Iranian Journal of Basic Medical Sciences

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Effect of neuregulin-1 on the auditory cortex in adult C57BL/6J mice

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ARTICLE INFO

Article type:
Original article

Article history:
Received: Jul 25, 2018
Accepted: Jul 27, 2019

Keywords:
Auditory cortex
Mice
Mouse neurotrophic factor
Neuregulin-1
Presbycusis

ABSTRACT

Objective(s): We sought to explore whether neuregulin-1(NRG1) would have a protective effect on the auditory cortices of adult C57BL/6J mice.

Materials and Methods: We used RTPCR and Western blot (WB) to detect the expression of NRG1 and ERBB4 (the receptor of NRG1) in the auditory cortices of C57BL/6J mice of different ages (6–8 weeks and 42–44 weeks). Three groups of 42–44 week-old C57BL/6J mice were intraperitoneally injected with mouse neurotrophic factor (m-NGF), NRG1, or saline for two months. We observed the ultrastructures of the auditory cortices of adult mice after treatment using transmission electron microscopy. Additionally, we observed expression of NRG1 in the auditory cortices by immunohistochemistry.

Results: Expression of NRG1 and ERBB4 in the auditory cortices of C57BL/6J mice at the age of 42–44 weeks was lower compared with 6–8 week-old mice. The ultra-structures of the auditory cortices, including the neurons and myelin sheaths, as revealed by transmission electron microscopy were healthier in the m-NGF and NRG1 treatment groups than those in the saline group. We found that expression of NRG1 in the auditory cortices after treatment in the m-NGF and NRG1 groups, especially in the NRG1 group, was higher than that in the saline group.

Conclusion: We concluded that with increasing age, NRG1 in the auditory cortices of C57BL/6J mice gradually decreased, and that NRG1 had a protective effect on the auditory cortices in adult C57BL/J mice.

► *Please cite this article as:*
Zhang YM, Yang ZhD, Yu YF. Effect of neuregulin-1 on the auditory cortex in adult C57BL/6J mice. Iran J Basic Med Sci 2020; 23:362-367. doi: 10.22038/IJBMS.2020.33656.8026

Introduction

Aging is an irreversible phenomenon that is often accompanied by the degeneration of physiological structures, including those of the auditory system. Age-related hearing loss is also termed presbycusis, which is typically progressive sensorineural hearing impairment caused by aging. The pathological manifestations of presbycusis are various. In addition to loss of hair cells, degeneration of other peripheral auditory organs, including the stria vascularis (1) and spiral ganglion cells (2) have also been observed. Decreased speech understanding in noisy environments and increased temporal processing deficits are indicative of functional declines of the central auditory system caused by aging (3). Elevated auditory brainstem response (ABR) thresholds in aged rats reflect the degeneration of auditory system (4). The deterioration of perineuronal nets has been found in the auditory cortices of senior C57BL/6J mice (5). Aging also reduces the response strengths at both the brainstem and cortical levels and increases response latency to a greater extent at the cortical level than the brainstem level.

Scientists have been working to find a cure for deafness. The regeneration of hair cells that has been found in the laboratory is a promising approach for replacing the cochlea, but what are the treatments for the degeneration of the central auditory system? The NRG1/ERBB4 signaling pathway plays an important role in synaptic transmission in the central nervous system (6). NRG mediates connections as a protein that signal between cells of the nervous system, heart, breast, and other organs. The central nervous system is the region in which NRG1 and ERBB4 (the main receptor of NRG1) are most highly expressed. The data show a widespread expression of NRG-1 in the adult human brain (7), and electrical activity controls the proteolytic processing of NRG-1 in a frequency-dependent fashion (8). In the central nervous system, the NRG1 signaling system regulates the formation of glial cells, migration of neurons (9), development of oligodendrocytes, myelination of axons (10), orientations of axons (11), development of dendrites (12), and expression of neurotransmitter receptors. In the peripheral nervous system, NRG1 regulates the differentiation of target cells (13), expression of neurotransmitter receptors (14), expression of synapses between neurons (15), and survival of Schwann cells (16).

The amplitude of wave I of brainstem evoked potentials and hearing have been found to be increased in mice overexpressing NRG1. These effects are due to the enhancing effect of NRG1 on synaptic transmission between hair cells and spiral ganglion neurons (17). We proposed that NRG1 may protect against degeneration of the auditory cortex in adult C57BL/6J mice. In the following experiment, we first observed the distributions of NRG1 and ERBB4 in adult mice. We then

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observed changes in the auditory cortices in adult mice after the use of NRG1.

