Auris Nasus Larynx xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Auris Nasus Larynx

journal homepage: www.elsevier.com/locate/anl

The effects of unilateral cochlear ablation on the expression of vesicular glutamate transporter 1 in the lower auditory pathway of neonatal rats

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ARTICLE INFO

Article history:
Received 30 November 2016
Accepted 26 January 2017
Available online xxx

Keywords:
Vesicular glutamate transpoter 1
Cochlear ablation
Auditory pathway

ABSTRACT

Objectives: Unilateral cochlear damage has profound effects on the central auditory pathways in the brain.
Methods: We examined the effects of unilateral cochlear ablation on VGLUT1 expression in the cochlear nucleus (CN) and the superior olivary complex (SOC) in neonatal rats.
Results: VGLUT1 expression in the CN subdivisions (the AVCN, the PVCN and the DCN-deep layers) and the SOC (the MnTB, the LSO and the MSO) ipsilateral to the ablated side was significantly suppressed by unilateral cochlear ablation. Interestingly, VGLUT1 expression in the PVCN and the DCN-deep layers contralateral to the ablated side was also reduced.
Conclusion: Our findings indicate that unilateral cochlear ablation affects VGLUT1 expression in the central auditory pathways not only ipsilateral but also contralateral to the ablated side.

1. Introduction

Cochlear damage causes morphological and functional changes in the central auditory pathways of the brain such as the cochlear nucleus (CN) and the superior olivary complex (SOC) [1–4]. Because, in addition to changes in cochlear function, changes in the central auditory pathways affect hearing ability, these changes have been intensively investigated [5,6]. It was reported that unilateral cochlear lesions induced abnormal axonal connections between the ventral CN (VCN) and the SOC, including the medial nucleus of the trapezoid body (MnTB), the medial superior olive (MSO) and the lateral superior olive (LSO) [7]. Although numerous morphological changes caused by unilateral cochlear damage in the central auditory pathways have been uncovered [8], the neurochemical changes caused by unilateral cochlear damage still remain largely unclear. Neurochemical changes caused by unilateral cochlear damage, especially those related to neurotransmission, are of great interest because they may indicate functional changes in the central auditory pathways.

Vesicular glutamate transporters (VGLUTs), such as VGLUT1 and VGLUT2, are responsible for the active transport of glutamate and play crucial roles in glutamatergic

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http://dx.doi.org/10.1016/j.anl.2017.01.007
0385-8146/

transmission in the central nervous system [9–11]. Because of the importance of VGLUTs in glutamatergic transmission, we examined the changes in VGLUT expression in the rat central auditory pathways in response to unilateral cochlear ablation. Among VGLUTs, it has been reported that VGLUT1 is widely expressed in the central auditory pathways [12–16]. Immunohistochemical studies showed that VGLUT1 was expressed in the MSO and the LSO, as well as in the VCNs [17]. We therefore examined the expression of VGLUT1 in the rat central auditory pathways.

Because it was demonstrated that earlier cochlear damage during development leads to stronger morphological changes in the central auditory pathways [8], we performed unilateral cochlear ablation soon after the birth of rat pups, and examined VGLUT1 expression using immunohistochemistry. We found that the expression of VGLUT1 in the rat central auditory pathways was markedly affected. This study therefore provides important insights regarding the neurochemical changes in the central auditory pathways in response to cochlear damage. It would be important to uncover the entire picture of the changes in the central auditory pathways in response to cochlear damage.

2. Materials and methods

2.1. Animals

All procedures were performed in accordance with protocols approved by the Animal Research Committee of Kanazawa University Graduate School of Medical Sciences. Pregnant Sprague-Dawley rats were purchased from Charles River Laboratories, Japan, and were reared on a normal 12 h light/dark schedule. The day of birth was counted as postnatal day 0 (P0). In total, twelve P60 rats were included this study.

2.2. Surgical procedures

Unilateral cochlear ablation was performed on newborn rat pups as described previously, with modifications [8]. Briefly, newborn rats (P3-5) were anesthetized with hypothermia, and a skin incision was made inferior to the left pinna. Using a post-auricular approach in microsurgery, the lateral semicircular canal and middle ear soft tissues were exposed by blunt dissection with a coagulator, with care taken not to damage the facial motor nerve and blood vessels. After removing the stapes, the cochlea was identified and ablated mechanically by crushing it with a forceps and aspirating the remaining tissues. The cochlear damage was confirmed by visual inspection at the end of the surgery. The skin incision was closed with glue. The rats were warmed under a heating lamp, and returned to their mothers after recovery. Brain samples were obtained at P60. In addition to these seven experimental animals, five non-ablated, age-matched naive control animals were used in this study. Naive control animals and ablated animals were used simultaneously along the entire procedure.

2.3. Immunohistochemistry

Immunohistochemistry was performed as described previously, with slight modifications [18–20]. Both ablated animals and naive control animals at P60 were deeply anesthetized and perfused transcardially with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde (PFA) in PBS at the same time. Brains were dissected, post-fixed in 4% PFA/PBS overnight, cryoprotected by two-day immersion in 30% sucrose, and embedded in optimal cutting temperature compound (Tissue-Tek OCT; Sakura, USA). To distinguish sides of the brain ipsilateral and contralateral to the cochlear ablation, the thin needle was used to make a tiny whole which indicated the right cortex or the right brainstem. Coronal sections of 40 μm thickness were made using a cryostat (CM 1850, Leica). After being treated with 2% skim milk and 0.5% Triton X-100 in PBS, the sections were incubated overnight with rabbit anti-VGLUT1 antibody (Synaptic Systems) at 4 °C. The sections were washed, and incubated with Alexa 488-conjugated secondary antibody (Molecular Probe) and red-fluorescent Nissl stain (Invitrogen) for 2 h. The sections were then washed and mounted. Epifluorescence microscopy was performed with an AxioImager A1 microscope (Carl Zeiss).

2.4. Quantification of the sizes of the CN subdivisions

Coronal sections were stained with red-fluorescent Nissl stain (Invitrogen), and sections that contained the largest size of each CN subdivision were used for quantification. The outlines of each subdivision were traced, and the areas within the outlines were measured using ImageJ software (NIH) for the analysis.

2.5. Quantification of VGLUT1 immunoreactivity

VGLUT1 signal intensities in the subdivisions of the CN (the AVCN, the PVCN and the DCN) and the SOC (the LSO, the MSO and the MnTB) were measured. Coronal sections containing the largest size of each subdivision were used for quantification. After background signal intensities were subtracted, the mean signal intensity in each subdivision was measured using ImageJ software as follows. First, the outline of each subdivision was traced manually. After the "Mean gray value" option was selected, the "Measure" command was performed. To minimize the variation of the signal intensities among different sections, the mean signal intensities in each subdivision were divided by those of the ventrolateral principal trigeminal nucleus (Pr5VL) or the oral part of the spinal trigeminal nucleus (Sp5O), which we used as reference nuclei, in the same sections. In this study, we analyzed VGLUT1 immunoreactivities ipsilateral and contralateral to the ablated side compared with those of naive control animals.

2.6. Statistical analysis

Statistical significances were analyzed with Student's *t*-test and were determined with a confidence limit of $p < 0.05$.

Please cite this article in press as: Hasegawa H, et al. The effects of unilateral cochlear ablation on the expression of vesicular glutamate transporter 1 in the lower auditory pathway of neonatal rats. Auris Nasus Larynx (2017), http://dx.doi.org/10.1016/j.anl.2017.01.007