The control group comprised 8 temporal bones from 8 subjects having presbycusis without tinnitus (5 males and 3 females; age range, 70–88 years; mean age ± SD, 76.1 ± 6.2 years; high-tone-loss pattern, 1 subject, and descending pattern, 7 subjects; mean bone-conductance threshold ± SD, 42.2 ± 16.4 dB).

Autopsy reports of 1000 patients from the temporal bone collection at the University of Minnesota (Minneapolis, MN) were screened to select cases with presbycusis. Patients whose temporal bones were selected for study fulfilled the following criteria for presbycusis: sensorineural hearing losses characterized by insidious onset, bilateral symmetry, progression into old age without clinical evidence of other ear disorders, and age of 60 years or older. Pure tone audiometry had been performed within 24 months of death for all of the selected subjects. On the basis of the medical records and otologic interview sheet, we excluded subjects with history of ear diseases, ototoxic drug use, head and acoustic trauma, central disease, mental disease, and systemic diseases such as diabetes or neurologic disease.

Thereafter, we divided the subjects into presbycusis with tinnitus (8 subjects) and without tinnitus (8 subjects) according to their detailed medical records. We used unilateral temporal bones from the 16 subjects. Cochlear changes were evaluated using light microscopy in both the groups. Temporal bones had previously been removed at autopsy less than 24 hours after death and fixed in a formalin solution. Each bone was decalcified, embedded in celloidin, and serially sectioned in the horizontal plane at a thickness of 20 μm. Every 10th section was stained with hematoxylin-eosin and mounted on a glass slide for observation under light microscopy.

2.2. Audiometric classification

Patterns of audiometric hearing loss were determined based on bone-conductance thresholds at frequencies of 250, 500, 1000, 2000, 4000, and 8000 Hz. Audiometric patterns with a threshold of less than 25 dB were considered to be normal [3,4]. The cases were divided into 2 groups based on the audiogram type [3]: patients with an abrupt high tone loss (high-tone-loss pattern) and those with a gradually sloping curve (descending pattern). The high-tone-loss pattern was defined as hearing loss with a threshold of more than 25 dB at 4000 and 8000 Hz and a difference in thresholds of more than 20 dB between 2000 and 4000 Hz. The descending pattern was defined as hearing loss with a threshold of more than 25 dB at 2000, 4000, and 8000 Hz, and a difference in thresholds between 2000 and 4000 Hz with an increase of less than 20 dB. In addition to the audiometric hearing loss patterns, mean bone-conductance thresholds were assessed at 500, 1000, 2000, and 4000 Hz. Mean bone-conductance thresholds were used as a parameter for perceptive hearing loss.

2.3. Criterion of tinnitus

We obtained otologic interview sheet including tinnitus during patients' lifetime. We therefore selected apparently subjects without tinnitus and excluded subjects with circumstance factors such as noise from subjects with tinnitus. Subjects with tinnitus had experienced tinnitus for the mean duration of 20.3 years (15.7–25.0 years).

2.4. Spiral ganglion cells

Rosenthal's canal was divided into 4 segments, as described previously [5], as I (from base to 6 mm), II (6–15 mm), III (15–22 mm), and IV (22 mm to apex). Nucleoli of spiral ganglion cells were counted using light microscopy. The number of ganglion cells was determined for each segment and for the cochlear as a whole by multiplying their summed counts by 10 to account for the unmounted sections

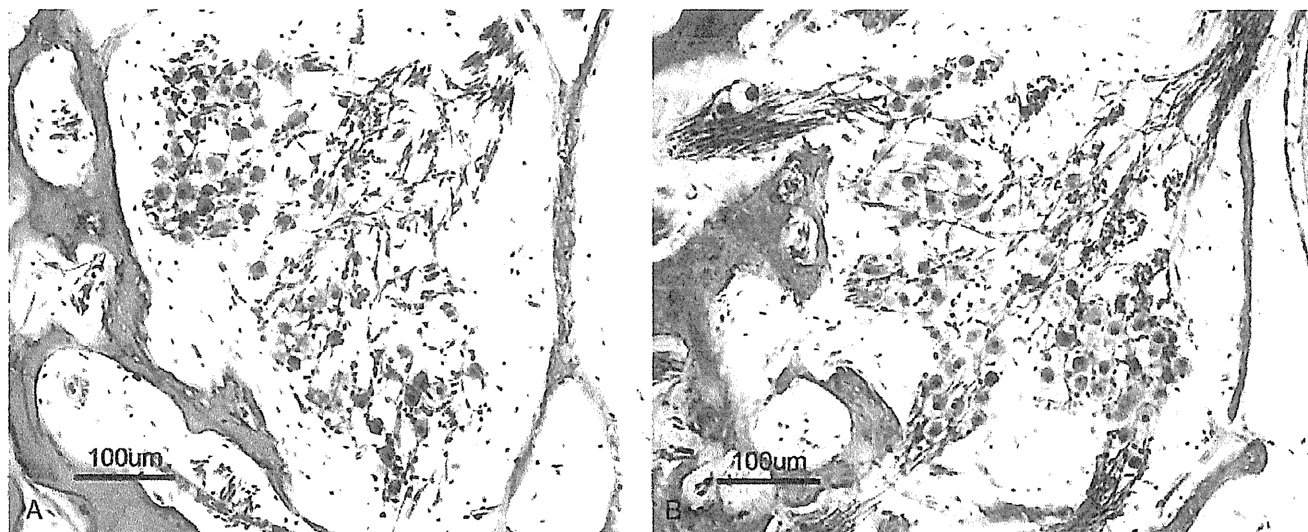

Fig. 1. Loss of spiral ganglion cells in the basal turn of the cochlea is of the same degree in the tinnitus group (A) and the control group (B) (hematoxylin-eosin staining, original magnification ×200).

and by a factor of 0.9 to account for the cells that would be counted because of their location at the interface between sections [6].

2.5. Cochlear hair cells

The cochleas were reconstructed by standard cytocochleograms [3]. In each section, the number of cochlear hair cells was assessed. The percentage loss of cochlear hair cells in each turn was counted to compare these 2 groups.

2.6. Stria vascularis and spiral ligament area

Morphometric measurements of the area counts of the stria vascularis and spiral ligament were made in all turns of the cochlea at the midmodiolar level and the 2 adjacent sections. The area of stria vascularis and spiral ligament in each turn was counted as the mean of these 3 sections. The image was acquired with a CCD camera connected to a computer. The calibrated image of the stria vascularis was obtained at a magnification of 200× and that of the spiral ligament was obtained at 40×. The areas of stria vascularis and spiral ligament were quantified by determining the areas of their cut surfaces using a computer. Measurements were made using a commercially available image analysis software Image-Pro Plus (version 3.0; Media Cybernetics, Silver Springs, MD).

2.7. Statistical analysis

Statistical evaluation was performed using the nonparametric Mann-Whitney test. A P value of less than .05 was considered significant.

3. Results

3.1. Spiral ganglion cells

There was no significant difference in the number of spiral ganglion cells in all the segments, as well as the total number of cells, between the tinnitus group (Fig. 1A) and the control group (Fig. 1B).

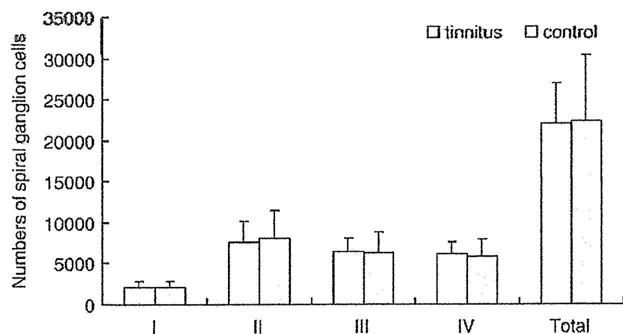

Fig. 2. No significant difference is observed in the loss of spiral ganglion cells in all the segments. Data are presented as mean ± SD.

