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난청 이후 중뇌 하구에서 유발되는 억제성 신경 전파에 대한 축삭 발아의 역할

The role of axonal sprouting for inhibitory transmission in the inferior colliculus after cochlear ablation

2019 년 07 월

서울대학교 대학원

의학과 이비인후과학 전공

서 유 주

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The role of axonal sprouting for inhibitory transmission in the inferior colliculus after cochlear ablation

by
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A Thesis submitted to the Department of Otorhinolaryngology in partial fulfillment of the requirements for the Degree of Master of Science in Otorhinolaryngology at Seoul National University College of Medicine

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Approved by Thesis Committee

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ABSTRACT

The role of axonal sprouting for inhibitory transmission in the inferior colliculus after cochlear ablation

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Introduction: Cochlear injury alters the pattern of neuronal activity and neurotransmission in central auditory pathway. Inferior colliculus (IC) plays a significant role in converging auditory input and then projecting it to the auditory cortex. The integration of auditory and somatosensory input also takes place in IC. I assumed that the neuroplastic change after the degeneration of cochlear nucleus contributes to the change of neurotransmission in IC, and Axonal sprouting is a major mechanism of neuroplasticity and losartan inhibits axonal sprouting by blocking TGF- β signaling, which is the primary pathway of axonal sprouting. This study aims to evaluate the change of neuronal transmission and the effect of axonal sprouting using losartan in IC after cochlear ablation.

Methods: The study was performed at one week and two weeks after cochlear ablation. Rats were randomly divided into two groups at one week; control group

and deaf group, and into three groups at two weeks; control group, deaf group, and losartan group. The right IC was harvested after surgery in all groups. Losartan was injected intraperitoneally for two weeks in losartan group. Western blotting was performed for GAP43, Synaptophysin, PDS95, pSmad2/pSmad3, NR2A, Calb, GABAAα1, GAD67, GAP43, ATRX, GDF10, Lingo1, and IGF1.

Results: ABR thresholds ranged from 20 to 35 dB sound pressure level (SPL) before surgery and became higher than 80 dB SPL after cochlear ablation. The proteins showed significant difference among control group, deaf group and losartan group at two weeks; GAP43, pSmad2, and pSmad3 were higher in deaf group than control group (p=0.004, p=0.007 and p=0.015), and decreased in losartan group than deaf group (p=0.006, p=0.003 and p=0.002). GABAAα1 and GAD-67 were lower in deaf group than control (p=0.003 and p=0.003), and were less decreased in losartan group than deaf group (p=0.010 and p=0.015). When comparing the proteins between control group and deaf group at one week, GAD-67 was decreased and IGF-1 was increased in deaf group (p=0.046 and p=0.046). Looking at the time-dependent changes in deaf groups, GAP43 and GDF10 showed a increase (p=0.037 and p=0.011), and synaptophysin and GABAAα1 showed a decrease (p=0.045 and p=0.037) at two weeks.

Conclusion: Axonal sprouting might occur in IC in response to cochlear ablation and have a more significant effect on inhibitory transmission than excitatory transmission. I concluded that axonal sprouting might enhance the reduction of

inhibitory transmission of auditory neuron after cochlear injury, considering inhibitory transmission was increased when losartan blocked axonal sprouting.

Keywords: hearing loss, inferior colliculus, axonal sprouting, inhibitory neurotransmission, Losartan

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LIST OF ABBREVIATIONS

IC : Inferior colliculus

ABR : auditory brainstem response

GAP43 : Growth Associated Protein 43

PDS95 : Postsynaptic Density 95

pSmad2/pSmad3 : phosphorylated Smad2/ phosphorylated Smad3

NR2A: N-Methyl-D-Aspartate receptor subunit 2A

Calb : Calbindin

GABAAα1 : GABAA receptor subunit α1

GAD-67 : Glutamic-Acid Decarboxylase-67

GDF10 : Growth and differentiation factor 10

Lingo1 : Human Leucine-rich repeat and

immunoglobulin-like domain-containing nogo

receptor-interacting protein 1

IGF1 : insulin-like growth factor-1

Introduction

Inferior colliculus (IC) has been known as a “hub” of the central auditory system, which connects the auditory pathway from the lower brainstem to the auditory cortex.(1,2) It has been suggested that peripheral hearing loss would decrease the inhibitory transmission of the central auditory system, leading to increased neuronal hyperactivity(3), and the hyperexcitability might be attributed to the inhibitory circuitry reduction in IC.(4-6) The disruption of inhibitory transmission in IC has been suggested to play a role in the development of tinnitus.(7) The fact that tinnitus persists after ablation of the dorsal cochlear nucleus implies that hyperexcitability of higher structures, such as IC, might be responsible for tinnitus maintenance.(8) Therefore, identifying the mechanism of the disruption of inhibitory transmission in IC is important for the treatment of acoustic disorders. However, to the best of our knowledge, there is no known mechanism for the disruption of inhibitory transmission in IC.

