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Research paper

Age-related decline of the cytochrome c oxidase subunit expression in the auditory cortex of the mimetic aging rat model associated with the common deletion

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ABSTRACT

The age-related deterioration in the central auditory system is well known to impair the abilities of sound localization and speech perception. However, the mechanisms involved in the age-related central auditory deficiency remain unclear. Previous studies have demonstrated that mitochondrial DNA (mtDNA) deletions accumulated with age in the auditory system. Also, a cytochrome c oxidase (CcO) deficiency has been proposed to be a causal factor in the age-related decline in mitochondrial respiratory activity. This study was designed to explore the changes of CcO activity and to investigate the possible relationship between the mtDNA common deletion (CD) and CcO activity as well as the mRNA expression of CcO subunits in the auditory cortex of D-galactose (D-gal)-induced mimetic aging rats at different ages. Moreover, we explored whether peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α), nuclear respiratory factor 1 (NRF-1) and mitochondrial transcription factor A (TFAM) were involved in the changes of nuclear- and mitochondrial-encoded CcO subunits in the auditory cortex during aging. Our data demonstrated that D-gal-induced mimetic aging rats exhibited an accelerated accumulation of the CD and a gradual decline in the CcO activity in the auditory cortex during the aging process. The reduction in the CcO activity was correlated with the level of CD load in the auditory cortex. The mRNA expression of CcO subunit III was reduced significantly with age in the D-gal-induced mimetic aging rats. In contrast, the decline in the mRNA expression of subunits I and IV was relatively minor. Additionally, significant increases in the mRNA and protein levels of PGC-1α, NRF-1 and TFAM were observed in the auditory cortex of D-gal-induced mimetic aging rats with aging. These findings suggested that the accelerated accumulation of the CD in the auditory cortex may induce a substantial decline in CcO subunit III and lead to a significant decline in the CcO activity progressively with age despite compensatory increases of PGC-1α, NRF-1 and TFAM. Therefore, CcO may be a specific intramitochondrial site of age-related deterioration in the auditory cortex, and CcO subunit III might be a target in the development of presbycusis.

Abbreviations: CcO, cytochrome c oxidase; mtDNA, mitochondrial DNA; CD, common deletion; D-gal, D-galactose; PGC-1α, peroxisome proliferator-activated receptor-γ coactivator 1α; NRF-1, nuclear respiratory factor 1; TFAM, mitochondrial transcription factor A; AHL, age-related hearing loss; OXPHOS, oxidative phosphorylation; ATP, adenosine triphosphate; ETC, electron transport chain; TEM, transmission electron microscopy; SEM, standard error of the mean; LSD, least significant difference; DPOAE, distortion product otoacoustic emissions; OHC, outer hair cells; ABR, auditory brainstem responses.

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1. Introduction

Aging is a universal phenomenon affecting all living organisms that is characterized by a progressive decline in the biochemical and physiological functions of many organs. Age-related hearing loss (AHL), also known as presbycusis, is observed in 40% of the population older than 65 years of age (Gates and Mills, 2005). Both the peripheral and central components of the auditory system were proposed to be implicated in the development of presbycusis (Frisina and Walton, 2006; Howarth and Shone, 2006). Some physiological mechanisms involved in presbycusis have been revealed (Frisina and Frisina, 1997; Frisina et al., 2007; Jacobson et al., 2003; Kim et al., 2002; Spongr et al., 1997; Tadros et al., 2007; Walton et al., 1997; Zettel et al., 2007; Zhu et al., 2007). However, the mechanisms involved in presbycusis are not fully clarified yet. During the aging process in humans, many stress factors besides the aging-related factors may also affect hearing, such as ototoxic drug utilization, noise exposure and otitis media (Hong and Kim, 2001; Kusunoki et al., 2004; Lin et al., 2012). Furthermore, the auditory tissues in humans are inaccessible during life. Thus, an investigation for the mechanisms involved in human presbycusis is extremely complex and difficult. And the rat is a common species that is widely used as an animal model in aging research. The life span of naturally aging rats is usually more than 2 years and various factors may affect hearing during such a long time. Therefore, the naturally aged rat as the study subject for presbycusis also has its limitation. The mitochondrial DNA (mtDNA) 4977-bp deletion in humans and 4834-bp deletion in rats, also known as the mtDNA common deletion (CD), have been reported to accumulate gradually in various tissues during aging (Cassano et al., 2004; Meissner et al., 2008; Nicklas et al., 2004). Previous investigations have suggested that the CD was associated with presbycusis (Markaryan et al., 2009). Until now, the role of the CD in presbycusis was still obscure. A suitable animal model of mimetic aging with the CD is necessary for the investigations in aging mechanisms. And long-term administration of D-gal induces a series of characteristics resembling those of natural aging process, including excessive reactive oxygen species (ROS) production (Kumar et al., 2010), diminished activity of antioxidant enzymes (Ho et al., 2003), mitochondrial dysfunction (Lei et al., 2008), neurotoxicity (Lu et al., 2006), low immune response (Uddin et al., 2010), cognitive deficit (Cui et al., 2006) and a shortened life span (Cui et al., 2004). D-gal overload model has been accepted as a mimetic aging model and widely used in research for aging mechanisms and anti-aging pharmacology (Kumar et al., 2011; Zhang et al., 2007). In our previous studies, we established a mimetic aging rat model harboring the mtDNA 4834-bp deletion in the inner ear by chronic administration of D-galactose (D-gal) (Kong et al., 2006a). Based on this model, we found that the mtDNA 4834-bp deletion may not directly cause hearing loss but may act as a predisposing factor that can greatly enhance the sensitivity of the inner ear to stress (Kong et al., 2006b). Further investigations demonstrated that the auditory central components of D-gal-treated rats also carried the mtDNA 4834-bp deletion (Chen et al., 2010a). Moreover, the age-related central auditory dysfunction and its corresponding pathological impairments in D-gal-treated rats were similar to those that occurred in naturally aging rats (Chen et al., 2010a). However, the effect of different levels of the mtDNA 4834-bp deletion loads on the morphology of the auditory cortex during aging remains unknown.