Materials and Methods

Animal and tissue preparation

In the first experiment, male C57BL/6J mice were divided into two groups according to age, i.e., 6–8 and 42–44 weeks (10 in each subgroup) (18, 19). The bilateral auditory cortices of mice were removed, placed in liquid nitrogen, and stored for real-time quantitative reverse transcriptase (RT)-polymerase chain reaction (PCR) and Western blot (WB) testing to detect the NRG1 and ERBB4 levels. In the next experiment, we selected 30 male C57BL/6J mice (aged 42–44 weeks) that were randomly divided into three groups (10 in each subgroup). Mice were intraperitoneally injected three times a week for two months with NRG1 (R&D systems, USA), m-NGF (Beida Road Bioengineering, Inc., China), or 0.9% saline. The NRG1 and m-NGF were dissolved in 0.9% saline for injections of 0.1 ml at concentrations of 0.1 mg/ml and 9 ug/ml, respectively. Mice were intracardially perfused with 0.9% saline followed by either 2% glutaraldehyde or 4% paraformaldehyde for transmission electron microscopy (TEM) or immunohistochemistry, respectively. For TEM the auditory cortex was removed and immersed in 2% glutaraldehyde for 4 hr at 4 °C. All experiments were performed in compliance with Chinese legislation on the use and care of laboratory animals, and all studies were approved by Soochow University Committee for Animal Care.

Real-time quantitative reverse transcriptase (RT)-polymerase chain reaction (PCR)

Total RNA was extracted using Trizol reagent (Jierui Bioengineering, Inc., China) according to the manufacturer's protocol. Reverse-transcribed cDNA was synthesized using a Revert Aid First Strand cDNA synthesis kit (Thermo Scientific Fisher, MA, USA). PCR was performed using a CFX Connect Real-Time System (Bio-Rad Laboratories, USA) according to the manufacturer's instructions. RT was conducted in a 20-µl reaction mixture containing 4 µl of 5X PrimeScript buffer, 1 µl of oligo(dT), 1 µl of random 6-mers, 1 µl of PrimeScript RT Enzyme Mix I, and 13 µl of total RNA. The reactions were performed at 37 ˚C for 1 hr and 85 ˚C for 5 min. PCR was performed using a RealMasterMix Probe (TIANGEN, China) according to the manufacturer's instructions, in a total volume of 20 µl. The mixture contained 10 µl of 2× SuperReal PreMix Plus, 1 µl of forward primer, 1 µl of reverse primer, and 8 µl of cDNA. The PCR cycling conditions were as follows: 50 °C for 3 min, 95 °C for 15 min, 95 ˚C for 10 sec, 55 ˚C for 20 sec, 72 °C for 30 sec for the plate read, followed by 40 cycles of 95 ˚C for 10 sec, and a melt curve of 70 ˚C to 95 ˚C with an increment of 0.5 ˚C per 5 sec and a plate read. Actin was used as an endogenous control for the quantification of PCR. The DNA sequences of the primers (forward and reverse) were as follows: Actin, 5'-GTGACGTTGACATCCGTAAAGA-3' and 5'-GCCGGACTCATCGTACTCC-3'; ERBB4, 5'-TCCCCCAGGC TTTCAACATAC-3' and 5'-GCTGTGTCCAATTTCACTCCTA-3'; and NRG1, 5'-TTCCCATTCTGGCTTGTCTAGT-3' and 5'-CCAGGGTCAAGGTGGGTAG-3'. The primers were designed using Primer Premier 5.0 software (Premier Biosoft International, Palo Alto, CA, USA) and synthesized at the Shanghai Institute of Cell Biology (Shanghai, China)

Western blot (WB)

The protein samples were obtained by cleavage and centrifugation, and the protein concentrations were determined with the BCA method. Electrophoresis was then performed on a polyacrylamide gel. The samples were transferred to PVDF membranes (Millipore, MA, USA). The membranes were blocked with 5% non-fat dry milk in Tris-buffered saline (pH 7.5) containing 0.1% Tween 20 (TBST) at room temperature for 1 hr. Then, the membranes were incubated in 1× TBST buffer with the relevant primary antibodies at 4 °C overnight. The anti-NRG1 (1/1000) and anti-ERBB4 (1/1000) antibodies were from Abnova (MA, USA). The anti-GAPDH (1/10000) antibody was purchased from Abcam (Cambridge, UK). After incubation with the appropriate horseradish peroxidase-conjugated secondary antibody in TBST at room temperature for 2 hr, enhanced chemiluminescence (ECL) reagents (BioRad, USA) were used to detect the interactions, and digital images were acquired using a Molecular Imager ChemiDoc XRS System (Bio-Rad, USA). The signals were quantified using the Image J2x 2.1.4.7 software (Rawak Software, Inc., Germany) and normalized using the total protein normalization (TPN) method provided by Stain-Free Technology.

