Available online at www.sciencedirect.com
ScienceDirect
Hearing Research 222 (2006) 54–61
Hearing Research
www.elsevier.com/locate/heares

Research paper

Prestin gene expression in the rat cochlea following intense noise exposure

Guang-Di Chen *

Center for Hearing and Deafness, 137 Cary Hall, 3435 Main Street, SUNY at Buffalo, Buffalo, NY 14214, United States

Received 12 April 2006; received in revised form 9 August 2006; accepted 11 August 2006
Available online 26 September 2006

Abstract

Noise-induced permanent loss of cochlear amplification was observed previously with the majority of outer hair cells (OHCs) still surviving in the cochlea and even with a normal OHC receptor potential, indicated by CM (cochlear microphonics) recording [Chen, G.D., Fechter, L.D., 2003. The relationship between noise-induced hearing loss and hair cell loss in rats. Hear. Res. 177(1–2), 81–90; Chen, G.D., Liu, Y., 2005. Mechanisms of noise-induced hearing loss potentiation by hypoxia. Hear. Res. 200, 1–9]. This study focused on effects of an intense noise exposure (10–20 kHz at a level of 110 dB SPL for 4 h) on the OHC motor protein (prestin) and structural proteins in the OHC membrane skeleton. The noise exposure significantly disrupted CM and CAP (cochlear compound action potential). The injured CM recovered after 1-week resting period. The impaired CAP at frequencies lower than the noise band also recovered. However, the CAP recovery at frequencies of the noise band stopped at a linear line one week after the noise exposure, indicating a permanent loss of cochlear amplification. Gene expression of prestin, β-spectrin, and β-actin was significantly up-regulated after the noise exposure. The elevated gene expression peaked at the 3rd post-exposure day and returned to baseline 4 weeks after the noise exposure. The up-regulated gene expression may be in response to injury of the proteins, which may be responsible for the loss of cochlear amplification.

Keywords: Prestin; OHC motor protein; Cochlear amplification; Noise-induced hearing loss; OHC cytoskeleton

1. Introduction

Hearing sensitivity in the mammalian cochlea is enhanced by about 40 dB by the cochlear amplifier (Davis, 1983; Gold, 1948). OHCs are considered to be the source of the cochlear amplifier since the isolated OHC has been found to be capable of length changes in response to alternating current stimulation (electromotility) (Ashmore, 1987; Brownell et al., 1985; Kachar et al., 1986). In the chinchilla, the first 40 dB noise-induced hearing loss (NIHL) was related to OHC loss (Hamernik et al., 1989). However, in the rat, noise-induced permanent threshold shifts (PTS) of up to 40 dB in the low-mid frequency region, resulting from a loss of cochlear amplification, were observed without a significant hair cell loss and even without a significant reduction of OHC receptor potential (Chen and Fechter, 2003; Chen and Liu, 2005). It is assumed that the surviving OHCs lost their electromotility. The OHC electromotility is believed to be generated in the OHC lateral cell wall and driven *in vivo* by the OHC receptor potential (Brownell et al., 1985; Dallos and Corey, 1991; Dallos et al., 1991; Holley and Ashmore, 1988; Jia and He, 2005). Since the noise-induced loss of cochlear amplification may occur in some cases with a normal OHC receptor potential, we believe that damage to the OHC lateral cell wall is primarily responsible for the loss of cochlear sensitivity.

Prestin is the motor protein in the OHC lateral cell wall responsible for the OHC motile activity (Liberman

Abbreviations: CAP, compound action potential; CM, cochlear microphonics; C_t, threshold cycle; I/O, input/output; IHC, inner hair cell; NIHL, noise-induced hearing loss; OHC, outer hair cell; PTS, permanent threshold shift

* Tel.: +1 716 829 2001x21; fax: +1 716 829 2980.
E-mail address: gchen7@buffalo.edu.

0378-5955/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.heares.2006.08.011

et al., 2002; Zheng et al., 2000). Damage to prestin may cause a loss of OHC electromotility without disruption of OHC mechano-electrical transduction (Liberman et al., 2002).

The OHC membrane skeleton has a higher circumferential stiffness (by F-actin) and lower axial stiffness (by spectrin), which facilitates OHC length change driven by the motor proteins in the OHC lateral membrane. Damage to the membrane skeleton may also affect OHC electromotility.