I focused on axonal sprouting, which is a phenomenon that forms new synapse after neural degeneration. It has been reported that the imbalance of auditory and somatosensory inputs occurred in the cochlear nucleus after cochlear ablation, and axonal sprouting contributes to this imbalance.(9-11) I evaluated the change of neural transmission after cochlear ablation and the effect of axonal sprouting on the neural transmission in IC. axonal sprouting related proteins (GAP43, Synaptophysin,

PSD95 and pSmad2/Smad3), Excitation and inhibition related proteins (NR2A, Calb, GABAAα1, and GAD-67), and post-stroke related proteins (ATRX, GDF10, Lingo1 and IGF1) were measured. GAP-43 is positioned in the growth cones of neurons(12), and an essential element of neuronal plasticity.(7) Synaptophysin and PSD95 have been known as useful markers for synaptic density.(13) Synaptophysin is an integral membrane protein, which is found in presynaptic neurosecretory vesicles.(14) PSD95 is localized to the postsynaptic density of synapses, where PSD plays an important role in modulating synaptic plasticity.(15) Smad2 and Smad3 are downstream transcription factors of TGF-β receptors.(16,17)

NR2A is the most widespread NMDA receptor subunit in brain(18), and plays pivotal roles in excitatory neurotransmission and synaptic plasticity.(19) Calb has a neuroprotective effect against excitotoxic damage caused by disruption of intracellular calcium homeostasis. GABAAα1 is a key inhibitory neurotransmitter receptor, which functions an important role in regulating neuronal excitability, and the large portion of GABAAα1 are widely expressed in the auditory system.(20) GAD-67 is a major enzyme for synthesis of GABA from intracellular glutamate.(21) .

ATRX is one of the most highly induced gene product in aged sprouting neurons after stroke, (22) which plays a critical role in modulating neuronal viability and migration.(23) GDF10 induces axonal outgrowth through TGF-β receptor I and II, and has been known as one of the most highly upregulated gene product when axonal sprouting is initiated in peri-infarct cortical neurons.(16) Lingo1 is functional

component of the Nogo receptor-1 complex and induces inhibition of axonal sprouting in spinal cord injury.(24) Given blocking the normal IGF-1 signaling induces neuronal death after stroke,(22) IGF1 mainly functions promoting neuronal survival.

Losartan has been reported to block axonal sprouting by inhibiting TGF-β signaling in the brain, and our previous study presented that systemic administration of losartan inhibited axonal sprouting in cochlear nucleus.(16,17,25) I evaluated that the effect of losartan also occurs in IC and the change of neural excitation and inhibition related proteins when axonal sprouting was blocked by losartan, . To the best of our knowledge, this is the first report that presents the role of axonal sprouting in IC.

Material and Methods

1) Experimental design

All experimental procedures were approved by the Institutional Animal Care and Use Committee of Chung-Ang University (2016-00092). Seven-week-old male Sprague–Dawley rats (200–250 g) were used. Thirty-four rats were randomly divided into three groups (Fig. 1): a control group(n=13) that underwent a sham operation and a deaf group (n=13) that underwent unilateral cochlear ablation on the left side, and a losartan group (n=8) that received losartan for two weeks after left cochlea ablation. Seven rats of the control group(n=13) were euthanized one week after the surgery, and the IC was harvested at the right side (control group at one week, n=7). The remaining six rats were euthanized two weeks after the surgery, and the IC was harvested at the right side (control group at two weeks, n=6). Deaf group (n=13) underwent the left cochlear ablation. Six rats of the deaf group were euthanized one week after the surgery, and IC was harvested at the right side (deaf group at one week, n=6). The remaining seven rats were euthanized in the second week without the administration of losartan (deaf group at two weeks, n=7). The eight rats were intraperitoneally administered Losartan (100 mg/kg) for two weeks and euthanized at two weeks after cochlear ablation (losartan group, n=8). The right IC was harvested after the surgery. Auditory Brainstem Responses (ABRs) before and after the surgery were used to estimate the hearing level of each rat. The level of axonal sprouting and neurotransmission related protein extracts from the right IC

was established using Western blot analysis.

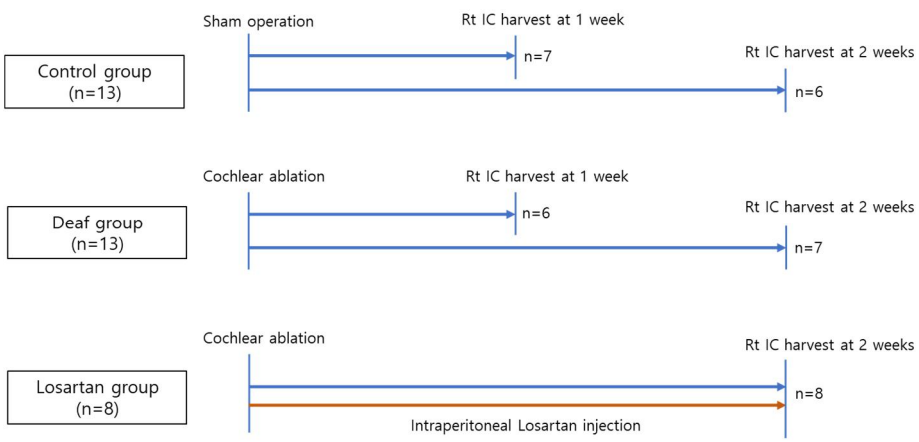

Fig. 1. Experimental design

2) Cochlear ablation

Tiletamine-zolazepam (40 mg/kg, Zoletil 50; Virbac, Bogotá, Colombia) mixed with xylazine (10 mg/kg, Rompun; Bayer-Korea, Seoul, Korea) was intramuscularly injected to each rat. 1% lidocaine hydrochloride was subcutaneously injected into the left retroauricular area for local anesthesia, and then the left retroauricular incision was made. After separating the muscle and fascia, the external auditory canal was exposed and the tympanic membrane was identified. The tympanic membrane and ossicles were extracted, excluding the stapes. The bony wall of the cochlea was identified and broken using a dental pick. The contents of the cochlea were also removed, then 100% ethanol was injected into the empty cochlear space.(Fig. 2) The right side was preserved from the surgery. In the sham

operation, the same procedure was performed before the opening of the external auditory canal, and the next procedure was omitted.