Transmission electron microscopy (TEM)

The auditory cortex was located using stereotactic coordinates according to the mouse brain atlas, removed and then immersed in 2% glutaraldehyde for 2 hr at 4 °C. The tissues were cut to approximately 1 mm^3 sized pieces, washed in phosphate buffer, and then post-fixed in 1% osmic acid. The tissues were dehydrated in a graded series of ethanol and then incubated 2× for 10 min in propylene oxide. The specimens were infiltrated with a mixture of epoxy resin 618 and propylene oxide (1:1 for 2 hr then 2:1 overnight) and then with pure epoxy resin 618 for 6 hr. After drying at 60 °C for 48 hr, the tissues were cut, stained with lead citrate and visualized using a H-600 transmission electron microscope (Hitachi, Japan).

Immunohistochemistry

The auditory cortices were removed and fixed with 4% paraformaldehyde for 4 hr. The paraffin-embedded slices were sectioned at a thickness of 4 um and mounted on poly-lysine-coated glass slides. The sections were washed 3× with 0.01 M PBS (5 min each) and blocked using 1% BSA for 30 min at room temperature. The sections were incubated overnight in a primary antibody (NRG1 1:100, Abnova Company). They were then rinsed 3× with 0.01 M PBS (5 min each) and incubated for 2 hr in biotinylated goat anti-rabbit antibody (1:200). The sections were incubated for another 2 hr in ABC solutions after identical rinsing steps. After washing with PBS as indicated above, the sections were stained using diaminobenzidine (DAB) and counterstained using hematoxylin. The slides were coverslipped using neural gum mounting medium. The data were quantified

by measuring the mean optical densities with Image-Pro Plus 6.0 software (Media Cybernetics, USA).

Statistical analysis

All data were expressed as the means±SD and were analyzed using the SPSS software package, version 19.0 (SPSS, USA). One-way analyses of variance (ANOVAs) with LSD post hoc tests were used to analyze the RT-PCR, WB, and immunohistochemistry data sets. The differences between groups were considered significant at $P \leq 0.05$.

Results

Expression of NRG1 and ERBB4 mRNA by real-time quantitative RT-PCR

The relative quantifications of mRNA were based on the Cq (numbers of PCR cycles) values. The relative expression levels of the NRG1 and ERBB4 mRNA were calculated using the formula $2^{-\Delta\Delta CT}$. The expression level of NRG1 in the auditory cortices of 6-8-W C57BL/6J mice was significantly higher than that in 42-44-W mice (Figure 1A). However, the expression levels of ERBB4 (B) were slightly high at 6–8 weeks of age. (Figure 1B).

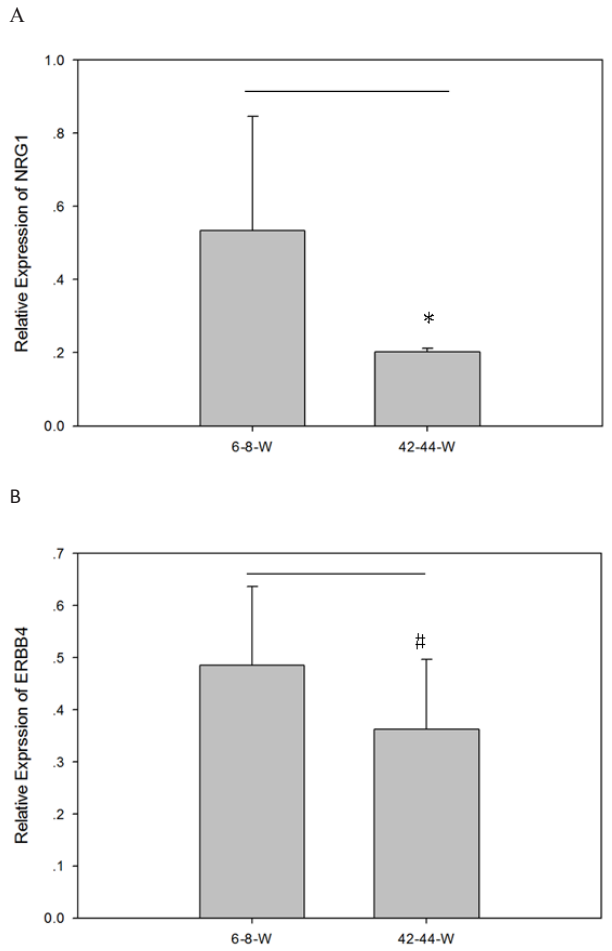

Figure 1. Quantified expression of NRG1 and ERBB4 via realtime quantitative RT-PCR analysis. The results revealed that the expression levels of NRG1 (A) in the auditory cortices of C57BL/6J mice were significantly higher at the age of 6–8 week old than at 42–44 weeks of age. However, the expression levels of ERBB4 (B) were slightly high at the age of 6–8 weeks. *P<0.05 6–8 weeks of age vs 42–44 weeks of age. #P>0.05 6–8 weeks of age vs 42–44 weeks of age

