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Dietary restriction reduces age-related degeneration of stria vascularis in the inner ear of the rat

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ABSTRACT

We report here beneficial effects of life-long dietary restriction on the progression of age-associated cochlear degeneration in female Sprague–Dawley rats. Thirty-month old rats on a 70% dietary restriction were compared to ad libitum fed age-matched rats, and three-month old adult rats. As expected, aged dietary restricted rats displayed about 20% higher survival rate than age-matched rats fed ad libitum. This difference was reflected also in the auditory system. In the dietary restricted group, 73% of the subjects had preserved auditory reflexes (Preyer), and only modest degeneration of the stria vascularis of the inner ear was observed. In contrast, aged ad libitum fed animals, of which only 15% had detectable Preyer reflexes, showed a marked thinning, cellular degeneration and loss of cell processes in the stria vascularis. The extent of loss of sensory hair cells (~24%) was similar in both the aged groups, and neither group showed a significant reduction in the number of spiral ganglion neurons across adult life-span. The observations thus demonstrate that dietary restriction delays age-related degradation of the auditory system. The results provide further insights into the mechanisms of strial presbycusis.

1. Introduction

A gradual loss of sensory perception is a prominent feature of the aging phenotype (Cowen and King, 2005). The effects of aging impact all sensory modalities but mostly those that rely on sophisticated peripheral receptors, such as the cochlea of the inner ear.

Age-related hearing loss, presbycusis, is one of the most common causes of hearing loss (Frisina, 2009; Jonsson et al., 1998; Ohlemiller, 2004; Schacht and Hawkins, 2005). In the developed world, presbycusis affects approximately 40% of the population at the age of 60, and the hearing loss progresses by approximately 1 dB per year thereafter. With an increasing elderly population, this becomes a problem for the individual as well as for society (Ciorba et al., 2012). Environmental factors such as previous exposure to noise and ototoxic drug treatment probably affect the onset and severity of the auditory impairment but genetic factors are likely to significantly influence the progression (Gates et al., 1999; Newman et al., 2012). Schuknecht (Schuknecht and Gacek, 1993) divided presbycusis into three different subtypes according to their suggested origin: 1) *sensory presbycusis* characterized by high-frequency hearing loss, caused by the degeneration of the sensory hair cells; 2) *neural presbycusis* with an equal hearing loss all over the frequency span and loss of word discrimination due to the degeneration of spiral ganglion neurons (SGN) and/or components of the central auditory system; and 3) *strial* (*metabolic*) *presbycusis* reflecting a hearing loss with an equal drop over the entire frequency range, but with relatively good speech discrimination. Histological examination of human temporal bones shows atrophy of the metabolic tissue of the cochlea, the stria vascularis (SV) and the underlying spiral ligament (Schuknecht and Gacek, 1993). The classification scheme has been disputed since a combination of different subtypes has been observed (Ohlemiller, 2004).

Understanding the natural history and the mechanisms of presbycusis rests in part on the use of animal models, in particular rodent models (Ohlemiller, 2004; Ohlemiller and Frisina, 2008). One cause of hearing loss in rodents is loss of sensory hair cells, primarily the outer hair cells in the most apical and basal parts of the cochlea. Other age-related changes described in mice, rats and Mongolian gerbils include degeneration of the hearing organ; the SV, the spiral ligament and loss of SGN (Buckiova et al., 2006; Hequembourg and Liberman, 2001; Keithley et al., 1992, 2004; Ohlemiller and Gagnon, 2004; Spicer and Schulte, 2002, 2005). Some strains, like the Fisher 344 rat and the DBA/2J mouse, are known for their early onset of age-related hearing loss; whereas other strains like the Sprague–Dawley rat, the CBA mouse and the BALB/c mouse show a later onset of this disability. Different cell types are affected to a variable extent in different strains (Keithley et al., 2004) and there are also differences in the degenerative pattern along the length of the cochlea reflecting the frequency variation of the hearing loss (Ohlemiller and Gagnon, 2004; Spongr et al., 1997). The observed

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strain differences in presbycusis highlight the importance of the genetic background.

The most successful means, yet known, to retard the negative impact of aging on the organism is to reduce dietary intake through caloric (CR) or dietary (DR) restriction. Although the precise mechanism by which CR/DR enhances health-span and extends life-span remains unresolved, it apparently involves multiple adaptations of cellular metabolism and energy production (Mair and Dillin, 2008; Masoro, 2003; Speakman and Mitchell, 2011). Only few studies have addressed to what extent CR/DR counteracts the deteriorating effects of aging on hearing (Someya et al., 2010a). In 27-month old Fisher 344 rats, CR was reported to impede both the age-associated increase in hearing threshold and the concomitant loss of hair cells observed in ad libitum (AL) fed animals (Seidman, 2000). Someya and coworkers reported that CR also suppressed apoptotic cell death of SGN and age-associated hearing loss in the C57Bl/6 mouse (Someya et al., 2007).

The aim of the present study was to examine in greater detail the end-point effects of DR compared to AL feeding (Altun et al., 2007) by unbiased stereological quantification of the total number of sensory hair cells and SGN, moreover, the volume and the fine structure of the SV, a fundamental metabolic structure of the inner ear. The outbred Sprague–Dawley albino rat strain was chosen as a model because of its similarity to humans regarding diversity in life span, body weight, sensory and motor functions during aging (Altun et al., 2007).