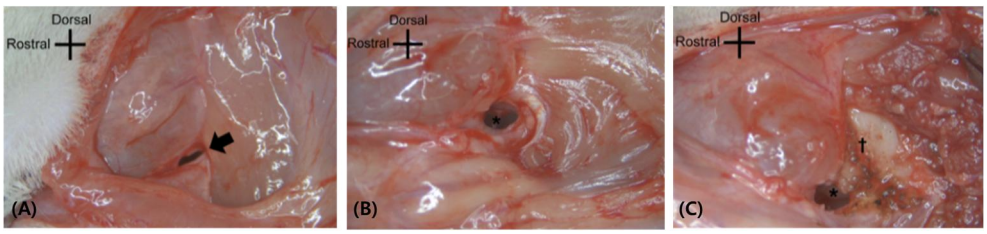

Fig. 2. The process of cochlear ablation. (A) A postauricular incision was made, and then, the external auditory canal (↑) was opened. (B) After opening the external auditory canal wide, the malleus (*) was seen beneath the tympanic membrane. (C) After the removal of the tympanic membrane and the ossicles with the exception of the stapes, the cochlear promontory (*) was identified and broken using a dental pick. The contents of the cochlea were also removed, then 100% ethanol was injected into the empty cochlear space. (†: the muscles on the lambdoidal ridge)

3) Auditory brainstem response recordings

The subdermal needle electrodes were inserted at the left mastoid, the nape of the neck and the right mastoid as the return. Sound stimuli (5-ms duration, cos shaping, 21 Hz) were generated using tone pips of 8, 16, and 32 kHz. ABRs were measured with high-frequency transducers (HFT9911–20–0035) and software (ver. 2.33) from SmartEP (Intelligent Hearing Systems, Glenvar Heights, FL, USA). The responses were amplified (100,000×), bandpass-filtered (100–1,500 Hz) and presented an average 512 times sequentially from lowest to highest. Stimulus intensity was decreased in 5-dB sound pressure level (SPL) decrements. The lowest

stimulus intensity that evoked a perceptible response was determined by two researchers with blinded to the experimental group, and that intensity was considered as the threshold.

4) Western blot analysis

I performed western blot analysis to quantify protein expressions of GAP43, Synaptophysin, PSD 95, pSmad2, pSmad3, NR2A, Calb1, GABAAα1, GAD-67, ATRX, GDF10, Lingo1, and IGF-1. Rats were euthanized one or two weeks after surgery, and the brain tissue was obtained from the IC on the right side. The location of IC of the midbrain was identified according to the atlas of Paxinos and Watso (2006) (−7.30 mm to −9.30 mm from the bregma).(26) The IC on the right side was harvested using a microscope, and frozen in cryopreservation tubes including liquid nitrogen and preserved at −80°C. The samples were homogenized with lysis buffer (Pro-Prep protein extraction solution; iNtRON Biotechnology, Seongnam, Korea) on ice, and were incubated on ice for at least one hour. Then, the extracts were centrifuged for 15 minutes at 13,000 rpm at 4°C (Microcentrifuges, 5424R; Eppendorf, Hamburg, Germany). The supernatant was collected and measured with a Bradford assay. Each protein was denatured at 95°C for 5 minutes in sample buffer (4×, Laemmli SDS-sample buffer; GenDEPOT, Barker, TX, USA), and loaded in each well. Proteins were electrophoresed on a10% SDS-PAGE gel. (WIDE OneStep Acryl Solution, #CBWL-1000; Chembio, Medford, NY, USA).

After electrophoresis, protein bands were transferred to PVDF membranes (Immune-Blot PVDF membrane for protein blotting;Bio-Rad, Hercules, CA, USA). The resulting membranes were blocked with blocking solution (2% bovine serum albumin [BSA] in Tris buffered saline with Tween 20 [TBST]) overnight and incubated overnight at 4°C with primary antibodies. The membranes were probed with primary antibodies for NR2A, Calb, GABAAα1, GAD-67, GAP-43, synaptophysin, PSD 95, IGF-1, pSmad 2, pSmad 3, GDF-10, Lingo 1, and ATRX. The primary antibodies were diluted in TBST containing 5% BSA using the following dilutions: mouse β-actin, 1:25000(A3854, Sigma); rabbit NMDAR2A, 1:500 (ab14596 , Abcam); rabbit Calb1, 1:500 (#ABN2192, Millipore); rabbit GABAA Receptor α1, 1:10000 (#06-868, Millipore); mouse GAD-67, 1:100 (sc-28376, Santa cruz); mouse GAP-43, 1:1000 (#MAB347, Millipore, Burlington, MA, USA); rabbit Synaptophysin, 1:1000 (#5461, Cell Signaling); mouse PSD95, 1:1000 (#36233, Cell Signaling); rabbit IGF-1, 1:500 (GTX64353, Genetax); rabbit p-Smad2/3, 1:1000 (#8828, Cell Signaling Technology, Danvers, MA, USA); rabbit GDF10, 1:1000 (ab235005, Abcam); rabbit Lingo1, 1:1000 (ab23631, Abcam) and mouse ATRX, 1:200(sc-55584. Santa Cruz).

After the membrane was washed repeatedly, it was proved with a species-specific secondary antibody conjugated to horseradish peroxidase for one hour at room temperature. The secondary antibodies were diluted in TBST containing 5% dry milk (anti-rabbit, 1:3000 [#7074, Cell Signaling Technology] and anti-mouse,

1:3000 [#7076, Cell Signaling Technology]). The chemiluminescence of the secondary antibodies was detected with enhanced chemiluminescence (ECL) detection (ECL Prime Western Blotting Detection Reagent; GE Healthcare, Little Chalfont, England) and analyzed using a chemiluminescence image analyzer system (ChemiDoc, Bio-rad).