Expression of the NRG1 and ERBB4 proteins by Western blot

Expression of the NRG1 protein relative to GAPDH in the auditory cortices of C57BL/6J mice at the age of 6–8 weeks was higher than that in 42–44 week old mice (Figures 2A, 2B). Moreover, expression of the ERBB4 protein relative to GAPDH in the auditory cortices of C57BL/6J mice at the age of 6–8 weeks was also higher than that in 42–44 week old mice (Figures 2A, 2C).

The ultrastructure of the auditory cortex observed by TEM

In the saline group, the ultra-structure of the auditory cortex exhibited neurons with deformed morphology and folding and wrinkling of the myelin sheaths with distortions (Figures 3A, 3B). The distortion of the myelin sheaths in the saline group was significant compared to

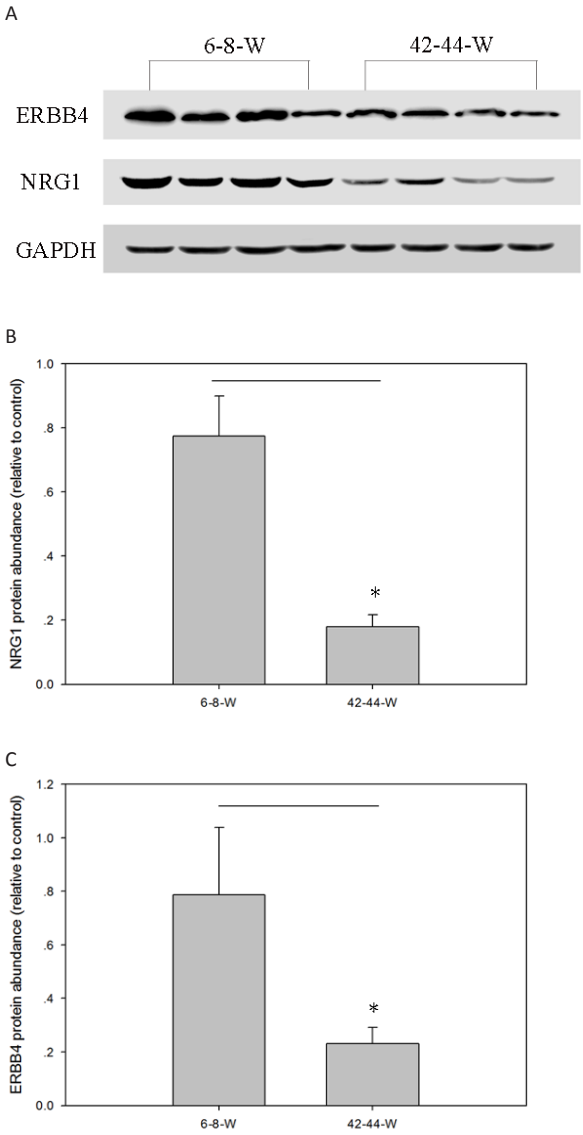

Figure 2. Western blot analyses of ERBB4 and NRG1. Western blot analyses were used to monitor expression of the ERBB4 and NRG1 proteins in two age groups (A). The results revealed that the protein abundances of NRG1 (B) and ERBB4 (C) relative to GAPDH in the auditory cortices of C57BL/6J mice were both significantly higher at the age of 6–8 weeks than at 42–44 weeks of age. *P<0.05 6–8 weeks of age vs 42–44 weeks

