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Research Report

Downregulation of N-methyl-D-aspartate receptor ζ1 subunit (*GluN1*) gene in inferior colliculus with aging

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ABSTRACT

Presbycusis is the impairment of auditory function associated with aging, which stems from peripheral cochlear lesions and degeneration of the central auditory process. The effect of age-induced peripheral hearing loss on the central auditory process is not fully understood. C57Bl/6 (C57) mice present accelerated peripheral hearing loss, which is well developed by middle-age and mimics the human presbycusis pattern. The aim of this study was to elucidate the molecular effects of peripheral hearing loss in the inferior colliculus (IC) with age between young and middle-aged C57 mice using cDNA microarray. Glutamate receptor ionotropic NMDA ζ1 (GluN1) exhibited the greatest decrease in the middle-aged group as determined using cDNA microarray and by further assessment using real-time PCR (qPCR). Histological assessment with in situ hybridization of GluN1 showed significantly decreased expression in all IC subdivisions of the middle-aged group. GluN1 is a receptor for excitatory neurotransmission, and significant downregulation of this gene may be subsequent to the decline of afferent input from the cochlea in aging C57 mice. Consequently, using the combination of microarray, qPCR, and in situ hybridization, we showed that the decline of GluN1 in the IC of aging animals might have a key role in the pathogenesis of presbycusis.

1. Introduction

Age-related hearing loss, or presbycusis, is one of the predominant problems associated with aging. Between 25% and 40% of the elderly population over 65 years old are hearing-impaired, and the prevalence increases with age (Yueh et al., 2003). By the age of 80, about half of the U.S. population is affected (Mulrow, 1991). Although hearing aids provide hearing assistance to many of those affected, they do not resolve the central nervous system components of presbycusis. To pro-

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Abbreviations: NMDA, N-methyl-D-aspartate; GluN1, glutamate receptor ionotropic NMDA ζ1; IC, inferior colliculus; ABRs, auditory brain stem responses; ISH, in situ hybridization; qPCR, real-time PCR; Gsn, gelsolin

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vide appropriate therapies for the central nervous system components of presbycusis, it is first essential to understand the underlying deficits.

The inferior colliculus (IC) is a major brainstem auditory structure that functions as a center of integration for the ascending and descending auditory pathways, and is involved in processing complex sounds, such as speech. The structure can be divided into three subregions: the central nucleus (CIC), dorsal cortex (DCIC), and external cortex (ECIC) (Meininger et al., 1986). The CIC receives most of the ascending input from the inferior auditory nuclei (cochlear nucleus, superior olive, and nuclei of the lateral lemniscus), and the cortex of the IC receives descending input from the auditory cortex.

Several lines of evidence suggest that both excitatory and inhibitory amino acid neurotransmitters and their receptors within the IC are involved in processing acoustic signals (Holt et al., 2005; Kelly and Zhang, 2002; Ma et al., 2002; Tadros et al., 2007; Wynne et al., 1995). Changes in these excitatory and inhibitory amino acid transmitters and their receptors occurring in association with deafness have been reported to result in altered evoked responses, synaptic strength, and inhibitory synaptic strength in the IC (Kotak and Sanes, 1997; Mossop et al., 2000; Vale and Sanes, 2002). Thus, loss of these neurotransmitters (e.g., GABA) and their receptors with aging alters the functions of the IC, resulting in poor speech understanding and a decreased ability to recognize acoustic signals among the elderly (Caspary et al., 1995).