5) Statistical analysis

Statistical analyses were carried out with IBM SPSS ver. 21.0 (IBM Corp., Armonk, NY, USA). The pre and post-operative ABR audiograms (specifically the SPLs) of the groups were compared at individual frequencies, using Mann-Whitney U test with a P value of 0.05 considered significance. Mann-Whitney U test and Kruskal Wallis test were used for comparisons of the western blot data between two and three groups, respectively, with a P value of 0.05 considered significant. After the performance of Kruskal-Wallis tests, post hoc Mann-Whitney U tests were performed, and a P value of 0.017 (Bonferroni-corrected alpha, 0.05/3) was determined as statistical significance.

Results

1. Comparison of Auditory brainstem response recordings before and after surgery

ABR thresholds in the control groups ranged from 20 to 30 dB SPL before and after surgery, which showed no significant difference across all frequencies, indicating that control groups had no acoustic damage. Baseline ABR thresholds of deaf groups ranged from 20 to 30 dB SPL before the cochlear ablation, and became higher than 80 dB SPL after the cochlear ablation, indicating that there was a significant threshold shift (Fig. 3).

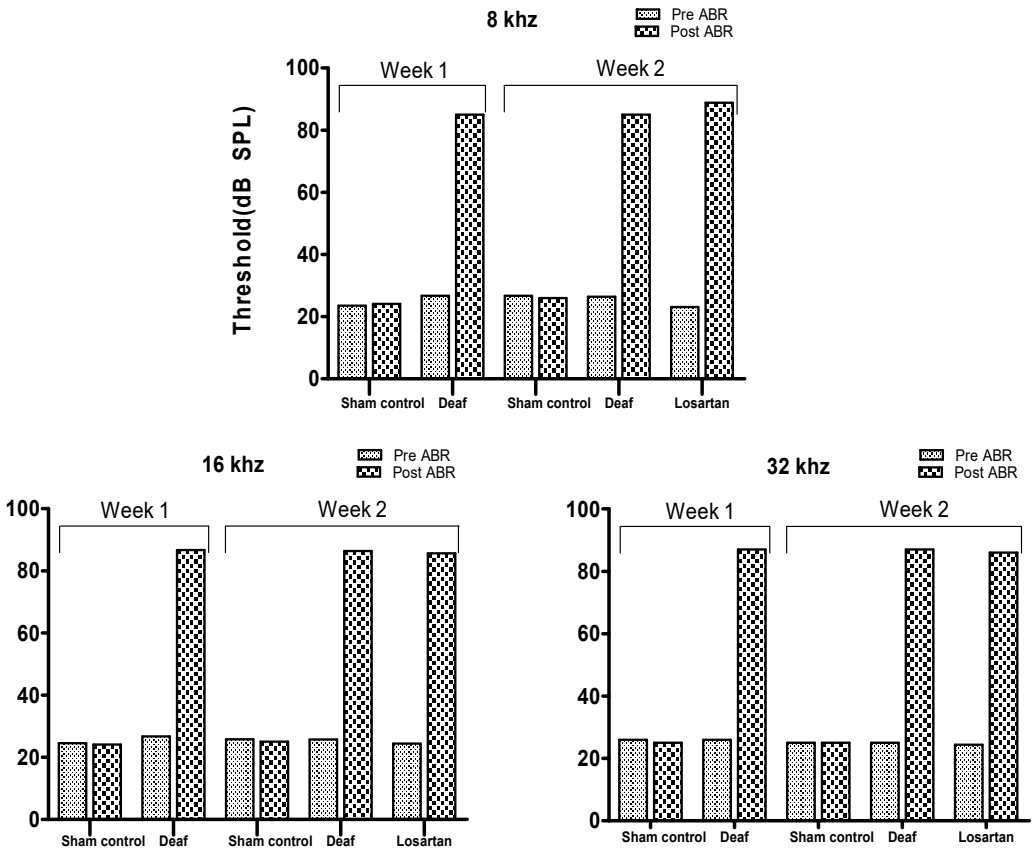

Fig. 3. ABR thresholds for the control group, deaf group and losartan group before and after the sham operation or cochlear ablation. ABR, auditory brainstem response

2. Comparison of the expression of axonal sprouting related proteins

The expression of axonal sprouting related protein was described in Fig. 4. When I compared the proteins between the W1 control group and W1 deaf, there were no significant differences between the groups. However when W2 control, W2 deaf and Losartan group were compared, GAP43, pSmad2, and pSmad3 were significantly higher in W2 deaf group than W2 control group ($p = 0.004$, $p=0.007$, and $p=0.015$, respectively), however they were much lower in Losartan group than W2 deaf group ($p=0.006$, $p=0.003$, and $p=0.002$, respectively). Looking at the time-dependent changes in deaf groups, GAP43 showed a significant increase ($p=0.037$) and synaptophysin showed a significant decrease ($p=0.045$) in W2 deaf group than W1 deaf group.