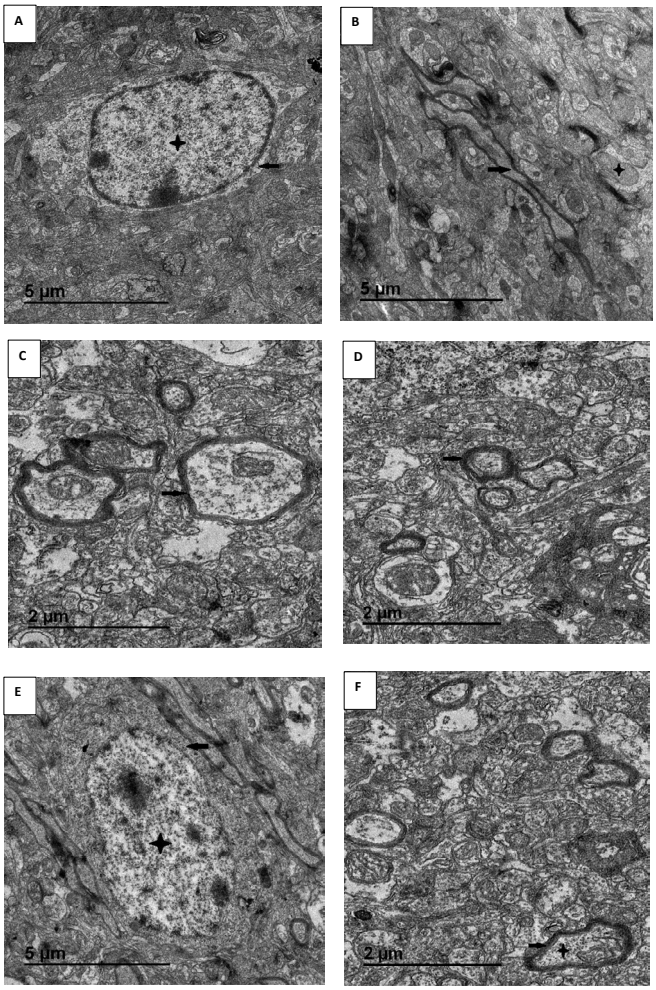

Figure 3. Ultrastructure of the auditory cortices of 42–44 week old C57BL/6J mice after treatment observed by transmission electron microscopy. In the saline group, degeneration of the neurons with abnormal morphology and folding, wrinkling and distortion of the myelin sheath is present (A, B). The distortion of the myelin sheath in the m-NGF group was better than that in the saline group (C, D). In the NRG1 group, the morphologies of the neurons were near normal, and the demyelination and distortion of the myelin sheaths were markedly improved. (E, F). The black asterisk indicates the neuron. The black arrow indicates the myelin. Bar=5 um

the m-NGF group (Figures 3C, 3D). In the NRG1 group, the morphologies of the neurons were near healthy, and the demyelination and distortion of the myelin sheaths were significantly changed (Figures 3E, 3F).

Expression of NRG1 based on immunohistochemistry

We observed the expression levels of NRG1 in the auditory cortices of 42–44 week old C57BL/6J mice after treatment (Figures 4A-C). The expression level of

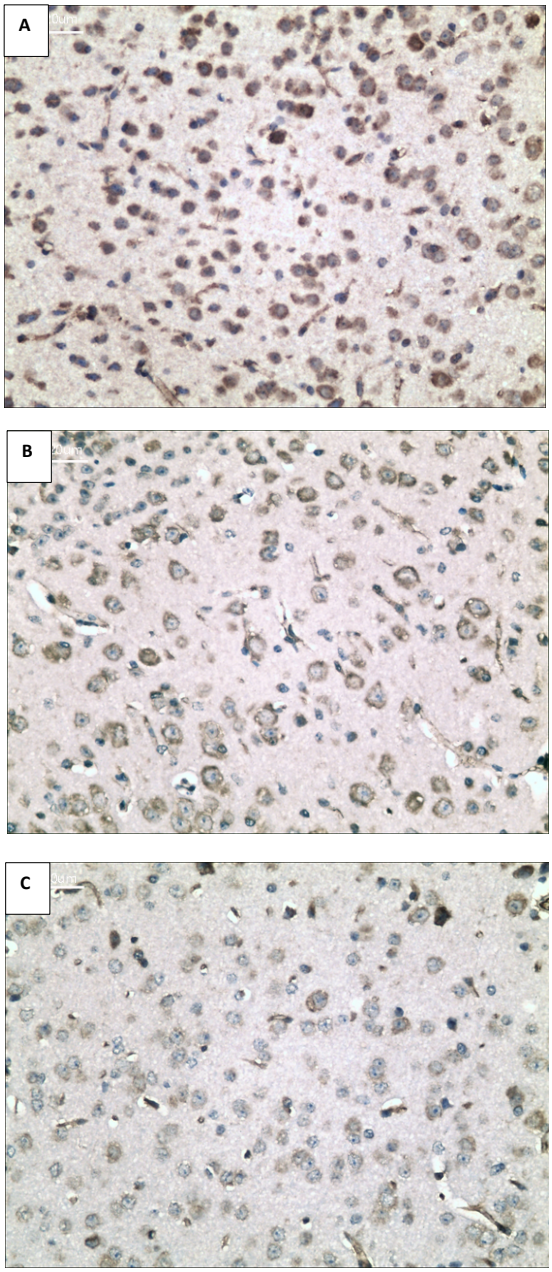

Figure 4. Expression of NRG1 in the auditory cortices of 42–44 week old C57BL/6J mice after treatment assessed with immunohistochemistry. Immunoreactive products appear as brown particles in the nuclei. The expression level of NRG1 was higher in the NRG1 group (A) than in the saline group (C). The difference in the NRG1 levels between the m-NGF group (B) and the NRG1 group was not significant. Bar=20 um

NRG1 was significantly greater in the NRG1 group than in the other two groups. The expression level of NRG1 in the m-NGF group was greater than that in the saline group (Table 1).