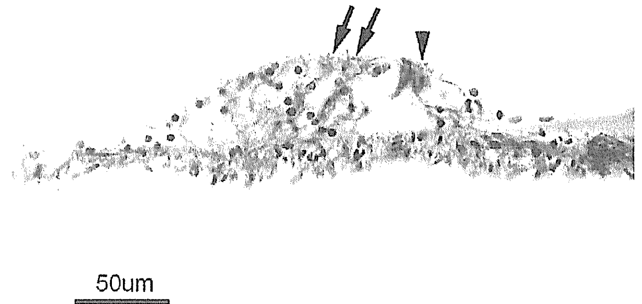

Fig. 3. The lower basal turn of the cochlea in the tinnitus group shows loss of outer hair cells. Inner hair cell (arrowhead) and 2 outer hair cells (arrow) can be observed (hematoxylin-eosin staining, original magnification ×400).

The mean number of spiral ganglion cells was as follows: segment I, 1916 in tinnitus and 1954 in control; segment II, 7371 in tinnitus and 7740 in control; segment III, 6049 in tinnitus and 6089 in control; segment IV, 5804 in tinnitus and 5804 in control; and total, 21 241 in tinnitus and 21 587 in control (Fig. 2).

3.2. Cochlear hair cells

There was a significantly greater loss of outer hair cells in the tinnitus group compared with the control group in the lower basal (P = .006), upper basal (P = .025), and upper middle (P = .018) turns (Fig. 3). No significant difference was found in the lower middle and apical turns.

The mean loss of outer hair cells in each turn was as follows: lower basal turn, 28.8% in tinnitus and 12.4% in control; upper basal turn, 31.0% in tinnitus and 12.5% in control; lower middle turn, 34.5% in tinnitus and 9.8% in control; upper middle turn, 36.7% in tinnitus and 7.6% in

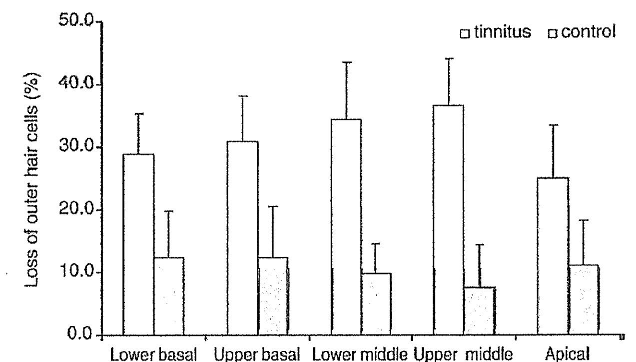

Fig. 4. A greater loss of outer hair cells occurred in the tinnitus group in the basal and upper middle turns.

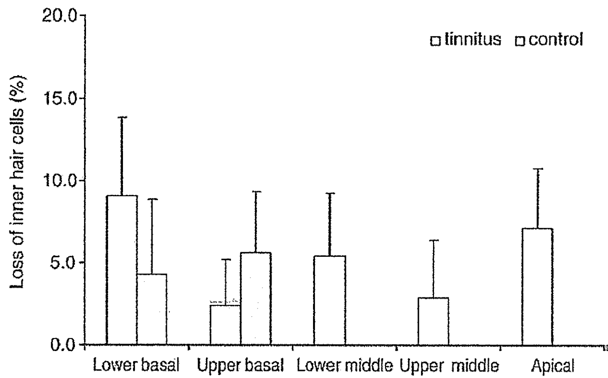

Fig. 5. No significant difference is observed in the loss of inner hair cells in all the turns. Data are presented as mean ± SD.

control; and apical turn, 25.0% in tinnitus and 11.1% in control (Fig. 4).

No significant difference was found between the tinnitus group and the control group in the loss of inner hair cells in any turn.

The mean loss of inner hair cells in each turn was as follows: lower basal turn, 9.1% in tinnitus and 4.3% in control; upper basal turn, 2.4% in tinnitus and 5.6% in control; lower middle turn, 5.4% in tinnitus and 0.0% in control; upper middle turn, 2.9% in tinnitus and 0.0% in control; and apical turn, 7.1% in tinnitus and 0.0% in control (Fig. 5).

3.3. Stria vascularis area

There was a significant loss of stria vascularis area in the tinnitus group (Fig. 6A) compared with the control group (Fig. 6B) in the lower basal (P = .016) and upper basal (P = .027) turns. No significant difference was found in the other turns.

The mean area of stria vascularis in each turn was as follows: lower basal turn, 6235 μm^2 in tinnitus and 7501 μm^2 in control; upper basal turn, 5998 μm^2 in tinnitus and 7419 μm^2 in control; lower middle turn, 5310 μm^2 in tinnitus and 5884 μm^2 in control; upper middle turn, 4053 μm^2 in tinnitus and 5022 μm^2 in control; and apical turn, 3346 μm^2 in tinnitus and 3943 μm^2 in control (Fig. 7).

3.4. Spiral ligament area

There was no significant difference in the spiral ligament area in all of the turns between the tinnitus group and control group.

The mean area of the spiral ligament in each turn was as follows: lower basal turn, 347 040 μm^2 in tinnitus and 310 368 μm^2 in control; upper basal turn, 247 753 μm^2 in tinnitus and 211 832 μm^2 in control; lower middle turn, 135 506 μm^2 in tinnitus and 135 197 μm^2 in control; upper middle turn, 82 804 μm^2 in tinnitus and 91 302 μm^2 in control; and apical turn, 53 428 μm^2 in tinnitus and 53 183 μm^2 in control (Fig. 8).

4. Discussion

The 2 most common causes of tinnitus, acoustic trauma and aging, are typically associated with impaired cochlear function [7]. We found that the degeneration of outer hair cells and stria vascularis could be the main cause of tinnitus generation in subjects with presbycusis. Some animal models of tinnitus due to noise and ototoxicity have been noted [8–10], but a study of tinnitus due to aging has not

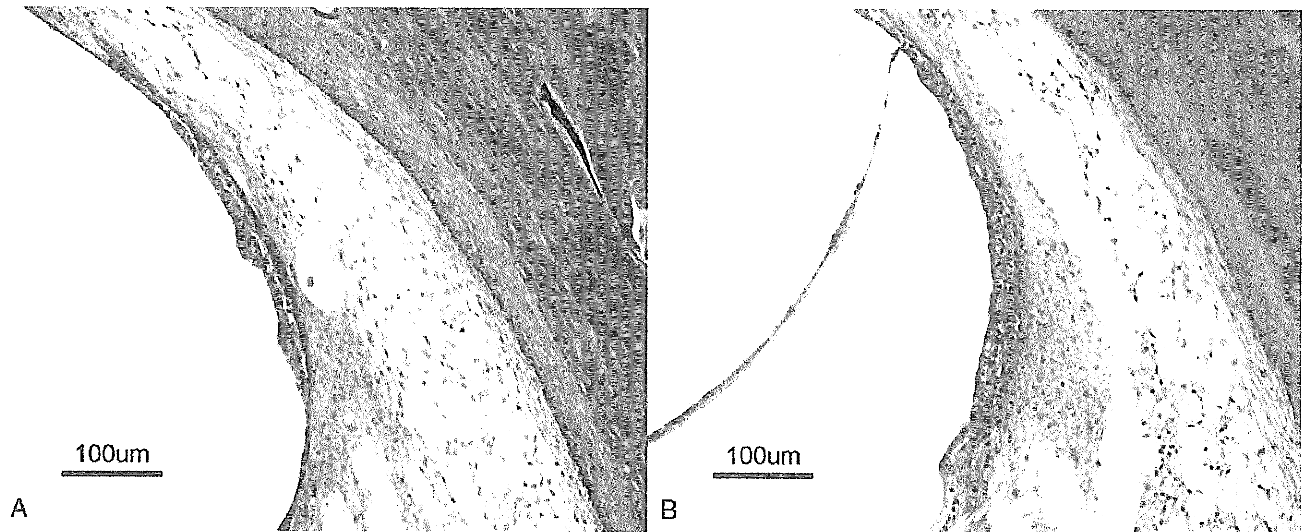

Fig. 6. The stria vascularis of the lower basal turn of the cochlea in the tinnitus group (A) shows more severe atrophy than in the control group (B) (hematoxylin-eosin staining, original magnification ×200).

been published so far in human temporal bones. The present study using human temporal bones could provide valuable information regarding the histopathologic changes that can indicate the underlying mechanism of tinnitus generation.