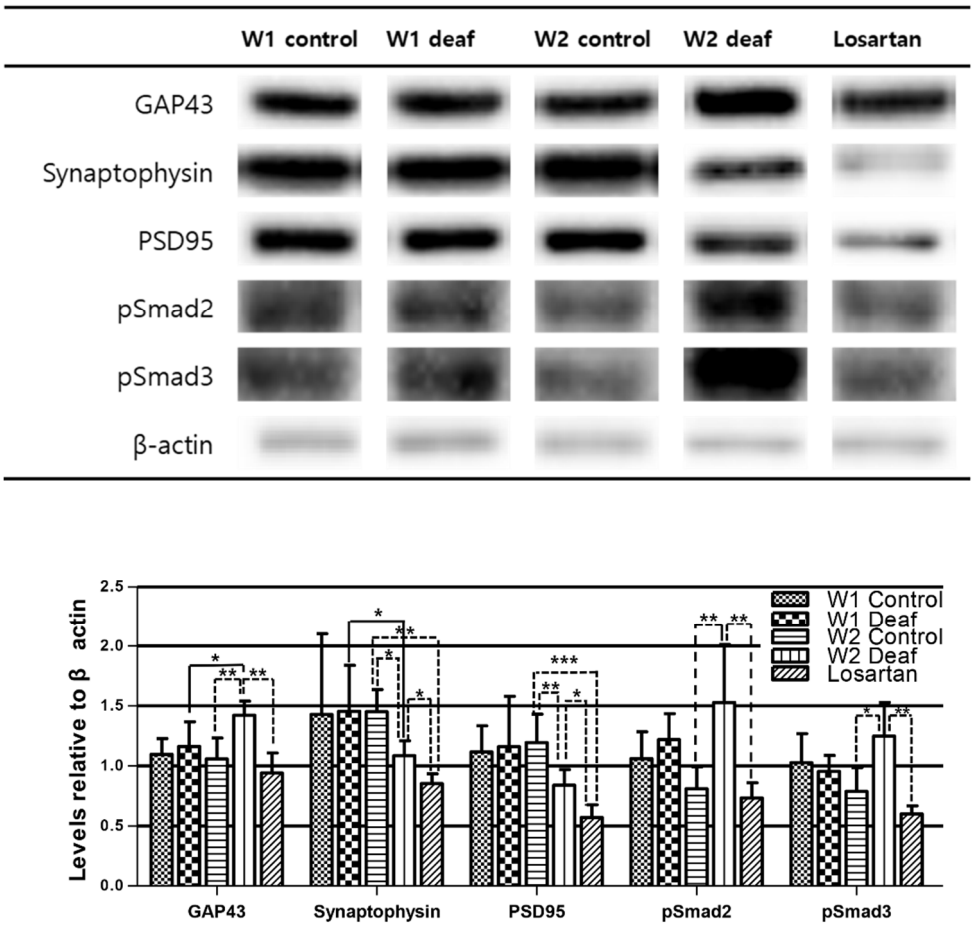

Fig. 4. Expression of axonal sprouting related proteins

Western blot assay was performed one and two weeks after the cochlear ablation. Representative Western blots and quantitative analysis of axonal sprouting related proteins. Data are presented as the mean ± SE. Mann-Whitney test and Kruskal Wallis test were used with a P value of 0.05 considered significant (*: p-value<0.05, **: p-value<0.01, ***: p-value<0.001). After the performance of Kruskal-Wallis tests, post hoc Mann-Whitney U tests were performed and a P value of 0.017 (Bonferroni-corrected alpha: 0.05/3). GAP-43, Growth Associated Protein 43; PSD 95, Postsynaptic Density 95; IGF-1, insulin-like growth factor-1

3. Comparison of the expression of excitation and inhibition-related proteins

The expression of excitation and inhibition-related protein was described in Fig. 5. When comparing W1 control group with W1 deaf group, GAD-67 showed a significant decrease in W1 deaf group ($p = 0.046$), while the other proteins showed no significant difference. When W2 control, W2 deaf and Losartan groups were compared, GABAAα1 and GAD-67 were significantly lower in W2 deaf group than W2 control ($p=0.003$ and $p=0.003$, respectively) and Losartan group ($p=0.010$ and $p=0.015$). There was no significant difference between W2 control and Losartan group. NR2A in W2 deaf group was significantly lower than in W2 control group ($p=0.004$), and there was no significant difference between W2 deaf and Losartan group. Calb showed no significant difference among the groups. Looking at the time-dependent changes in deaf groups, GABAAα1 showed a significant decrease in W2 deaf group than W1 group ($p=0.037$), while the other proteins showed no significant difference.