Table 1. Expression of NRG1 in the auditory cortices of 42–44 week old C57BL/6J mice after treatment (mean optical density±SEM)

	NRG1	m-NGF	Saline
NRG1	0.2641±0.0198*,#	0.2477±0.0136#	0.1946±0.0200*

*P<0.05, #P>0.05

Discussion

Schuknecht divided presbycusis into four categories according to the inner ear pathology, i.e., sensory, neural, metabolic, and mechanical deafness (20). Sensory presbycusis is caused by hair cell loss and subsequent neural degeneration. Neural presbycusis is caused by the primary degeneration of cochlear neurons. Metabolic presbycusis is caused by atrophy of stria vascularis. Mechanical deafness involves changes in the basilar membrane that affect its properties and function. Additional types have been described and include mixed and indeterminate types in which multiple influences interact (21). The central type was later supplemented based on the degeneration of the central auditory system (22). It is also known as central auditory process dysfunction (CAPD). The prevalence of presbycusis and CAPD in populations older than 65 years are 64.1% and 14.3%, respectively (23). Impairments of the peripheral auditory system, such as impairments of the cochlea, can induce homeostatic changes in the central auditory system from the brainstem to the cortex (24). Imaging of the central auditory pathways has demonstrated decreased fractional anisotropy in the lateral lemniscus and inferior colliculus in individuals with hearing impairment (25). Neuronal loss of nearly 20% has been discovered in the vestibulocochlear nerve in rats, and the superior olivary complex is particularly affected (26). Another study demonstrated that the auditory cortex exhibited atrophy in cases of presbycusis with high-frequency hearing loss. The auditory cortex might be a target for evaluating the efficacy of interventions for age-related hearing loss (27).

Although therapies have been explored, there has been no effective method for preventing or treating presbycusis until now. NRG is believed to play critical roles in inner ear synapse density and synaptic regeneration after injury (28). We explored the use of nerve regulatory factor to determine its effect on the central auditory system. Many studies have demonstrated that NRG1 played a key role in synaptic transmission through both forward and backward signaling pathways. The forward signaling pathway of NRG1 regulates expression of synaptic-associated proteins, such as neurotransmitters and ion channels (29). The backward signaling pathway up-regulates apoptosis and expression of synaptic genes through the nuclear translocation of the cytoplasmic region (30).

Neuroprotective effects of NRG1 on the central nervous system have been reported. NRG1 can protect damaged dopaminergic neurons by reducing neuronal apoptosis in Parkinson's disease (31). NRG1 has been found to be significantly expressed in both the cochlea and spiral ganglion (32). Recent experiments have demonstrated that transgenic mice overexpressing NRG1 in spiral ganglion neurons (SGNs) showed improvements in hearing thresholds (17). What is the effect of NRG1 on the central auditory system? Our real-time fluorescence quantitative RT-PCR experiments revealed that expression of NRG1 in the auditory cortices of adult C57BL/6J mice was significantly decreased. This finding suggested that synaptic transmission was reduced in the auditory cortices of adult mice, which would affect the function of the auditory cortex. NRG1, as a neuroprotective factor, has been reported to protect mouse cerebellar neurons from oxidative stress and neuro-inflammation, which suggests a potential therapeutic use against neuronal inflammation associated with neuro-degenerative diseases (33).

To explore the protective effect of NRG1 on the auditory cortices of adult mice, we used the m-NGF group as the comparison group and the saline group as a control group. NGF has been found to promote the proliferation and differentiation of mouse cochlear neural stem cells *in vitro*, and this process plays a protective role in the treatment of neuronal hearing loss (34). M-NGF is a common exogenous nerve growth factor, is also widely believed to be capable of promoting the survival and growth of neurons, and has been used clinically for many years. M-NGF can act on spiral ganglion neurons and outer hair cells to preserve hearing (35).

