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Research Paper

Neuroplastic change of cytoskeleton in inferior colliculus after auditory deafferentation

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ABSTRACT

Neural plasticity is a characteristic of the brain that helps it adapt to changes in sensory input. We hypothesize that auditory deafferentation may induce plastic changes in the cytoskeleton of the neurons in the inferior colliculus (IC). In this study, we evaluated the dynamic status of neurofilament (NF) phosphorylation in the IC after hearing loss. We induced auditory deafferentation via unilateral or bilateral cochlear ablation in rats, aged 4 weeks. To evaluate cytoskeletal changes in neurons, we evaluated mRNA fold changes in NF heavy chain expression, non-phosphorylated NF protein fold changes using SMI-32 antibody, and the ratio of SMI-32 immunoreactive (SMI-32-ir) neurons to the total neuronal population in the IC at 4 and 12 weeks after deafness. In the bilateral deafness (BD) group, the ratios of SMI-32-ir neurons significantly increased at 4 weeks after ablation in the right and left IC ($6.1 \pm 4.4\%$, $5.0 \pm 3.4\%$, respectively), compared with age-matched controls ($P < 0.01$, $P < 0.01$). At 12 weeks after ablation, the ratio of SMI-32 positive neurons was higher (right, $3.4 \pm 2.0\%$; left, $3.2 \pm 2.3\%$) than that in the age-matched control group, albeit not significant in the right and left side ($P = 0.38$, $P = 0.24$, respectively). Consistent with the results of the ratio of SMI-32-ir neurons, SMI-32-ir protein expression was increased at 4 weeks after BD, and the changes at 12 weeks after bilateral ablation were not significant in the right or left IC. The age-matched control fold changes of NF mRNA expression after bilateral deafness were not significant at 4 and 12 weeks after deafness in right and left IC. Unilateral deafness did not induce significant change of NF mRNA expression, SMI-32-ir protein expression, and the ratio of SMI-32-ir neurons in the IC at 4 and 12 weeks after hearing loss. Bilateral auditory deafferentation induces structural changes in the neuronal cytoskeleton within the IC, which is prominent at 4 weeks after BD. The structural remodeling of neurons stabilized at 12 weeks after BD. Unlike BD, unilateral auditory deafferentation did not affect the dynamic status of NFs in the IC.

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1. Introduction

Neural plasticity is the remarkable capacity of the juvenile and adult brain to adapt and reorganize in response to environmental experience and change. Numerous studies have showed that sensory deprivation resulted in changes to neuronal connections, neurochemical properties, and metabolic activities (Nelson, 1999). Prolonged deafness induces the hyperactivity of auditory cortex via visual stimuli in deafness (Nishimura et al., 1999; Petitto et al., 2000; Finney et al., 2001), accompanied by the improvement of functional performance in visual behaviors in the deaf (Neville and Lawson, 1987). In animal model using congenital deaf cat, the auditory cortex exhibited increased field potentials containing neurons that responded to somatosensory or visual stimulation after deafness (Hunt et al., 2006; Meredith and Lomber, 2011). Visual localization in the peripheral field and movement detection ability improved with recruitment of the posterior auditory field and dorsal zone of the auditory cortex, respectively (Lomber et al., 2010). The expansion of secondary auditory cortex and ventral auditory field was also reported (Wong et al., 2014).

In addition to long-term developmental adaptations, late onset

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sensory deprivation has also been reported to induce plastic change of neuron in adult brain. Visual deprivation with dark exposure induced widespread changes in synaptic function in auditory and visual cortices in mice (Keck et al., 2013; Lee and Whitt, 2015; Petrus et al., 2015). The rapid and early plastic changes of preexisting neural circuit were related with improved performance of Braille reading and increase in BOLD signal of occipital cortex after temporary visual deprivation (Merabet et al., 2008). When the sensory deprivation was prolonged, the structural change of neuron and reorganization of neural connectivity was resulted (Merabet and Pascual-Leone, 2010). The prolonged bilateral deafness in adult rats induced the cytoskeletal change of neuron in auditory cortex which related with dendritic sprouting and axonal regeneration (Park et al., 2016).

During the reorganization of neocortex after sensory deprivation, plastic changes in the midbrain might also be expected. The inferior colliculus (IC) is the principal midbrain nucleus of the auditory pathway and play a role in integration of information from lower brainstem nuclei of auditory pathway, opposite IC, and descending input from auditory cortex (Casseday et al., 2002). The IC was reported to be related with cross-modal interaction of auditory and somatosensory input, which resulted in increased sensitivity of dorsal cochlear neurons to trigeminal stimulation (Dehmel et al., 2008; Shore and Zhou, 2006).