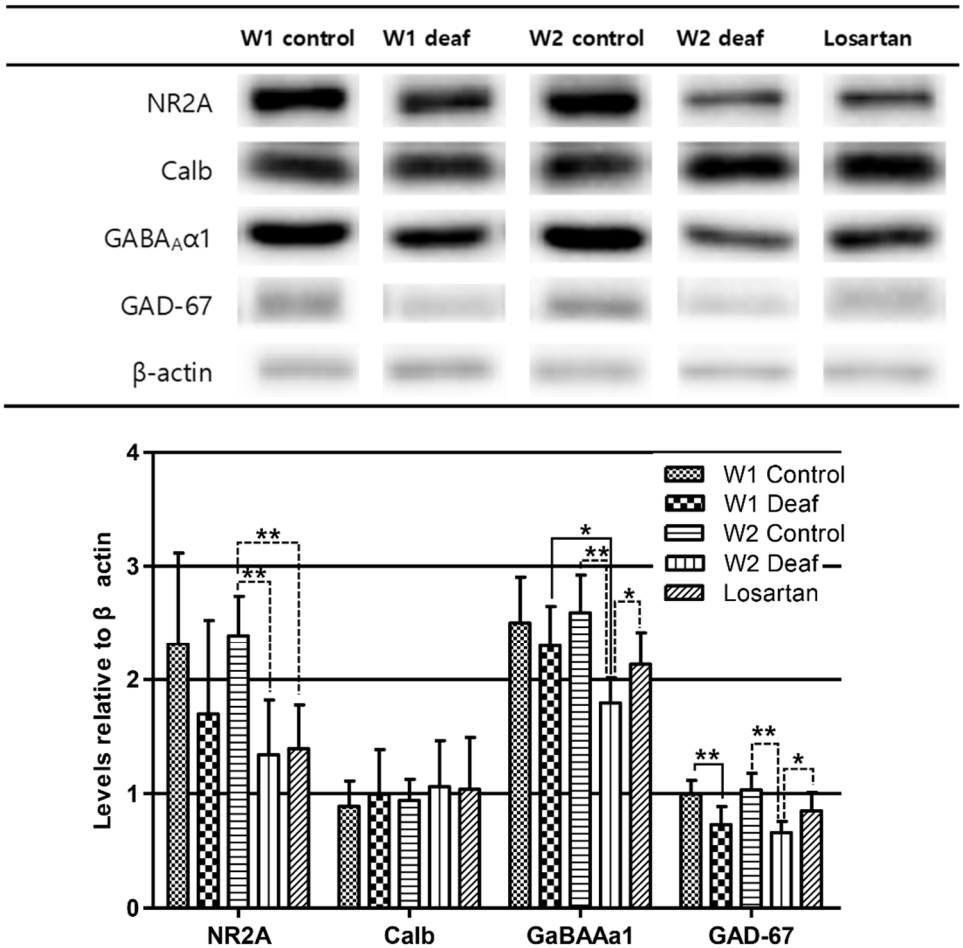

Fig. 5. Expression of excitation and inhibition-related proteins.

Western blot assay was performed one and two weeks after the cochlear ablation. Representative Western blots and quantitative analysis of excitation and inhibition-related proteins. Data are presented as the mean ± SE. Mann-Whitney test and Kruskal Wallis test were used with a P value of 0.05 considered significant (*: p-value<0.05, **: p-value<0.01, ***: p-value<0.001). After the performance of Kruskal-Wallis tests, post hoc Mann-Whitney U tests were performed and a P value of 0.017 (Bonferroni-corrected alpha: 0.05/3). NR2A, N-Methyl-D-Aspartate receptor subunit 2A; Calb1, Calbindin; GABAAα1, GABAA receptor subunit a1; GAD-67, Glutamic-Acid Decarboxylase-67

4. Comparison of the expression of proteins related to post-stroke axonal sprouting

The expression of proteins related to post-stroke axonal sprouting was described in Fig. 6. When W1 control group was compared with W1 deaf, IGF1 showed a significant increase in W1 deaf group ($p= 0.046$) and other proteins showed no significant difference. When W2 control, W2 deaf and Losartan groups were compared, GDF10 was significantly increased in W2 deaf group than W2 control group ($p =0.004$). The level of GDF10 in losartan group did not significantly differ from that of W2 deaf group, however, showed a significant increase than that of W2 control group ($p=0.004$). The level of ATRX and Lingo1 were not significantly different among the three groups. Looking at the time-dependent changes in deaf groups, GDF10 showed a significant increase in W2 deaf group ($p = 0.011$) and no significant difference was found in other proteins.