Tinnitus may be associated with abnormalities in any level of the auditory pathway, but these abnormalities can often start in the cochlea [11,12]. In the present study, cochlear changes in the tinnitus group were more severe than those in the control group. Our findings suggest that the generation of tinnitus in subjects with presbycusis results from further damage of the cochlear function.

Previous clinical study showed that normal hearing subjects with tinnitus have a significantly higher percentage of abnormal transient- and distortion-evoked otoacoustic emissions than normal hearing subjects without tinnitus [13]. The author concluded that dysfunction in the outer hair cells may be important in the generation of tinnitus [13]. According to the discordant theory of inner and outer hair cells by Jastreboff [12], the reduction or lack of input from outer hair cells belonging to a portion of the basilar membrane may result in decreased activity within efferent fibers and consequently might cause increased negative dampening (through outer hair cells) and decreased inhibition on afferents coming from inner hair cells. This will yield enhanced activation of normal inner hair cells, resulting in an abnormal activity perceived as tinnitus. In the present study, loss of outer hair cells in the basal and upper middle turns was significantly greater in the tinnitus group than in the control group, but there was no significant difference in the loss of inner hair cells between them. These findings are consistent with those of previous physiologic studies [12,13].

The stria vascularis and spiral ligament are essential for generating positive endocochlear potential, which is necessary for auditory hair cell function [14,15]. According to Johnsson and Hawkins [16], strial atrophy causes degeneration of hair cells by alteration of endolymph composition. Ascertaining whether atrophy of the strial vascularis or loss of outer hair cells came first in our histologic study is difficult, but we fully considered the relationship between these 2 entities. In addition, the extent to which the primary degeneration of one cochlear element will lead to secondary degeneration of another cochlear element remains unclear [17].

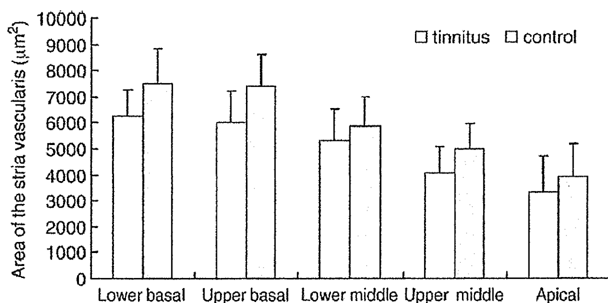

Fig. 7. Atrophy of the stria vascularis in the basal turn in the tinnitus group was significantly higher than in the control group. Data are presented as mean ± SD.

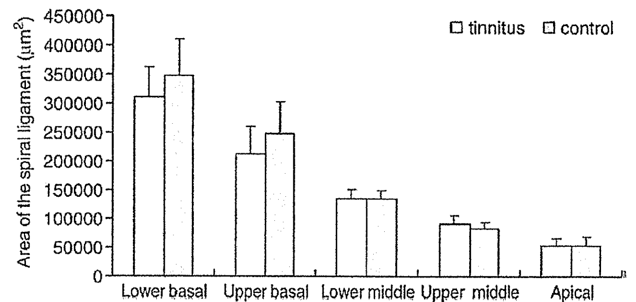

Fig. 8. No significant difference is observed in the spiral ligament in all the turns. Data are presented as mean ± SD.

5. Conclusions

The present study clearly showed that atrophy of the stria vascularis and loss of outer hair cells are more common in patients with presbycusis with tinnitus than in patients with presbycusis without tinnitus. Further studies with a larger patient population are necessary to confirm the result.

Acknowledgment

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BRAIN RESEARCH

Research Report

Ras/p38 and PI3K/Akt but not Mek/Erk signaling mediate BDNF-induced neurite formation on neonatal cochlear spiral ganglion explants

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ABSTRACT

Neurotrophins participate in regulating the survival, differentiation, and target innervation of many neurons, mediated by high-affinity Trk and low-affinity p75 receptors. In the cochlea, spiral ganglion (SG) neuron survival is strongly dependent upon neurotrophic input, including brain-derived neurotrophic factor (BDNF), which increases the number of neurite outgrowth in neonatal rat SG *in vitro*. Less is known about signal transduction pathways linking the activation of neurotrophin receptors to SG neuron nuclei. In particular, the p38 and cJUN Kinase (JNK), mitogen-activated protein kinase (MAPK) pathways, which participate in JNK signaling in other neurons, have not been studied. We found that inhibition of Ras, p38, phosphatidyl inositol 3 kinase (PI3K) or Akt signaling reduced or eliminated BDNF mediated increase in number of neurite outgrowth, while inhibition of Mek/Erk had no influence. Inhibition of Rac/cdc42, which lies upstream of JNK, modestly enhanced BDNF induced formation of neurites. Western blotting implicated p38 and Akt signaling, but not Mek/Erk. The results suggest that the Ras/p38 and PI3K/Akt are the primary pathways by which BDNF promotes its effects. Activation of Rac/cdc42/JNK signaling by BDNF may reduce the formation of neurites. This is in contrast to our previous results on NT-3, in which Mek/Erk signaling was the primary mediator of SG neurite outgrowth *in vitro*. Our data on BDNF agree with prior results from others that have implicated PI3K/Akt involvement in mediating the effects of BDNF on SG neurons in vitro, including neuronal survival and neurite extension. However, the identification

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Abbreviations: ANOVA, one-way analysis of variance; BDNF, brain-derived neurotrophic factor; CNS, central nervous system; HC, hair cells; JNK, cJUN Kinase; MAPK, mitogen-activated protein kinase; NT-3, neurotrophin-3; PI3K, phosphatidyl inositol 3 kinase; PVDF, polyvinylidene difluoride; SCs, supporting cells; SG, spiral ganglion

[1] Drs. Ryan and Brand contributed equally to the supervision of this work.

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of p38 and JNK involvement is entirely novel. The results suggest that neurotrophins can exert opposing effects on SG neurons, the balance of competing signals influencing the generation of neurites. This competition could provide a potential mechanism for the control of neurite number during development.

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1. Introduction

Neurotrophins play a critical role in neural development, regulating differentiation, neurite extension, target innervation and survival (Bibel and Barde, 2000). Brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) are well known to influence neurons in the inner ear.

In particular, mice deficient in BDNF exhibit reduced cochlear neuronal populations, especially in the apical turn (Bianchi et al., 1996; Ernfors et al., 1994, 1995; Farinas et al., 2001; Fritzsch et al., 1997a, 1997b). We, and others, have noted a dramatic effect of BDNF on developing spiral ganglion (SG) neurons in culture. BDNF treatment enhances survival of dissociated SG neurons (Malgrange et al., 1996), dramatically increases neurite number on SG explants (Pirvola et al., 1994) and promotes SG neurons survival *in vivo* (Leake et al., 2011).

Recently, Leake et al. (2011) demonstrated in neonatally deafened kittens and Landry et al. (2011) in adult deafened guinea pigs that chronic BNDF delivery from a miniosmotic pump improved electrically evoked auditory brainstem response thresholds. The authors therefore concluded that BDNF may have potential therapeutic value for the use with cochlear implants in the future. Furthermore, increasing reports are available on the potential therapeutic role of BDNF in a range of central nervous system (CNS) disorders such as amyotrophic lateral sclerosis, Parkinson's disease, peripheral neuropathy, Alzheimer's disease, Huntington's disease and stroke (reviewed by Nagahara and Tuszynski, 2011).

Neurotrophins signal primarily via high-affinity tyrosine kinase receptors in the cochlea, TrkB and TrkC (Pirvola et al., 1994), with some contribution from the low-affinity p75 receptor (Schecterson and Bothwell, 1994). BDNF signaling is mainly mediated via TrkB receptors and TrkB and p75 receptors are expressed by SG neurons throughout the inner ear (Knipper et al., 1996; Pirvola et al., 1994; Sano et al., 2001). Mice null for TrkB are reported to lose 15–20% of SG neurons (Fritzsch et al., 1997a, 1997b). BDNF increases neurite number on SG explants *in vitro* throughout the entire length of the cochlea with no difference in the responses from different cochlear turns (our own unpublished data).