This experiment was design to determine gene expression of prestin, β-actin, and β-spectrin in the cochlea with a noise-induced permanent loss of cochlear amplification, but without significant OHC loss in the rat model. Our results demonstrate that damage to the proteins in the OHC lateral cell wall is primarily responsible for the noise-induced permanent loss of cochlear amplification.

2. Methods

2.1. Subjects

Long Evans pigmented rats (male) approximately 2.5 months of age were acquired from Harlan Sprague Dawley and housed in the University of Oklahoma Health Sciences Center animal facility after delivery. All animal facilities in OU are registered with the US Department of Agriculture and are inspected semiannually by the members of the Institutional Animal Care and Use Committee (IACUC). Background noise level in the colony room was 50 dB (A-weighting). Temperature was maintained at 21 °C. Lights were on from 6:30 am to 6:30 pm. All procedures regarding the use and handling of animals were reviewed and approved by the IACUC serving the University of Oklahoma Health Sciences Center.

2.2. Noise exposure

An octave band noise (OBN, 10–20 kHz) was generated using a TDT RP2 Real time signal processor (TDT, Gainesville, FL). The noise was amplified and delivered to two speakers in the exposure chamber. Noise intensity was measured using a Quest sound level meter at the approximate level of the animals' ears. Rats were exposed to the noise at 110 dB SPL for 4 h.

2.3. Cochlear functional assessment

Rats were deeply anesthetized with ketamine (50 mg/kg, i.m.) and xylazine (6 mg/kg, i.m.). The right cochlea was surgically exposed using a ventro-lateral approach and a silver wire electrode was carefully placed on the round window for eliciting cochlear responses. A silver chloride reference electrode was placed in the neck muscles. Tone bursts at different frequencies (2, 4, 6, 8, 12, 16, 20, 24, 30, 35, and 40 kHz) were generated in a real time processor (TDT RP2.1, system-3, TDT, Gainesville, FL). The signals with 10-ms duration and 1-ms rise/fall time were attenuated by a TDT PA5 programmable attenuator and then amplified using the HVA-1 High Voltage amplifier and delivered to a high frequency earphone (made from an ACO 1/2″ microphone, 7013) placed within a speculum that opens to the ear drum. Sound levels at all testing frequencies were calibrated using a probe microphone located near the eardrum. The cochlear potentials were amplified with a Grass A. C. preamplifier (Model P15). The gain of the preamplifier was set at 1000, and the band of the filter was set from 0.1 Hz to 50 kHz. The cochlear potentials were averaged 50 times using the TDT RP2.1 real time processor and stored in a computer. The CAP and CM components were obtained from the cochlear response by off-line analysis. CM was obtained by high-pass filtering at a frequency 1 kHz lower than the stimulation frequency. Since CAP energy mainly distributes within 3 kHz, CAP component was obtained by low-pass filtering at 3 kHz. Amplitudes of CAP and CM were measured and plotted as a function of the stimulation level (input/output (I/O) function). CAP thresholds were obtained from the CAP I/O functions at 5 μV level.

2.4. Hair cell loss examination

Three of the 10 cochleae from each group ($n = 5$ rats) were fixed in 10% formalin. The cochleae were dissected and stained with FITC labeled phalloidin (Sigma, St. Louis, MO, Cat# P5282) for hair cell counting.

2.5. Gene expression determination

2.5.1. Tissue sampling

Rats were deeply anesthetized and the cochleae were removed quickly after decapitation and transferred into the RNA stabilization reagent (RNAlater, Qiagen, #76104), which preserves mRNA. The round window, oval window, and the apex of the cochleae were opened immediately to let the RNA stabilization reagent enter the cochlea as soon as possible. The basilar membrane with the sensory epithelium (the organ of Corti) was sampled and frozen in liquid nitrogen immediately. The samples were stored at −80 °C before RNA extraction.

2.5.2. RNA extraction

Since one cochlea did not provide enough RNA for cDNA reverse transcription, samples from 7 of the 10 cochleae in each group ($n = 5$ rats) were pooled into one tube and homogenized in 0.5 ml tri-reagent solution with 0.05% polyacryl carrier (Molecular Research Center, Inc., cat#: PC152). The homogenized solution with addition of 0.1 ml chloroform was centrifuged (12,000*g*) for 15 min at 4 °C. The top aqueous phase was transferred into a new tube and mixed with an equal amount of isopropanol and centrifuged (12,000*g*) again for 10 min. The bottom pellet was the isolated RNA. The liquid phase from the