The immunohistochemistry results revealed that expression of NRG1 in the auditory cortex neurons of older mice increased significantly after treatment with NRG1 or m-NGF. We observed the ultra-structure of the auditory cortex using transmission electron microscopy. The saline group exhibited degeneration of the neurons with deformed morphology and wrinkling and distortion of the myelin sheaths. The demyelination was improved in the m-NGF group. The structures of the neurons in the NRG1 group were near healthy, and the demyelination and distortion of the myelin sheath were markedly improved. These results suggested that neurotrophic factors, especially NRG1, had a protective effect on the auditory cortices of adult mice. In light of past results and our experimental results, we concluded that NRG1 had a protective effect on both the peripheral auditory system and the central auditory system. However, presbycusis is a multifactorial disease that involves age- and/or disease-related changes in the auditory system and in the brain. NRG1 may be a means of prevention, and further research in this area is needed.

Conclusion

The results of the present study demonstrated that with increasing age, NRG1 in the auditory cortices of C57BL/6J mice, gradually decreased, and that NRG1 had a protective effect on the auditory cortex in adult C57BL/J mice.

Acknowledgment

This study was supported by Suzhou's Key Discipline of Medicine (no. SZXK201503), grants from Science and Technology Bureau of Suzhou (no. SYS201449), grants from Jiangsu Provincial Commission of Health and Family Planning (no. H2017061), grants from Science and Technology Bureau of Suzhou (no. SS201861), and a grant from Jiangsu Provincial "Double Innovation Doctors" Program.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

1. Gratton MA, Schulte BA, Smythe NM. Quantifcation of the stria vascularis and strial capillary areas in quiet-reared young and aged gerbils. Hear Res 1997; 114: 1-9.