We previously found that Ras or Mek/Erk inhibition blocked NT-3 effects on SG neurites, while p38 inhibition had no effect (Aletsee et al., 2001). Mice with mutations in the docking site for the Shc adaptor protein on the TrkB receptor, which would be expected to reduce both Ras/MAPK and phosphatidyl inositol 3 kinase (PI3K) signaling, showed modest reduction in SG neuron survival (Postigo et al., 2002).

To explore BDNF signal transduction in SG neurons, SG explants were treated with BDNF in the presence of specific inhibitors of intracellular signaling pathways involved in TrkB signaling in the inner ear and other neuronal systems, and activation of signaling proteins was assessed by Western blotting.

2. Results

2.1. *BDNF increases SG neurite number but not length*

Consistent with previous studies (Hartnick et al., 1996; Hegarty et al., 1997), treatment of neonatal SG explants with BDNF resulted in a significant increase ($p<0.05$) in the number of SG neurites present on each explant (Figs. 1 and 2). In contrast, and also consistent with prior results (Malgrange et al., 1996), there was no effect of BDNF treatment on the length of SG neurites (Figs. 1 and 3).

2.2. *Inhibitors of several signal transduction pathways alter BDNF-induced increases in SG neurite number*

The influence of signaling inhibitors on the BDNF-induced increase in neurites on SG explants is illustrated in Figs. 1 and 2. When BDNF treatment occurred in the presence of the pan-G-protein inhibitor GDPßS, there was no significant influence ($p>0.06$). In contrast, the specific Ras inhibitor FTI-277 virtually eliminated the BDNF-induced increase in SG neurite number at all inhibitor doses ($p<0.03$). While the MEK/Erk inhibitor UO126 had no effect ($p>0.08$), the p38 inhibitor SB203580 reduced the BDNF response at all doses ($p<0.02$). Interestingly, the Rac/cdc42 inhibitor C *difficile* toxin B significantly increased the BDNF effect on neurite number, but only at the lowest dose employed ($p<0.04$). The PI_3 kinase inhibitor Wortmannin reduced the BDNF effect, but only at the highest dose employed ($p<0.0001$). Akt inhibitor II significantly attenuated the BDNF effect at 100 nM ($p<0.0001$) and 1 nM ($p<0.01$), but not at 0.1 ($p<0.08$). The PKA inhibitor KT5720 did not alter BDNF effects on SG neurites. When applied alone at the effective dose, or at the highest dose used when no effect was observed, none of the inhibitors influenced SG neurite number.

2.3. *Signal transduction inhibitors influence SG neurite length*

As discussed above, BDNF alone did not affect SG neurite length. Nevertheless, some signaling inhibitors in the presence of BDNF significantly altered neurite length (Fig. 3). The Rac/cdc42, G protein, Mek/Erk and Akt inhibitors each decreased SG neurite length at all doses employed ($p<0.04$). The PI3K inhibitor Wortmannin decreased length at the highest dose ($p<0.04$). The PKA inhibitor KT5720 increased neurite length at all doses ($p<0.04$). When explants were

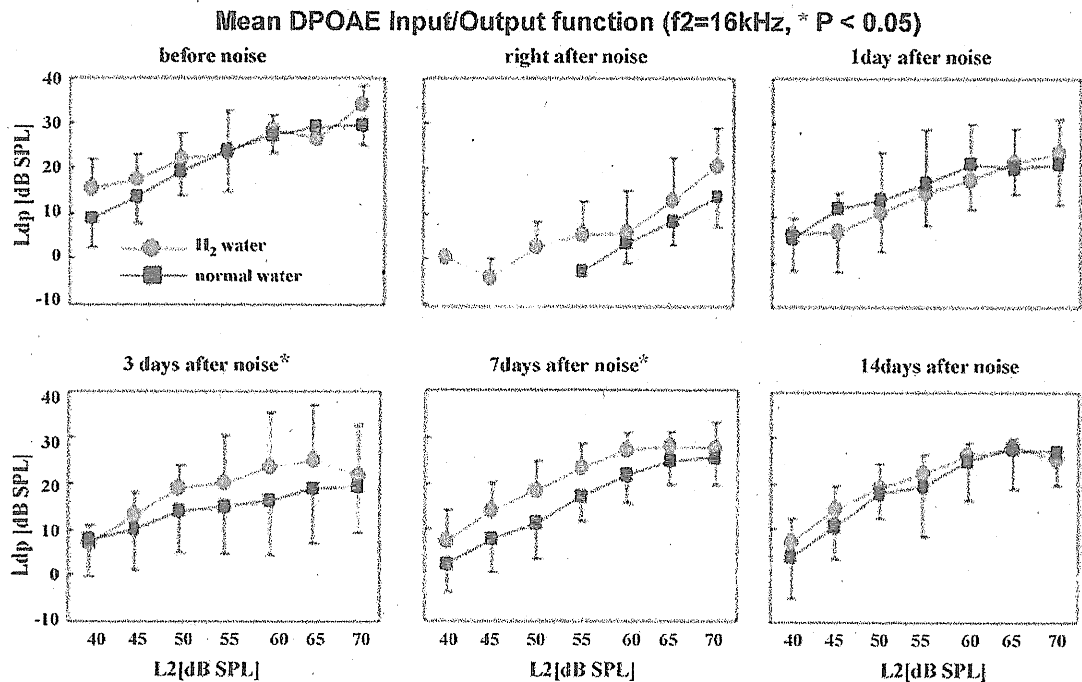

Fig. 3. Mean DPOAE input–output function at different time points at f2 = 16 kHz in normal water-treated controls and hydrogen-rich water-treated animals ($n = 5$ in each group). There is a statistical significance on post-noise days 3 and 7 days. *$p < 0.05$.

hydrogen as an adjuvant agent for noise-induced hearing loss in humans.

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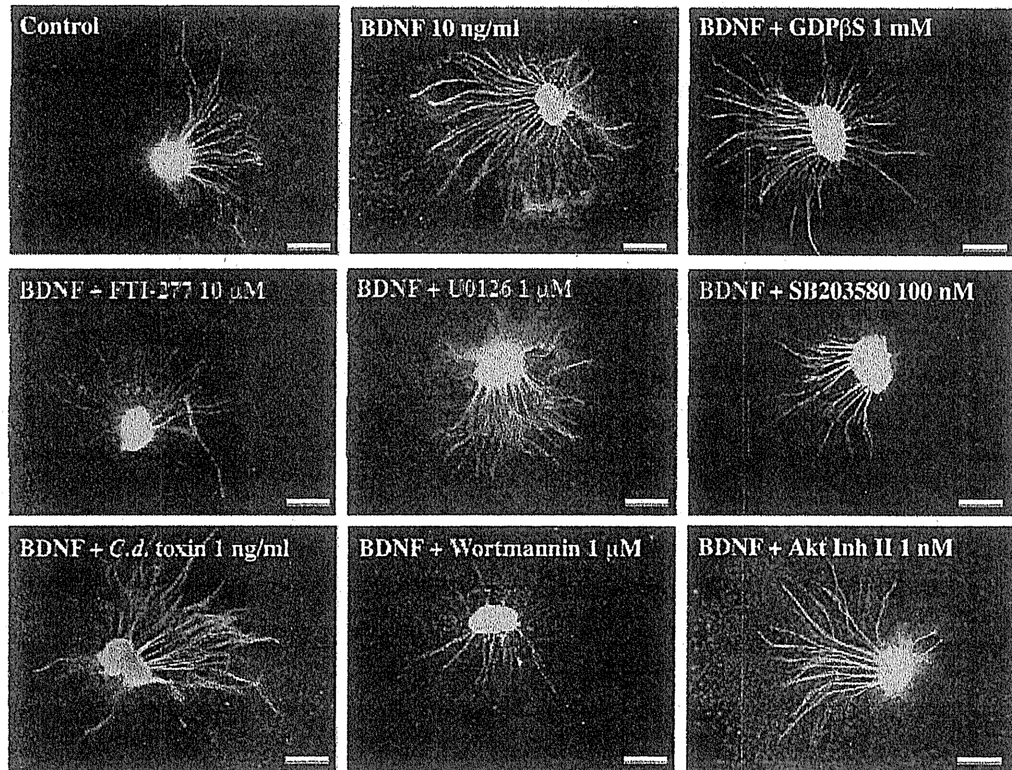

Fig. 1 – Representative SG explants stained with anti-200 kDa neurofilament antibody, for each experimental condition. Scale bar 300 μm.

exposed to the inhibitors alone, neurite numbers were increased by UO126 at 1000 nM ($p<0.05$), and decreased by Akt inhibitor II at 1 nM ($p<0.02$). None of the other inhibitors used affected neurite length when applied alone.