Thus, in this study, we hypothesized that auditory deafferentation would induce neuroplastic changes within the IC as well as the auditory cortex, accompanied by changes in neuronal cytoskeleton structure. To demonstrate this, we investigated the expression and phosphorylation status of neurofilaments (NF) in the IC after hearing loss.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats (4–16 weeks of age) underwent auditory brainstem response (ABR) testing using a click stimulus to confirm normal hearing (threshold < 30 dB SPL) in both ears (smartEP; Intelligent Hearing Systems, Miami, FL, USA). The rats were divided into the unilateral deafness (UD, n = 24), bilateral deafness (BD, n = 24), or control group (n = 36). Deafness was induced at the age of 4 weeks on right side (UD group) or both sides (BD group) using the cochlear ablation method (Park et al., 2016). The middle ear bullae were opened via the retroauricular approach under anesthesia with an intramuscular injection of a mixture of Zoletil (5 mg/kg; Virbec, Carros, France) and xylazine (5 mg/kg Rompun; Bayer, Wuppertal, Germany). Under direct vision, the lateral wall of the cochlea was identified and destroyed using a micro-drill and a sharp pick. Auditory brainstem responses were measured to confirm deafness before sacrificing at 4 and 12 weeks after deafening. At 4 or 12 weeks after cochlear ablation (8 and 16 weeks of age, respectively), the animals were sacrificed for immunohistochemistry, Real-time quantitative reverse transcription-PCR (RT-qPCR), and western blot analysis. The age-matched control group was sacrificed with the BD or UD group at 4, 8, and 16 weeks of age. All experimental procedures and use of animals were approved by institutional animal care and use committee, and followed their guidelines.

2.2. Tissue preparation

For immunocytochemistry, rats were transcardially perfused with heparin (1000 IU/L) in phosphate-buffered saline (PBS, pH 7.2), followed by fixation with 4% paraformaldehyde (PFA) in PBS at room temperature. The brains were harvested and dissected using a brain matrix, post-fixed overnight at 4 °C in 4% PFA in PBS. The brain tissue was embedded in paraffin, and serial coronal 10 μm thick sections from caudal end of IC (−8.72 mm from bregma) to rostral end of IC (−9.30 mm from bregma) were cut using a microtome (Paxinos and Watson, 2013). For western blotting and RT-qPCR, animals under deep anesthesia were decapitated and the whole brain was quickly harvested.

2.3. Real-time quantitative reverse transcription-PCR (RT-qPCR)

The mRNA expression level of neurofilament heavy chain (NF-H) was evaluated using RT-qPCR. Rats were decapitated under deep anesthesia and the right and left IC were dissected separately into RNAlater solution (Qiagen, Hilden, Germany). Total RNA was extracted using the RNeasy Mini Kit (Qiagen, Hilden, Germany). Complementary DNA was synthesized using the amfiRivert cDNA synthesis platinum master mix for reverse transcription-PCR (Gendepot, Barker, Tx, USA) according to the manufacturer's specifications. Real-time quantitative PCR was performed on a LightCycler 480 Instrument II using the LightCycler 480 probes master kit (Roche, Indianapolis, IN, USA) and Taqman probes (Applied Biosystems, Waltham, MA, USA). We used a probe set specific for NF-H (Gene ID: 24587, NM_012607; NF 200, Taqman probe assay ID Rn00709325-m1) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; NM_017008.3, Taqman probe assay ID Rn.01775763_g1). The relative NF-H level of each IC sample was calculated using the 2−ddCt analysis method with GAPDH as the endogenous control (Schmittgen and Livak, 2008).

2.4. Western blot analysis

Rats were decapitated under deep anesthesia and brains were harvested within 5 min. The IC was identified using a reference brain atlas (Paxinos and Watson, 2013), quickly harvested, and frozen in liquid nitrogen. The tissues were homogenized in protein extraction solution (PRO-PREP; iNtRO, Seong-Nam, Korea) and the supernatant was collected after centrifugation at 13 000 rpm for 15 min at 4 °C. Protein concentrations of the extracts were measured using a Pierce BCA protein assay kit (Thermo Scientific, Rockford, IL, USA). Approximately 30 μg of protein was separated on a 6% Tris-glycine gel and electrophoretically transferred onto an Immobilon - P transfer membrane (Millipore, Billerica, MA, USA). Prior to immunoblotting, the membranes were blocked with 5% bovine serum albumin in Tris-buffered saline containing 0.1% Tween 20 (TBST) and incubated with the NF-H non-phosphorylated monoclonal antibody (SMI-32, 1:1000, BioLegend, San Diego, CA, USA) or rabbit anti-β tubulin (1:1000, Cell Signaling Technology, Danvers, MA, USA) antibody overnight at 4 °C. The blots were washed with TBST and incubated with horseradish peroxidase-conjugated rabbit anti-mouse immunoglobulin IgG (1:15 000, Abcam, Cambridge, UK) or goat anti-rabbit IgG (1:6 000, Cell Signaling Technology, Denver, MA, USA) secondary antibody for 60 min at room temperature. After another wash in 0.05% Tween-PBS, the membrane underwent a reaction with a chemiluminescent agent (enhanced ECL substrate; ATTO, Tokyo, Japan) for visualization. The blots were exposed in a LAS-4000 (GE healthcare life sciences, Pittsburgh, PA, USA) and band intensity was measured using Image J program ver. 1.49 (National Institutes of Health, Bethesda, MD, USA). The quantities of the SMI-32 target protein (180, 200 kDa) in all groups were normalized using the structural protein β-tubulin (55 kDa).

2.5. Immunohistochemistry

Among about 50 sections from caudal to rostral part of IC, 4 sections with 100–150 μm interval were selected for staining using

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