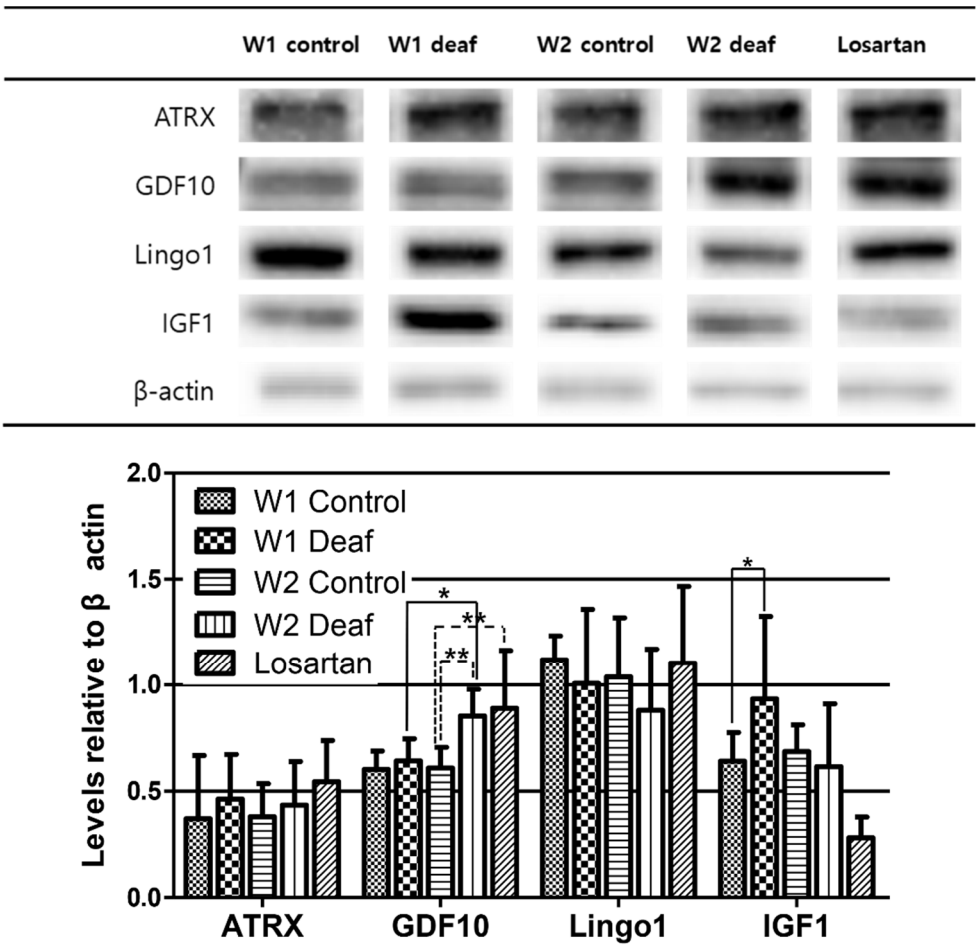

Fig. 6. Expression of proteins related to post-stroke axonal sprouting

Western blot assay was performed one and two weeks after the cochlear ablation. Representative Western blots and quantitative analysis of proteins related to post-stroke axonal sprouting. Data are presented as the mean ± SE. Mann-Whitney test and Kruskal Wallis test were used with a P value of 0.05 considered significant (*: p-value<0.05, **: p-value<0.01, ***: p-value<0.001). After the performance of Kruskal-Wallis tests, post hoc Mann-Whitney U tests were performed and a P value of 0.017 (Bonferroni-corrected alpha: 0.05/3). GDF10, Growth and differentiation factor10; LINGO1, Human Leucine-rich repeat and immunoglobulin-like domain-containing nogo receptor-interacting protein 1; IGF1, insulin-like growth factor-1

Discussion

The degeneration of cochlear nucleus could alter the activity in neural circuits projecting to the IC and induce various changes including gene expression, protein synthesis, and synaptic activity.(27,28) These changes affect neural transmission and trigger neuronal plasticity in IC.(28) Neuronal plasticity is mainly mediated by axonal sprouting, which takes place in the deafferented neuron in response to neuronal damage after brain injury.(29) It was reported that over 80% of the synapses were degenerated after brain injury and subsequently replaced by axonal sprouting from adjacent neurons.(30) Axonal sprouting was also reported to induce neuronal hyperexcitability and contribute to the development of epileptic activity.(31,32) Based on these knowledge, I hypothesized that axonal sprouting would occur in response to neuronal degeneration after hearing loss and affect the change of neural transmission in IC.

First, I investigated how axonal sprouting-related proteins changed in IC after the unilateral cochlear ablation and after the systemic administration of losartan. GAP43, Smad2, and Smad3 showed a significant increase at two weeks after the cochlear ablation compared with W2 control group (Fig. 4). GAP43 plays a major role in axonal sprouting,(33) and was used as a marker of axonal sprouting in several studies.(33-35) TGF-β is the main signaling pathway to initiate axonal sprouting,(36) and Smad2 and Smad3 are downstream transcription factors of TGF-β receptors.

(37) Therefore, the increased level of GAP43, pSmad2 and, pSamd3 after the cochlear ablation implies that axonal sprouting is induced in IC after hearing loss. Then, I examined whether the systemic administration of losartan could inhibit axonal sprouting in IC. It was reported that systemic administration of losartan suppressed TGF-β signaling and, consequently, inhibited axonal sprouting in brain.(16,17) In our previous study, I found that the systemic administration of losartan blocked TGF-β signaling in dorsal cochlear nucleus of deafness rat.(25) Based on these results, I expected that losartan inhibits TGF-β signaling, and blocks axonal sprouting in IC. In fact, our data showed that GAP43, Smad2, and Smad3 significantly decreased after systemic administration of losartan (Fig. 4), implying that losartan prevents TGF-β signaling, and consequently inhibits axonal sprouting in IC.

In the next step, I investigated the effect of axonal sprouting on excitatory and inhibitory activities by blocking axonal sprouting with losartan. NR2A, GAD-67, and GABAAα1 were decreased in W2 deaf group than W2 control group. There was no significant change in the level of Calb. However, GABAAα1 and GAD-67 were significantly increased after the administration of losartan, compared with W2 deaf group (Fig 5). I assumed that the decrease of NR2A was due to the degeneration of excitatory transmission by decreased auditory input after the cochlear ablation and axonal sprouting does not appear to have a significant effect on excitatory transmission. Considering the increased level of GABAAα1 and GAD-67 after

blocking axonal sprouting with losartan, axonal sprouting appears to affect more inhibitory transmission than excitatory transmission. To the best of our knowledge, this is the first report that presents the role of axonal sprouting for inhibitory transmission in IC.

IC receive both auditory and somatosensory input.(38) Somatosensory input originates in the dorsal root ganglia and trigeminal ganglia and transmits to the dorsal cochlear nucleus and IC.(39) The ventrolateral border region of IC is the primary region that receives convergent projections from the cochlear nucleus and the somatosensory system, and integrates multimodal systems.(40) Several studies reported that somatosensory-auditory integration is occurred after hearing loss, suggesting decreased auditory input lead to increased responsiveness of the somatosensory system on auditory system.(41,42) Similarly, I assumed that cochlear injury would degenerate auditory input, and consequently increase the interaction of somatosensory and auditory system in IC. Furthermore, axonal sprouting may have contributed to the increase of somatosensory responsiveness. As somatosensory input is mainly inhibitory to auditory neuron, the increased somatosensory responsiveness may reduce the inhibitory transmission of auditory neuron.(38,43) Blocking axonal sprouting with losartan may inhibit the synaptogenesis of somatosensory neuron, and thus the inhibitory action of somatosensory neurons may have less impact on auditory neurons. As a result, the inhibitory transmission of auditory neuron showed less decrease after administration of losartan than that of

W2 deaf group. However, further research would be needed on the influence of somatosensory input on auditory pathway following hearing loss and the axonal sprouting from somatosensory region to auditory region in IC.