2.4. *BDNF increases both SG neuron survival and neurites/neuron*

The methods used above could not distinguish whether BDNF-induced increases in the number of neurites on SG explants were due to increased SG neuron survival, neurite branching within the explant, or both. We therefore explored alternate methods, and found that a different fixation and staining regimen combined with clearing allowed visualization of SG somata in explants larger than those used for the studies above. The results of culture and BDNF treatment on SG neuron survival in this model are illustrated in Fig. 4. Freshly dissected SG explants contained an average of 0.466 SG neurons/μm of ganglion. Control samples cultured without BDNF for 72 hours showed 0.050 (±.010) neurons/μm, while explants cultured with BDNF showed 0.131 (±.014) neurons/μm. Thus, BDNF resulted in a 162% increase in SG neuron survival compared to untreated explants. Of course, no neurites were observed on freshly dissected explants. However, control explants cultured without BDNF for 72 hours showed 0.020 (± .006) neurites/μm. Thus, neurites extending from the explants represented only 40% of surviving neurons. BDNF resulted in a 520% increase in the number of neurites that extended from the explant when compared to control explants, representing both increased survival and increased neurites/neuron.

2.5. *BDNF activates p38 and Akt in SG*

Western blotting revealed specific activation of cell signaling in SGNs by BDNF. Using Actin as an internal control, normalized phospho-38, phospho-Akt and phospho-Erk levels were expressed as % of control. In three replicates, the relative intensity of phosho-p38 and phosho-Akt was increased in BDNF treated tissue compared to tissue in culture media only. In contrast, only a modest not statistically significant increase in activated Erk MAPK was noted (Fig. 5).

3. Discussion

In the current study, we show that Ras/P38 and PI3K/Akt but not Mek/Erk signaling mediate BDNF-induced neurite formation on neonatal cochlear SG explants. In order to assess the signaling pathways mentioned above, we first evaluated the effects of BDNF alone on SG neurites *in vitro*. Then, SG explants were treated with BDNF in the presence of specific inhibitors of the intracellular signaling pathways involved downstream from TrkB signaling. Finally, we confirmed activation of signaling proteins by Western blotting.

The observation that BDNF treatment results in substantially more neurites on SG explants is consistent with increases

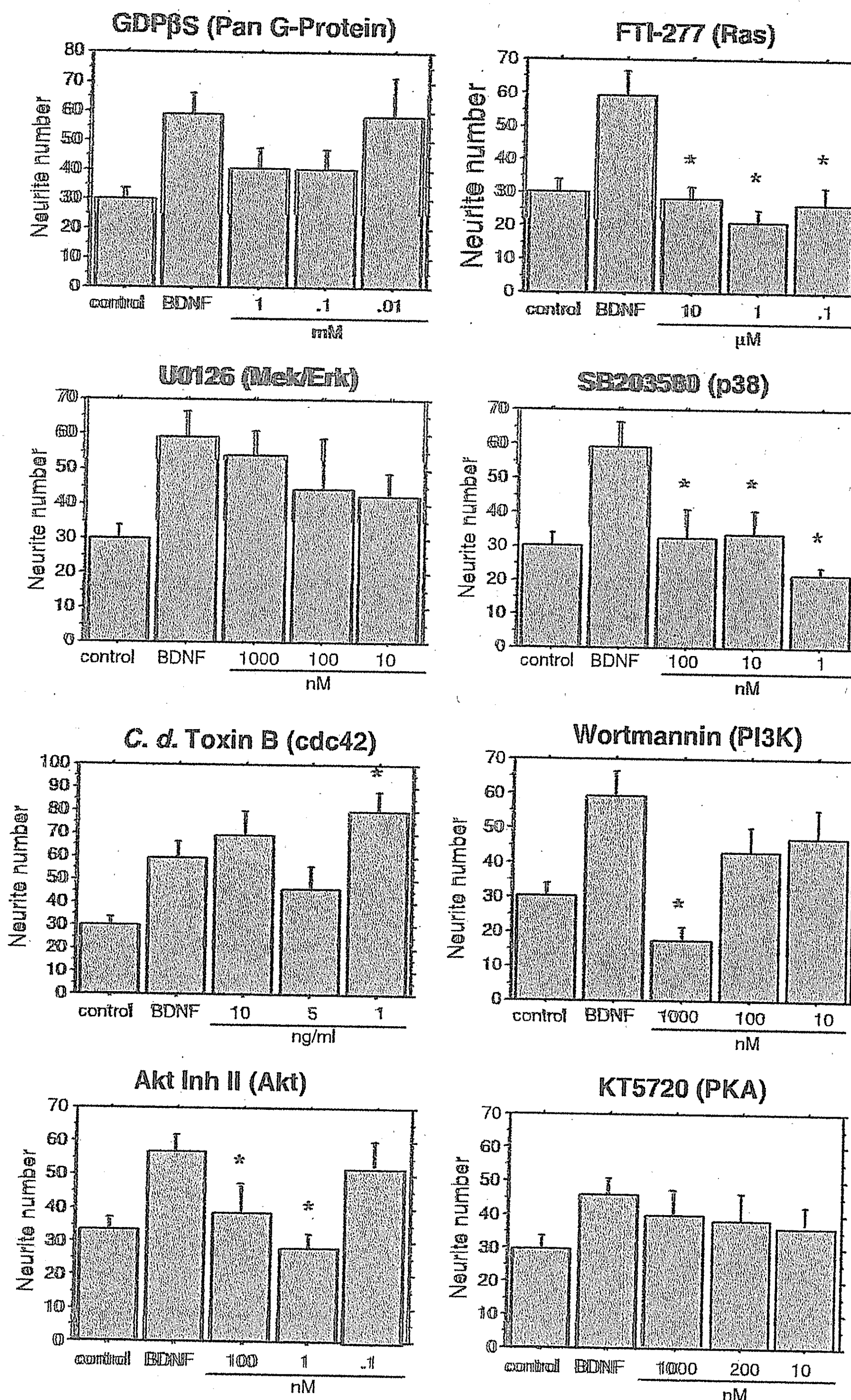

Fig. 2 – Average number of SG neurites observed on SG explants. The number of neurites observed on control and BDNF-treated explants are compared to that seen with three different levels of each signaling inhibitor in addition to BDNF. Lines represent one SEM. BDNF was significantly different from control in all cases. Asterisks denote statistical difference of inhibitor plus BDNF groups from the BDNF-alone group. $n = 12$ for each experimental condition, except Rac/cdc42 inhibitor *C. difficile* toxin B $n = 18$.

in neuronal survival that have been observed with dissociated SG neurons (e.g. Hartnick et al., 1996). However, when survival and neurite number were compared directly, we noted an even greater increase in the number of neurites/neuron following BDNF treatment. This was not associated with an obvious branching of the fibers, nor did the number of neurites exceed one per neuron, indicating that BDNF also increased the production of individual, unbranched neurites on SG neurons. Thus, BDNF appears to be both a survival promoting and neuritogenic factor for SG neurons. The lack of effect of BDNF on neurite length also agrees with several previous studies (Hartnick et al., 1996; Malgrange et al., 1996).