The mitochondrion is an essential organelle for energy transduction within the cell, and mitochondrial oxidative phosphorylation (OXPHOS) is the main source of adenosine triphosphate (ATP) for the eukaryotic cell. Intact mtDNA molecules are necessary to maintain normal OXPHOS function (Shoffner, 2001). Cytochrome c oxidase (CcO), the terminal component of the mitochondrial electron transport chain (ETC), is possibly the rate-limiting enzyme complex in the ETC (Helling et al., 2008). Additionally, CcO can transfer electrons from cytochrome c to an oxygen molecule and simultaneously create a proton gradient across the mitochondrial inner membrane that can provide the force to produce ATP. Thus, CcO exerts a crucial role in cellular energy transformation. In our recent study, we found that the CcO activity in the inner ear of D-gal-induced mimetic aging rats was significantly lower than in the control animals (Zhong et al., 2011). However, there is no literature available concerning the observation of CcO activity in the auditory cortex during aging. Also, the relationship between the CD and CcO activity in the auditory cortex is still unknown.

Mitochondrial transcription factor A (TFAM), a key activator of mitochondrial transcription, is involved in directly regulating the mtDNA copy number and mtDNA transcription in mammals (Ekstrand et al., 2004). Nuclear respiratory factor 1 (NRF-1), a transcriptional activator of nuclear genes, plays a significant role in coordinating the transcriptional regulation of all ten nuclear-encoded COX subunits in neurons (Dhar et al., 2008). Moreover, NRF-1 is known to activate TFAM (Piantadosi and Suliman, 2006). Peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α), a member of a family of transcription coactivators, can interact directly with and coactivate NRF-1 on the TFAM gene promoter and thus participate in the physiological control of mitochondrial function (Finck and Kelly, 2006). As mentioned above, a decrease of CcO activity was observed in the inner ear of D-gal-induced mimetic aging rats in our recent study (Zhong et al.). However, whether PGC-1α, NRF-1 or TFAM, the regulatory factors for the expression of nuclear- and mitochondrial-encoded CcO subunits, have been affected in the auditory cortex of D-gal-induced mimetic aging rats is still unclear.

The present study was designed to utilize the D-gal-induced mimetic aging rat model with the CD to explore the long-term changes of the ultrastructural morphology of the auditory cortex with aging, the possible relationship between the CD and CcO activity as well as the mRNA expression of CcO subunits in the auditory cortex at different ages and whether PGC-1α, NRF-1 and TFAM are involved in the changes of CcO activity.

2. Methods

2.1. Animals procedures

One hundred and thirty-two male Sprague–Dawley rats, which were 3 weeks old, were obtained from the experimental animal center of Tongji Medical College, Huazhong University of Science and Technology. After 7 days of acclimation, 12 1-month-old rats were used as baseline controls for the various biochemical assays. The other rats were randomly divided into the following two groups: group A (D-gal group; $n = 60$) and group B (control group; $n = 60$). The D-gal group was injected subcutaneously with 500 mg/kg of D-gal daily for 8 weeks, and the control group was given the same volume of vehicle (0.9% saline) for 8 weeks. Then, both groups were divided into 5 age subgroups [3 months old (just after the injection was finished), 6 months old, 9 months old, 12 months old and 15 months old (12 months after injection)], with each subgroup consisting of 12 rats. All animals were housed at a temperature of 24 °C ± 2 °C in a light-controlled environment with a 12-h light–dark cycle and provided with free access to food (standard rodent chow) and water. All animal experiments were carried out under the supervision of the Institutional Animal Care and Use Committee of Huazhong University of Science and Technology.