Lastly, I assessed whether the proteins related to axonal sprouting after stroke were expressed in IC after hearing loss. ATRX, IGF1, Lingo1, and GDF10 were gene products significantly upregulated in peri-infarct neurons.(44) GDF10 induces axonal outgrowth through TGF- β receptor in peri-infarct cortical neurons.(16) Our data showed that GDF10 was significantly increased at two weeks after the surgery, however, did not decrease after blocking of TGF-β signal by losartan (Fig. 6). GDF10 could be induced not only by TGF-β pathway but also by SMAD-independent pathway, so that GDF10 may not be decreased even if TGF-β signal is blocked.(43) IGF-1 plays a role in neuroprotection after neuronal injury, and blocking IGF-1 signaling induced neuronal death after stroke.(13,45) Our data showed that IGF1 was significantly increased only at one week and decreased at two weeks after the surgery (Fig. 4). I assumed that IGF1 initially increased to protect against neuronal injury following cochlear ablation, and then gradually reduced when compensatory axonal sprouting occurred. ATRX promotes axonal sprouting and Lingo1 suppresses axonal sprouting after spinal cord injury(22,24), however, ATRX and Lingo1 did not show any significant change after the cochlear ablation and after losartan administration. These results imply that ATRX and Lingo1 would not be involved in axonal sprouting in IC after hearing loss (Fig. 6).

In conclusion, Axonal sprouting might occur in IC in response to cochlear injury and have a more significant effect on inhibitory transmission than excitatory transmission of auditory neuron. Change in somatosensory-auditory interaction after cochlear injury might be contributed to these neural changes. I conclude that axonal sprouting may enhance the reduction of inhibitory transmission after cochlear injury.

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국 문 초 록

난청 이후 중뇌 하구에서 유발되는

억제성 신경 전파에 대한 축삭 발아의 역할

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서론: 중뇌의 하구(inferior colliuculus)는 대부분의 청각 정보를 수렴하고 청각 피질에 투사하는 데 중요한 역할을 한다. 여러 문헌에서 청력상실 이후에 하구의 신경 세포 활동 및 신경 전달의 패턴이 변화되었다고 보고되었다. 청력 상실로 인해 입력되는 청각 반응의 감소는 하구에서 과흥분성을 유도하며, 이는 억제성 회로의 감소와 시냅스 생성을 유도한다. 우리는 transforming growth factor-β (TGF-β) 신호 전달에 의해 촉진되는 축삭 발아(axonal sprouting)는 난청 후 발생하는 하구에서의 흥분과 억제의 불균형을 보상하기 위해 증가한 시냅스의 생성을 반영한다고 추정했다. 이 연구의 목적은 첫째, 난청 이후에 하구에서 흥분 및 억제 관련 단백질의 변화를 평가하고 둘째, 난청 이후 하구에서 축삭 발아가 일어나는지 및 축삭 발아가 흥분성 및 억제성 신경전달에 미치는 영향을 조사하는 것이다.

방법: 본 연구는 시간 경과에 따른 하구에서의 신경세포의 활동 변화를 평가하기 위해 실험쥐에서 좌측 달팽이관 제거술(cochlear ablation)을

시행 이후 각각 1주째와 2주째 우측 하구를 채취하였다. losartan은 좌측 달팽이관 제거술 이후 2주동안 복강 내로 주사 되었다. 실험쥐는 모의수술(sham operation)을 받은 control군, 좌측 달팽이관 제거술을 시행받은 deaf 그룹, 좌측 달팽이관 제거술 시행 이후 2주간 losartan을 투여받은 losartan 그룹으로 무작위 배정되었다. 수술 후 1주째에는 Control군과 Deaf군으로 무작위 배정되었고, 수술 후 2주째에는 Control군, Deaf군 과 Losartan 그룹으로 무작위 배정되었다. 청력은 청성뇌간반응(auditory brainstem response)를 이용하여 측정되었다. 웨스턴블롯법(Western blot)을 이용하여 축삭 발아완 관련된 GAP43, Synaptophysin, PDS95, ATRX, GDF10, Lingo1, IGF1, pSmad2와 pSmad3, 신경의 흥분성을 반영하는 NR2A, Calb, 억제성을 반영하는 GaBAAα1, GAD67 단백질의 정량적 평가를 시행하였다.

결과: 수술 전 청성뇌간반응 역치는 20~35dB(SPL)이었으며, 달팽이관 절제술 이후에는 80dB 이상으로 측정되었다. 수술 이후 2주째에 control 그룹, deaf group, losartan 그룹의 단백질량 변화는 유의한 차이를 보였다. GAP43, p-Smad2, p-Smad3은 control 그룹보다 deaf 그룹에서 유의하게 증가하였으며 ($p=0.004$, $p=0.007$, $p=0.015$), deaf group보다 losartan 그룹에서 유의하게 감소하였다. ($p=0.006$, $p=0.003$, $p=0.002$). GaBAAα1과 GAD-67은 control 그룹보다 deaf 그룹에서

낮았으며(p=0.003, p=0.003), deaf그룹보다 losartan그룹에서 덜 감소하였다. (p=0.010, p=0.015). 수술 이후 1주째 각 그룹간 단백질량의 변화를 비교하였 때, control 그룹보다 deaf 그룹에서 GAD-67은 감소하였으며 IGF-1은 증가하였다. (p=0.046, p=0.046). 시간 경과에 따른 deaf group간 단백질량의 변화를 비교하였을 때, GAP43과 GDF10은 좌측 달팽이관제거술 이후 1주째보다 2주째에 유의하게 감소하였다. (p=0.045, p=0.037)

결론: 난청 이후 하구에서 축삭 발아가 일어나며 이는 흥분성 신경전파보다 억제성 신경전파에 더 효과적으로 작용한다. Losartan을 이용한 축삭 발아 억제시, 억제성 신경전파가 증가함을 확인하였다. 따라서 축삭 발아는 난청 이후 억제성 신경전파의 감소를 촉진시키는 역할을 할 것임을 알 수 있다.

주요어 : 난청, 하구, 축삭 발아, 억제성 신경전파, Losartan

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