NON-*H2*^{nbl} mouse is albinism. Strial intermediate cells normally produce melanin pigment, which is posited to protect the stria, and possibly organ of Corti, from injury caused by noise and ototoxins (Meyer zum Gottesberge, 1988; Tachibana, 1999). Protective properties of melanin may derive from its ability to bind cations and metals and to scavenge free radicals (del Marmol and Beermann, 1996; Schraermeyer and Heimann, 1999 #1633; Riley, 1997). Melanin comes in two major forms, brown–black eumelanin and yellow–red pheomelanin. Pheomelanin, the less common form, may be less protective and may even promote injury (Barrenäs and Holgers, 2000; Barrenäs, 1997). Once synthesized by strial intermediate cells, melanin is exported to the intrastrial space in melanosomes, where it may remain, or be taken up by marginal or basal cells (Wright and Lee, 1989). Largely owing to its ultra violet-absorbing properties, melanin in skin may chiefly serve to protect against skin cancer, while melanin in the iris and retina may reduce light injury to the eye (Riley, 1997; Trachimowicz et al., 1981; Schraermeyer and Heimann, 1999). Support for a protective role of melanin against cochlear pathology due to noise, ototoxins, or aging, has been equivocal, however. Positive results have often been questionably based upon temporary threshold shifts as an index of noise susceptibility, have applied unreliable external indicators of strial melanin type and density, or have utilized suboptimal genetic controls (Attias and Pratt, 1985; Barrenäs, 1993; Barrenäs and Lindgren, 1991; Conlee et al., 1988, 1989, 1991, 1995; Cunningham and Norris, 1982). Comparison of pigmented versus albino guinea pigs or rabbits has typically meant different genetic backgrounds. In such cases, any differences found cannot be assumed to reflect pigmentation alone. Comparisons of pigmented versus albino mouse strains are subject to the same confound, except in cases where new mutations have led to pigmented and albino animals on the same background. One such case is the C57BL/6-*Tyr*$^{c\text{-}2J}$ mouse. These mice possess normally functioning melanocytes in all tissues, but produce no melanin due to a natural inactivating mutation of the tyrosinase locus on chromosome 4 (Eppig et al., 2007). In all other respects, these mice are identical to the well studied C57BL/6J (B6) mouse, and share *Cdh23*^{Ahl} (Chr. 10) and *Ahl3* (Chr. 17), alleles known to promote age-related hearing loss (Johnson et al., 1997, 2000; Nemoto et al., 2004). Two recent studies have compared noise resistance in these models. Bartels et al. (2001) examined permanent noise-induced hearing loss (NIHL) in C57BL/6-*Tyr*$^{c\text{-}2J}$ (henceforth, albino B6) and found no difference versus pigmented B6 mice. Ohlemiller and Gagnon (2007b) compared basal turn EPs within hours after a severe noise exposure, and observed a modest (~9 mV) but significant reversible EP reduction only in the albinos. These results suggest that melanin may indeed exert a protective effect, but protection may be limited to the stria itself, and may not be manifested in reduction of NIHL *per se*.

Recent reports of age-related EP decline in the Fischer 344/NHsd rat, BALB mouse, and NOD.NON-*H2*^{nbl} mouse could not address the significance of albinism in these models, because appropriate pigmented controls were not available. Some investigators, noting seemingly abnormal buildup of melanin in the aging stria, have suggested that melanin may actually promote age-related pathology (Covell and Rogers, 1957). A comparison of young pigmented versus albino guinea pigs—albeit of different strains—indicated that intermediate cell replacement is slower in albinos (Conlee et al., 1994a). This may translate into more rapid net loss of intermediate cells with age in albinos. The age-related EP reduction found in *Tyrp1*$^{B\text{-}lt}$ mice (Cable et al., 1993) might bear on the importance of melanin in aging, since Tyrp1 (tyrosinase-related protein 1) is involved in melanin synthesis. Mice carrying at least one copy of the *B-lt* allele show pigment anomalies, yet they appear to retain a normal complement of strial intermediate cells as the EP declines. However, Cable et al. (1993) proposed that the mutated protein undergoes a deleterious gain-of-function that may be unrelated to any role of melanin.

Despite much speculation and a plausible basis as to *why* melanin might help preserve the EP with age, we know of no direct published evidence for this. We recently noted (Ohlemiller and Gagnon, 2008) that albino B6 mice tend toward EP reduction after 24 mos of age. Here we describe the cellular correlates of that EP reduction. As in our recent study of BALB mice (Ohlemiller et al., 2006), we exploited the fact that pigmented B6 mice show little or no EP decline out to 33 mos of age, reasoning that anatomical differences between the two strains might reveal the cells and processes that are most dependent on melanin. Based on our results, we propose that the concentration, form, or distribution of strial melanin may act with other factors to accelerate net strial marginal cell loss with age, and thereby increase the probability of EP decline. Absence of melanin may represent just one of many genetic or environmental factors that promote marginal cell loss with age.

2. Methods

Findings are based on comparison of round window compound action potential (CAP) thresholds, basal turn EP, and histologic measures encompassing cochlear sensory cells, stria vascularis, and spiral ligament. Except as noted, strial and ligament measures focused on the lower basal turn.

2.1. Animals

Mice were derived from sib mating of breeders purchased from The Jackson Laboratory (JAX). Roughly equal numbers of male and female mice of inbred strains C57BL/6J (JAX #000664) ($N = 157$) and C57BL/6-*Tyr*$^{c\text{-}2J}$ (recently renamed B6(Cg)-*Tyr*$^{c\text{-}2J}$/J, JAX #000058) ($N = 105$), ranging in age from 2 to 33 mos were analyzed. By 33 mos of age, survival rates in B6 males and females are below 25% (Eppig et al., 2007). Our sample therefore encom-