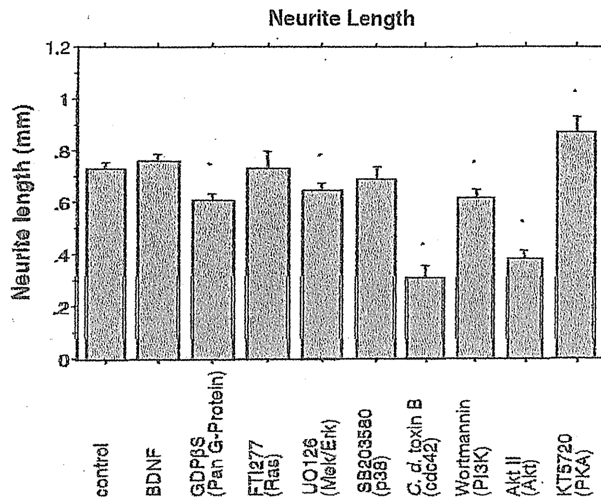

Fig. 3 – The average length of SG neurites observed on SG explants. The length of neurites observed on control and BDNF-treated explants is compared to that seen with signaling inhibitors in addition to BDNF. Lines represent one SEM. Asterisks denote statistical difference of inhibitor plus BDNF groups from the BDNF-alone group. $n = 12$ for each experimental condition, except Rac/cdc42 inhibitor *C. difficile* toxin B $n = 18$. Since BDNF did not alter neurite length, the inhibitors are presumably affecting alternative signaling, perhaps integrin activation by the fibronectin substrate.

It should be noted that we could not distinguish between the dendrites and axons of SG neurons, since we have not found markers that distinguish between the two in explants. Similarly, we could not distinguish between type I and type II SG neuron neurites, since peripherin labeling does not distinguish these two classes of neurons in the rat in culture, due to up-regulation of peripherin in type I neurons *in vitro* (Lallemend et al., 2007). However, since 95% of SG neurons are type I cells, it seems likely that this class of neuron dominates our results.

Our *in vitro* data on neuronal survival can also be related to *in vivo* observations of the SG. The endogenous expression of BDNF in the cochlea appears to vary during the period under study. At birth, BDNF is seen in rat inner and outer hair cells (HC) and along the length of the cochlea (Pirvola et al., 1992) and is present in the supporting cells (SCs) of the mouse organ of Corti only in the apical turn (Farinas et al., 2001). Wheeler et al. (1994) and Wiechers et al. (1999) reported that BDNF mRNA in HCs declined to background levels by P3–P4. Wiechers et al. (1999) observed BDNF mRNA in SCs and outer HCs at P6–P8, while Ylikoski et al. (1993) noted BDNF mRNA in both inner HCs and outer HCs at P7. Weichers et al. (1999) evaluated the expression of BDNF at the protein level during the first two postnatal weeks in mice, using immunohistochemistry. They found that BDNF is present in inner HCs and outer HCs at P1, and then disappears at P3. However, at P3 BDNF is found in some SG neurons. BDNF then reappears in HCs and SCs at P6, and is observed at high levels in SG neurons. At P10, BDNF is only present in some SCs and in scattered SG neurons. These results suggest that HCs produce BDNF during the first few days after birth, with a decline around P3–P4, but recovery by P6–P7. SG neurons also transiently express BDNF, beginning around P6. Rüttiger et al. (2007) showed that BDNF is not expressed in the organ of Corti, but in the SG in adult gerbils. While there was no change in BDNF expression in the apical turn, a moderate

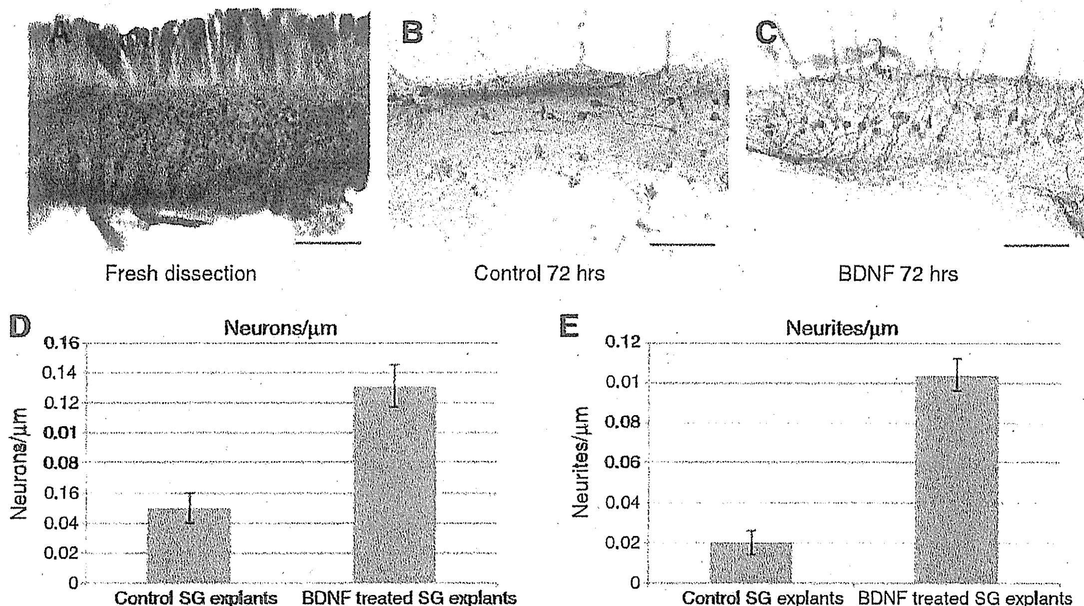

Fig. 4 – Effects of BDNF treatment on neuronal survival and extension of neurites from half-turn SG explants. (A) Explant from a fresh dissection. (B) Explant after 72 hours in culture without BDNF treatment. (C) Explant after 72 hours in culture with BDNF treatment. (D) BDNF resulted in an increased SG neuron survival and (E) increased number of neurites that extended from the explant compared to untreated explants. Scale bar 100 µm. $n = 12$ in each experimental condition. Lines represent one SEM.

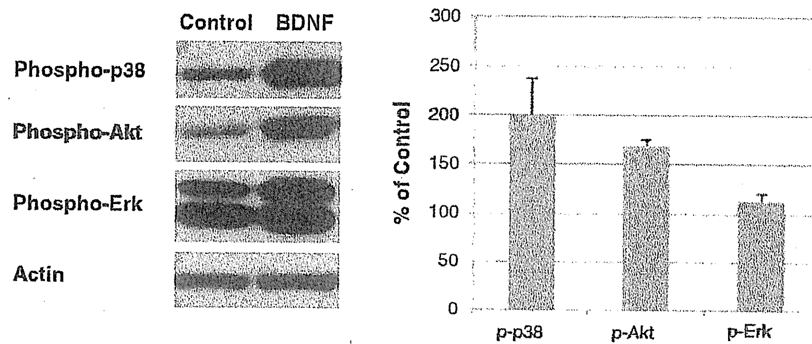

Fig. 5 – Representative Western blots of phosphorylated p38, phosphorylated Akt and phosphorylated Erk. SG explants were exposed either to culture media alone or with 25 ng/ml BDNF. Six whole SG were used per individual blot. Actin was used as an internal control. Phospho-protein levels were determined by densitometry and were normalized against actin. BDNF treated levels are expressed as % of control values. Phospho-p38 and phospho-Akt were significantly increased by BDNF treatment ($p<0.05$), whereas phospho-Erk levels were not. Bars show the mean ± one SEM of 3 independent experiments.

decrease in expression was seen in midbasal turns during aging. In contrast, a recent study by Liu et al. (2011) on adult surgical human cochlear specimens showed no expression of BDNF protein either in the organ of Corti or in the SG.

Our data indicate that SG neurons and neurites are highly sensitive to BDNF during the period in which declines in production are observed, around P3–P5. This is in line with electrophysiological experiments on P3–P8 neonatal mouse SG. Adamson et al. (2002) demonstrated that BDNF alters the endogenous membrane properties and channel types in such a way as to generate faster accommodation and kinetics. It can be speculated that Akt and/or p38 signaling may contribute to these effects.