2. Material and methods

2.1. Experimental design

A total of 53 female albino Sprague–Dawley rats, raised and maintained in-house (colony founders delivered by Scanbur BK, Sollentuna, Sweden), that were alive at end-point time, were used for the study. All animals were kept in the same holding unit on a 12/12 h day and night cycle, with a room temperature at 21 ± 0.2 °C and relative humidity at 50 ± 10%. Post-weaning off springs were arranged in sibling-groups and kept 3–5 in open type 4 cages (MakrolonTM, M4, Techniplast, Buguggiate (Va) Italy). Asp-woodchip was used as bedding material (Tapvei, Kortteinen, Finland) and as enrichment; the cages were equipped with plastic-tubes and nesting material (paper). As the animal aged and siblings died with advancing age the number of animals per cage decreased, but no animals were single-housed. Commercially available food-pellets (Lactamin R34, Lantmännen, Sweden) and water were served ad libitum. To avoid single-housing, the food was served once a day, a regime that allows all animals to fed and brings down the animal-to-animal body weight variation among cage-litter members (Altun et al., 2007). Animals were weighed at regular intervals (every other week or once a month). The chosen design for the study was a cross-sectional two-point study comparing young adults (age: 2–3 months) with the end-point age, i.e., 30 month old subjects. To challenge normal aging, a group of young females (n = 20; starting weight 50.4 +/− 5 g) derived from the same colony were post-weaning maintained on a life-long dietary restriction, corresponding to 70% of the food intake of the AL fed colony mates (n = 30; starting weight 48 +/− 9 g). It has previously been shown that, this restriction extends life-span by about 20% (Altun et al., 2007). Thus, at sacrifice the DR group (65% survival, n = 13; end-point weight 311 +/− 13 g) had a better survival (Supplementary Fig. S1) than the AL fed animals (survival 43%, n = 13; end-point weight 445 +/− 19 g). Preyer's reflex test was used to indicate auditory status of the animals. A qualitative non-blinded yes/no assessment of reflexes (ear contraction or body movement) was performed after presentation of a sound burst of 60 dB SPL above the cage. The test was repeated once if no reflexes were obtained after the first presentation. All young animals (n = 10) had positive reflexes, while in the aging groups, 73% of the DR animals (n = 11) and 15% of the AL animals (n = 13) displayed positive reflexes.

The animals were sacrificed and cochleae from the different groups were used for stereological estimation of total cell number and volume as well as morphological evaluation using transmission electron microscopy.

All experimental procedures followed Swedish regulations for the care and use of laboratory animals (ethical permissions N122/06 and N394/09).

2.2. Estimation of cell numbers and volumes

Cochleae from young rats (n = 8), old AL rats (n = 9) and old DR rats (n = 9) were perfused with a fixative containing 2.5% glutaraldehyde in phosphate buffer (pH 7.2) and then decalcified in 0.1 M EDTA until the bone became soft. The cochleae were post fixed with 1% osmium tetroxide, dehydrated, infiltrated and randomly oriented before being embedded in a 2-hydroxyethyl metacrylate-based resin (Technovit 7100, Heraeus, Germany). Serial sections (24-μm thick) from the whole cochlea were stained with hematoxylin and eosin. A stereological design was established for estimating the complete three-dimensional volume of the SV, a structure extending from the base to the apex along the lateral wall of the cochlea. Total number of sensory hair cells as well as total number and soma cell volume of SGN (Gundersen, 1988; Moller et al., 1990; Tandrup, 1993; Watanabe et al., 2010) were also estimated in each cochlea using the Cavalieri principle (Gundersen et al., 1988b) and the optical fractionator technique (Gundersen et al., 1988a). A microscope (Axioplan Zeiss) with a motorized stage and an electronic microcator (Prior ProScan II) with digital readout for measuring movements in the z-direction was interfaced to a digital camera (Pixel link) and a personal computer running newCAST software program (Visiopharm, Denmark) that randomly placed counting grids over the live image of the sections; for details see supplementary information. In short, volume estimation of stria vascularis was performed by placing a counting grid with test points over the section at 20× magnification. All test points that covered the SV were counted. To estimate the total number of cells, a counting frame was randomly placed over the section at 100× magnification and all inner and outer hair cells as well as both types of SGN within the frame were counted. Type I neurons were at the same time assessed for cell soma volume using the nucleator technique, where the average distance from the most centrally placed nucleolus to the cell membrane were calculated in four randomly placed test lines per neuron. Type I neuron was defined as having a large cell soma with one or several small scattered nucleoli in contrast to the smaller type II neuron, which has one large, centrally placed nucleolus.

2.3. Electron microscopy

The basal and apical parts of SV from three cochleae per group were analyzed at the cellular level using electron microscopy. The decalcified whole cochleae were dehydrated, cleared with propylene oxide and embedded in Agar 100, (Agar 100 Resin kit, Agar Scientific Limited, England). Basal and apical turns from approximately the same place of the cochlea were remounted on a new block of Agar 100. The SV was dissected out and 1-μm thick sections, stained with toluidine blue, were examined with a light microscope to control the orientation of the specimen before thin-sectioning. Thin-sections were put on formvar coated copper grids and stained with uranyl acetate and lead citrate. Sections were examined with a transmission electron microscope (JEOL 1230, JEOL GmbH, Germany) and digital images were captured at 2500× and 10,000× magnification.

2.4. Statistics

Data analysis was made using the SigmaPlot for Windows Version 11.0 and results are presented as mean values with standard deviations