The C57BL/6(C57) mouse strain has been used frequently in previous studies as an experimental model of progressive sensorineural hearing loss (SHL) (Bartolome et al., 2001; Gong et al., 2006; McFadden and Willott, 1994; Willott et al., 1988, 1994). Mutation of the Ahl allele causes this SHL by disrupting the stereocilia of the organ of Corti (Di Palma et al., 2001; Johnson and Zheng, 2002). This strain of mice shows relatively rapid progression of cochlear pathology, which begins from 2 to 3 months of age and is well developed by the age of 6–-12 months (Willott et al., 1988). The SHL initially affects the high-frequency range, but eventually affects all frequencies, similar to age-related hearing loss in humans. Thus, the C57 is a good model for the study of SHL, and investigation of the changes occurring in the IC in association with SHL in this mouse model may provide important information to understand the changes that occur in the central auditory system in human presbycusis.

Previous studies attempted to determine the pathogenesis of presbycusis by investigating the molecular changes occurring in the peripheral or central auditory systems in association with this phenomenon, using a cDNA microarray. Gene expression profiling of the cochlear tissues of C57 mice (Gong et al., 2006; Someya et al., 2007, 2008) and of the IC of CBA mice (Tadros et al., 2007) has been reported. Changes in the expressions of various genes have been described in these reports and further confirmed by PCR and/or histological examination. In this study, we attempted to investigate the cumulative changes in the IC occurring in association with presbycusis, by comparing the gene expression profiles in the IC of middle-aged hearing-impaired C57 mice with those in young C57 mice with unimpaired hearing using a cDNA microarray. We confirmed the results of the microarray analysis by real-time PCR (qPCR) and in situ hybridization (ISH).

2. Results

2.1. *Auditory brain stem responses (ABRs)*

To evaluate the sensorineural hearing loss in C57 mice, we assessed the ABRs of young (1 month of age) and middle-aged (12–15 months of age) mice. The ABR thresholds in the young and middle-aged groups of mice are shown in Fig. 1.

ABR thresholds of two groups were compared using Mann–Whitney's *U* test and middle-aged group showed significant change ($^{**}p<0.01$,$^{*}p<0.05$, n=5). The middle-aged group showed well-established sensorineural hearing loss for all frequencies, consistent with the genetically determined trait of C57 mice (Willott et al., 1988).

2.2. *Microarray expression profiling*

To determine the differences in the mRNA expression profiles in the IC of young and middle-aged C57 mice, we performed gene expression profiling using microarrays (Intelligene II Chip, Takara, Japan) that contained 4277 cDNA fragments of mouse-identified genes. We repeated the array analysis three times and assessed the median value. The results revealed 6 downregulated genes (Table 1) and 6 upregulated genes (Table 2) in the IC of the middle-aged mice, with a fold-change of more than 2.0 compared with the expression levels in the young group.

Among the downregulated genes, the most profound downregulation (116.67-fold) was observed for GluN1. Genes related to glutamate receptors, such as Gsn and Vamp2, also showed downregulation by middle-age. Among the upregulated genes, Ywhaz (14-3-3 protein subtype zeta) showed the greatest upregulation; similarly, genes such as Bmp4, Stam, and Mcm2 were also upregulated by middle-age.

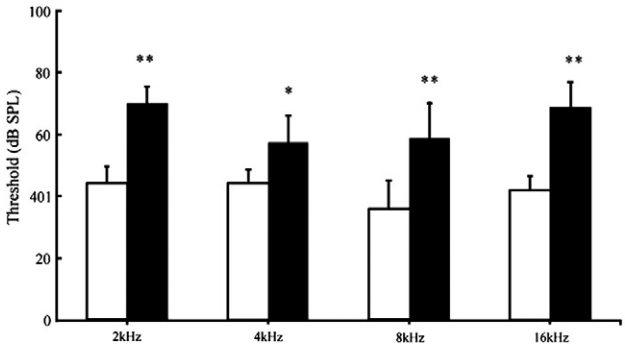

Fig. 1 – ABR thresholds were measured in young and middle-aged mice. The average ABR thresholds were measured (means±S.D.) in young (white bars, n=5) and middle-aged C57 mice (black bars, n=5) from 2 to 16 kHz. Student's *t*-test showed significant changes in the threshold with age at all frequencies ($^{**}p<0.01$).