It is possible that early postnatal production of BDNF in the organ of Corti maintains SG neurons and neurites during the period of reorganization of innervation. The decline in production may then induce apoptosis, with those neurons that ultimately survive having successfully innervated HCs, while neurons that fail to synapse on HCs die from lack of trophic support. SG neurons are reported to undergo substantial apoptosis during the first postnatal week in rodents (Echteler and Nofsinger, 2000).

Our signaling results suggest that a number of pathways participate in transmitting the effects of TrkB receptor activation to the nucleus. Our conclusions are summarized in Fig. 6. The strong effects of FTI-277 on neurite number suggest a major role for Ras in mediating the survival- and neuritogenesis-promoting effects of BDNF. The reduction in neurite number was observed at all FTI-277 dosages employed, including the lowest (0.1 μM). This implies that the effect is mediated at least in part by H-Ras, since other isoforms of Ras such as N- or K-Ras are only inhibited at higher levels (5 and 10 μm, respectively; Lerner et al., 1995). Also, a combination of p38 MAPK and PI3K-Akt signaling appears to stimulate SG neurites, while the UO126 data suggest that the promotion of SG neurite number by BDNF does not involve the canonical Ras-Mek-Erk MAPK survival pathway. This conclusion is supported by our Western blotting data, which demonstrated strong activation of p38 and Akt, but not Erk, in SG neurons after BDNF treatment. Similarly, in sympathetic neurons, NGF promotes survival via a Ras-PI3K-Akt pathway rather than Mek-Erk (Vaillant et al., 1999). Other studies have also shown BDNF mediated activation of PI3K-Akt signaling in SG *in vitro* (Lallemend et al., 2005; Hansen et al., 2001). However, our observation that BDNF does not involve the canonical Ras-Mek-Erk MAPK survival pathway is in contrast to a report by Lallemend et al. (2005) who found that BDNF enhancement of dissociated SG neuron survival was decreased by UO126. Since they used rat SG neurons of a similar age, the difference may be related to dissociation of the ganglion.

The p38 and cJUN kinase (JNK) mitogen-activated protein kinase (MAPK) families have not yet been investigated in BDNF signal transduction in the SG. Our findings that Ras/p38 promotes BDNF mediated effects on SNG while Rac/cdc42/JNK signaling reduces the BDNF mediated formation of neurites are novel.

While signal transduction pathways that mediate BDNF effects have received little attention in the inner ear, several pathways have been implicated in other neuronal systems. Results from pharmacological studies suggest that both MAPK and PI3K pathways mediate BDNF-induced neurite outgrowth from retinal ganglia (Bonnet et al., 2004), while Erk5 activation is critical to BDNF-promoted survival of developing cortical neurons (Liu et al., 2003). Activation of the PI3K target Akt (also known as protein kinase B), mediates BDNF effects on hippocampal neurons (Lee et al., 2002). It has been shown that p38 and JNK MAPK pathways can also be activated by Trk receptors in the nervous system. While in general they promote apoptosis (Mielke and Herdegen, 2000; Ishikawa et al., 2003), several examples of survival enhancement by these pathways have been documented (Nishina et al., 1997; Du et al., 2004). The p75 receptor can also be involved in BDNF signaling. As a dependence receptor (Mehlen and Bredesen, 2004), p75 requires neurotrophin binding to prevent cleavage of its intracellular domain and release of an apoptosis-promoting fragment. Alternatively, neurotrophin binding to p75 can induce apoptosis. This is thought to be Trk-dependent when a neurotrophin binds to a mismatched Trk in association with p75 (Bredensen and Rabizadeh, 1997).

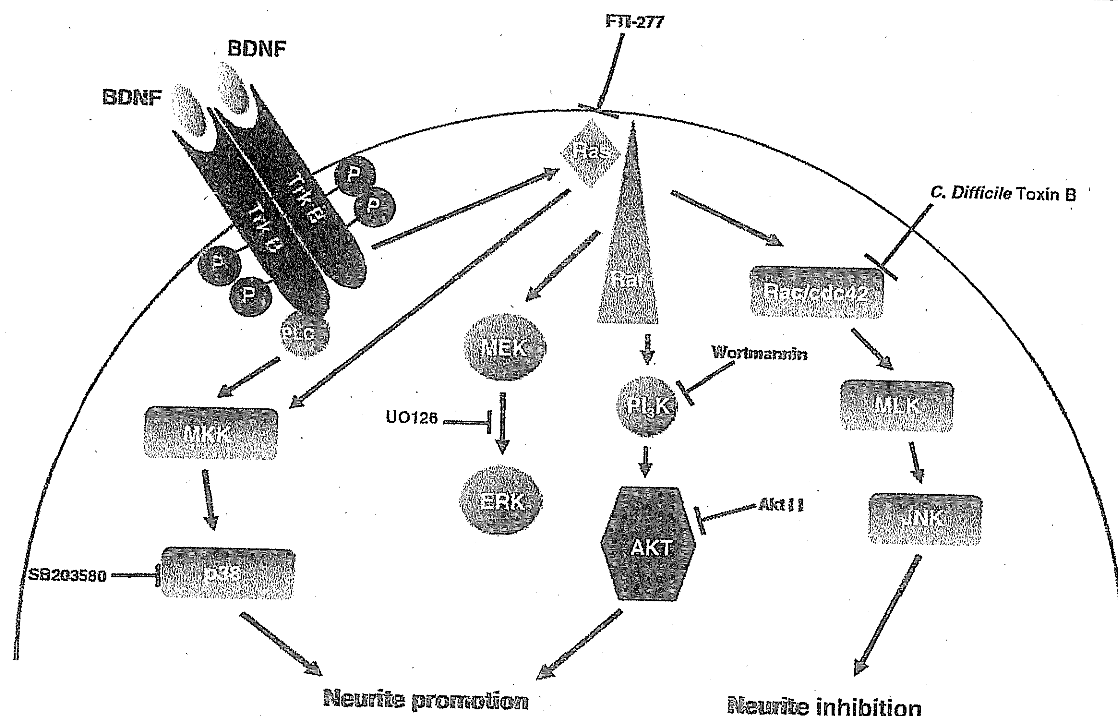

Fig. 6 – Schematic representation of possible signal transduction pathways involved in BDNF effects on SG neuritis, and the inhibitor used in the present study.

It is intriguing that Rac/cdc42 inhibition enhanced the neurite-promoting effects of BDNF. This observation suggests that BDNF may have a complex effect on SG neurons, with neurite number being promoted by p38 and Akt signaling, while being opposed by a Rac/cdc42/JNK pathway. However, the neurite-promoting effects of BDNF were only enhanced at the lowest concentration of the Rac/cdc42 inhibitor applied. A BDNF-independent effect seems unlikely, since Brors et al. (2003a) showed that Rac/cdc42 inhibition led to a dose-dependent decrease of SG neurite number cultured on laminin. The idea that BDNF may activate competing survival and death signals is consistent with current theories of apoptosis regulation in which it is the balance of such competing signals that determine a cell's fate (Salvesen, 2002).

The general G protein inhibitor GDPβS did not influence BDNF effects at any dosage. However, specific inhibition of the G protein Ras reduced BDNF effects, while inhibition of the Rho family G protein Rac/cdc-42 enhanced BDNF. The simplest explanation for the lack of effect of GDPβS is that inhibition of Ras and Rac/cdc42 signaling cancelled each other, resulting in no net effect. While this may well be the case, the very large number of G proteins that might potentially be involved in SG neurons suggests that there may well be a more complex explanation.

Agerman et al. (2003) replaced the coding sequence of the BDNF gene in mice with that of NT3, to analyze the selective roles of BDNF and NT3 during inner ear development. They found that NT3 largely replaced the actions of BDNF in the cochlea, indicating that these two neurotrophins have common and redundant functions. Interestingly, our data indicate that despite the fact that NT3 can largely replace the effects of BDNF in the cochlea, the signaling pathways activated by these neurotrophins are quite different. Aletsee et al. (2001) demonstrated that Ras/Mek but not p38 signaling mediates NT3-induced effects on SG neurons *in vitro*. This implies that the different signaling pathways activated by BDNF versus NT3 nevertheless converge on similar cell functions. The reason for the utilization of different signaling cascades is unclear. However, this might relate to the evolutionary history of the two receptors involved. It might also be speculated that different opportunities for regulation are provided by the two patterns of intracellular signaling.