2. Frisina RD, Ding B, Zhu X, Walton JP. Age-related hearing loss: prevention of threshold declines, cell loss and apoptosis in spiral ganglion neurons. Aging (Albany NY) 2016; 8: 2081-2099.
3. Ouda L, Profant O, Syka J. Age-related changes in the central auditory system. Cell Tissue Res. 2015; 361: 337-358.
4. Costa M, Lepore F, Prévost F, Guillemot JP. Effects of aging on peripheral and central auditory processing in rats. Eur J Neurosci 2016; 44: 2084-2094.
5. Brewton DH, Kokash J, Jimenez O, Pena ER, Razak KA. Age-related deterioration of perineuronal nets in the primary auditory cortex of mice. Front Aging Neurosci 2016;8:270.
6. Mei L, Xiong WC. Neuregulin 1 in neural development, synaptic plasticity and schizophrenia. Nat Rev Neurosci 2008; 9: 437-452.
7. Law AJ, Shannon Weickert C, Hyde TM, Kleinman JE, Harrison PJ. Neuregulin-1 (NRG-1) mRNA and protein in the adult human brain. Neuroscience 2004; 127: 125-136.
8. Ozaki M, Itoh K, Miyakawa Y, Kishida H, Hashikawa T. Protein processing and releases of neuregulin-1 are regulated in an activity-dependent manner. J Neurochem 2004; 91: 176-188.
9. Anton ES, Marchionni MA, Lee KF, Rakic P. Role of GGF/neuregulin signaling in interactions between migrating neurons and radial glia in the developing cerebral cortex. Development 1997; 124: 3501-3510.
10. Calaora V, Rogister B, Bismuth K, Murray K, Brandt H, Leprince P, Marchionni M, Dubois-Dalcq M. Neuregulin signaling regulates neural precursor growth and the generation of oligodendrocytes *in vitro*. J Neurosci 2001; 21: 4740-4751.
11. Lopez-Bendito G, Cautinat A, Sanchez JA, Bielle F, Flames N, Garratt AN, Talmage DA, Role LW, Charnay P, Marin O, Garel S. Tangential neuronal migration controls axon guidance: a role for neuregulin-1 in thalamocortical axon navigation. Cell 2006; 125: 127-142.
12. Gerecke KM, Wyss JM, Carroll SL. Neuregulin-1beta induces neurite extension and arborization in cultured hippocampal neurons. Mol Cell Neurosci 2004; 27: 379-393.
13. Hippenmeyer S, Shneider NA, Birchmeier C, Burden SJ, Jessell TM, Arber S. A role for neuregulin1 signaling in muscle spindle differentiation. Neuron 2002; 36: 1035-1049.
14. Buonanno A, Fischbach GD. Neuregulin and ErbB receptor signaling pathways in the nervous system. Curr Opin Neurobiol 2001; 11: 287-96.
15. Wolpowitz D, Mason TB, Dietrich P, Mendelsohn M, Talmage DA, Role LW. Cysteine-rich domain isoforms of the neuregulin-1 gene are required for maintenance of peripheral synapses. Neuron 2000; 25: 79-91.
16. Escher P, Lacazette E, Courtet M, Blindenbacher A, Landmann L, Bezakova G, Lloyd KC, Mueller U, Brenner HR. Synapses form in skeletal muscles lacking neuregulin receptors. Science 2005; 308: 1920-1923.
17. Jin D, Ohlemiller KK, Lei D, Dong E, Role L, Ryugo DK, Bao J. Age-realted neuronal loss in the cochlea is not delayed by synaptic modulation. Neurobiol Aging 2011; 32: 2321, e13-23.
18. Johnson KR, Erway LC, Cook SA, Willott, JF, Zheng, QY. A major gene affecting age-related hearing loss in C57BL/6J mice. Hear. Res 1997; 114: 83-92.
19. Yu YF, Zhai F, Dai CF, Hu JJ. The relationship between age-related hearing loss and synaptic changes in the hippocampus of C57BL/6J mice. Experimental Gerontology 2011; 46:716-722.
20. Schuknecht HF. Presbycusis. Laryngoscope 1955; 65: 402-419.
21. Nelson EG, Hinojosa R. Presbycusis. A human temporal bone study of individuals with downward sloping audiometric patterns of hearing loss and review of the literature. Laryngoscope 2006; 116: 1-12.
22. Welsh LW, Welsh JJ, Healy MP. Central presbycusis. Laryngoscope 1985; 95: 128-136.
23. Quaranta N, Coppola F, Casulli M, Barulli MR, Panza F, Tortelli R, Capozzo R, Leo A, Tursi M, Grasso A, Solfrizzi V, Sobbà C, Logroscino G. The prevalence of peripheral and central hearing impairment and its relation to cognition in older adults. Audiol Neurootol 2014; 19: 10-14.
24. Fetoni AR, Troiani D, Petrosini L, Paludetti G. Cochlear injury and adaptive plasticity of the auditory cortex. Front Aging Neurosci 2015; 7:8.
25. Chang Y, Lee Sh, Lee YJ, Hwang MJ, Bae SJ, Kim MN, Lee J, Woo S, Lee H, Kang DS. Auditory neural pathway evaluation on sensorineural hearing loss using diffusion tensor imaging. Neuroreport 2004; 15: 1699-1703.
26. Hoeffding V, Feldman ML. Changes with age in the morphology of the cochlear nerve in rats: light microscopy. J Comp Neurol 1988; 276: 537-546.
27. Eckert MA, Cute SL, Vaden KI Jr, Kuchinsky SE, Dubno JR. Auditory cortex signs of age-related hearing loss. J Assoc Res Otolaryngol 2012; 13: 703-713.
28. Wan G, Gomez-Casati ME, Gigliello AR, Liberman MC, Corfas G. Neurotrophin-3 regulates ribbon synapse density in the cochlea and induces synapse regeneration after acoustic trauma. Elife 2014; 3: e03564.
29. Li B, Woo RS, Mei L, Malinow R. The neuregulin-1 receptor erbB4 controls glutamatergic synapse maturation and plasticity. Neuron 2007; 54: 583-597.
30. Bao J, Wolpowitz D, Role LW, Talmage DA. Back signaling by the Nrg-1intracellular domain. J Cell Biol 2003; 161: 1133-1141.
31. Carlsson T, Schindler FR, Höllerhage M, Depboylu C, Arias-Carrión O, Schnurrbusch S, Rösler TW, Wozny W, Schwall GP, Groebe K, Oertel WH, Brundin P, Schrattenholz A, Höglinger GU. Systemic administration of neuregulin-1 β1 protects dopaminergic neurons in a mouse model of Parkinson's disease. J Neurochem 2011; 117: 1066-1074.
32. Stankovic K, Rio C, Xia A, Sugawara M, Adams JC, Liberman MC, Corfas G. Survival of adult spiral ganglion neurons requires erbB receptor signaling in the inner ear. J Neurosci 2004; 24: 8651-8661.
33. Xu J, Hu C, Chen S, Shen H, Jiang Q, Huang P, Zhao W. Neuregulin-1 protects mouse cerebellum against oxidative stress and neuroinflammation. Brain Res 2017; 1670: 32-43.
34. Han Z, Wang CP, Cong N, Gu YY, Ma R, Chi FL. Therapeutic value of nerve growth factor in promoting neural stem cell survival and differentiation and protecting against neuronal hearing loss. Mol Cell Biochem 2017; 428: 149-159.
35. Gao L, Ge R, Xie G, Yao D, Li P, Wang O, Ma X, Han F. Hearing improvement in A/J mice via the mouse nerve growth factor. Clin Exp Otorhinolaryngol 2017; 10: 303-308.