In the current study, BDNF treatment alone did not affect neurite length. Therefore, the effects of signaling inhibitors on neurite extension without BDNF presumably reflect an influence independent of this neurotrophin. One candidate for the mediation of length effects is alteration of extracellular matrix signaling via integrins. We have previously shown that extracellular matrix molecules enhance neurite outgrowth at the level used to coat the culture wells in the present experiment (Aletsee et al., 2001; Evans et al., 2007). It should be noted that integrin signaling is unlikely to mediate the effects of BDNF on SG neuron survival of neuritogenesis as discussed above, as we have not found in past experiments that ECM molecules influence SG neurite number (Aletsee et al., 2001). In the case of inhibitors that only influenced length in the presence of BDNF, it is possible that BDNF has both positive and negative influences upon neurite length, that on balance result in no effect. Inhibitors may upset this balance. While this hypothesis is perhaps too complex to be attractive without additional supporting data, it is at least consistent with our observations.

4. Experimental procedures

4.1. *Culture of spiral ganglion neurons*

Surgical procedures were approved by the animal subject committee of the San Diego VA Medical Center in accordance with the guidelines laid down by NIH regarding the care and use of animals for experimental procedures. Three to five day old Sprague–Dawley rat pups (P3–P5) were decapitated and the skulls were opened midsagitally under sterile conditions. The membranous labyrinth was exposed by peeling off the cartilaginous cochlear capsule under a dissecting microscope. The stria vascularis and the organ of Corti were removed to expose the SG. The ganglion was excised from the entire length of the cochlea and divided into explants that were approximately 300×300 μm. These individual explants were cultured in 24-well plates previously coated with fibronectin (Sigma-Aldrich, St. Louis, MO) and poly-L-lysine (Sigma-Aldrich). The tissue was incubated in 170 μl of an attachment media consisting of DMEM (Invitrogen/Gibco, Grand Island, NY, USA), 10% FCS (Invitrogen/Gibco), 5% HEPES (Invitrogen/Gibco) and 30 units/ml penicillin (Sigma-Aldrich) for 24 hours at 37 °C, 5% CO_2. After 24 hours, the culture medium was changed to 200 μl of a maintenance media consisting of DMEM supplemented with 1X N2 and 5 g/L glucose (Invitrogen/Gibco). For neurotrophin stimulation, the maintenance media contained BDNF (10 ng/ml; Calbiochem, La Jolla, CA, USA). BDNF control cultures received maintenance media alone. It should be noted that hearing in the rat cochlea begins on about postnatal day 10 (Henley et al., 1989; Rybak et al., 1992). Prehearing neurons were studied since older neurons are more difficult to culture and neurite development is ongoing at this age (Ernfors et al., 1995; Echteler and Nofsinger, 2000).

Experimental cultures contained BDNF with different concentrations of signaling inhibitors: 0.01, 0.1 or 1 mM of the general G-protein inhibitor GDPßS (Sigma-Aldrich); 0.1, 1 or 10 μM of the Ras inhibitor FTI-277 (Calbiochem); 10, 100 or 1000 nM of the MEK/Erk inhibitor UO126 (Calbiochem); 1, 10 or 100 nM of the p38 inhibitor SB 203580 (Calbiochem); 1, 5, or 10 ng/ml of the Rac/cdc42 inhibitor *C. difficile* toxin B (an upstream activator of JNK; Calbiochem); 10, 100 or 1000 nM of the PI3K inhibitor Wortmannin (Calbiochem); 0.1, 1.0, or 100 nM of the Akt inhibitor Akt inhibitor II (Calbiochem: 124008); 10, 200 or 1000 nM of the PKA inhibitor KT5720 (Cell Signaling Technology, Beverly, MA). Inhibitor control media contained the lowest effective dosage of the inhibitor alone. For each condition, 12 explants were studied, except Rac/cdc42 inhibitor *C. difficile* toxin B 18 explants were studied.

4.2. *Fixation and immunohistochemistry*

After 3 days of incubation, cultures were fixed with 4% paraformaldehyde for 20 min and then washed with PBS. The samples were blocked with 1% donkey serum (Sigma-Aldrich) for 10 min at room temperature to reduce nonspecific binding. Specimens were incubated with rabbit polyclonal anti-200 kDa neurofilament antibody (Sigma-Aldrich) diluted 1:500 at 4 C overnight. Explants were then incubated in FITC-conjugated donkey anti-rabbit secondary antibody (Jackson ImmunoResearch, West Grove, PA) diluted 1:100 in PBS. Immunolabeling controls in which rabbit serum was substituted for the primary antibody exhibited no labeling.

The explants were digitally imaged on a fluorescence inverted microscope (Olympus, IX 70) and the number and length of neurites were determined by image analysis software (Spot) as previously described (Brors et al., 2003b). Briefly neurites were traced from the edge of the explant to the tip. All neurites on all explants were measured.

4.3. *Quantitation of neuronal survival*

To assess BDNF effects on neuronal survival, half-turn SG explants were cultured as above with and without 25 ng/ml BDNF for 72 hours, except that the explants were grown on glass cover slips. In order to provide higher penetration and potential for effects on the ganglion body, we used 25 ng/ml in our Western Blot and neuronal studies. The explants were fixed as above, treated with 0.5% peroxide in methanol to block endogenous peroxidases, reacted with a mouse monoclonal antibody IgG against rat neurofilament 200 (Sigma-Aldrich), followed by a biotinylated secondary anti-mouse IgG and developed by an avidin and DAB procedure (Vector Laboratories, Burlingame, CA). The tissue was cleared with citrosol (Fischer Scientific, Waltham, MA, USA) to allow visualization of the cell soma and mounted for evaluation of neuronal survival and neurite number. Soma survival results from cultured explants were compared to those from freshly dissected explants.

4.4. *Assessment of signaling protein activation*

To assess the activation of signaling pathways, intact SG were harvested and placed in attachment media for 24 hours. They were then placed in maintenance media, with or without 25 ng/ml BDNF for 5 min. Explants were collected from media, and lysed with 100 μl T-Per Tissue Protein Extraction Reagent (Thermo Scientific, Rockford, IL) in 1X phosphatase/proteases inhibitors (Roche, Indianapolis, IN) and sonicated for 10 min to shear chromosomal DNA. Samples were centrifuged at 10,000×*g* for 10 min to separate the cytosolic from the membranous components. Equal quantities of these lysates were separated by Bis-Tris Mini Gels 4–12% gels, and electrotransferred to polyvinylidene difluoride (PVDF) membranes (Bio-Rad, Hercules, CA). The membranes were blocked with 5.5% nonfat dried milk in TBS-Tween [50 mM Tris–HCl (pH 7.4), 150 mM NaCl, 0.05% Tween 20] for 60 min at room temperature. Blots were incubated with primary antibodies in blocking buffer overnight at 4 °C and then incubated with horseradish peroxidase-linked secondary antibodies (Jackson ImmunoResearch) followed by chemiluminescent detection (GE Healthcare, Piscataway, NJ). Blots were evaluated with antibodies against phosphorylated Akt (Cell Signaling Technology), phosphorylated p38 (Cell Signaling Technology), phosphorylated Erk (Santa Cruz Biotechnology, Santa Cruz, CA) and to an internal control protein actin (BD Transduction Laboratories, San Diego, CA). After chemiluminescent exposure each membrane was placed inside a dark chamber, an autoradiography film 5×7 was laid over the membrane to capture light emission and scanned with an Agfa Arcus

II scanner. The intensity of the bands corresponding to phosphorylated-p38, phosphorylated-Akt and phosphorylated-Erk were quantified using Image J software. Band intensity for the phosphoproteins was corrected for intensity of our internal control protein (actin) and then expressed as the percentage increase, compared with non-treated tissue. Western blotting was replicated three times with independent biological replicate. With each biological replicate, Western blotting was performed twice. Six whole SG were used per individual blot. Ratio data were analyzed using the Mann–Whitney nonparametric statistical test.

4.5. *Quantitation of neurite outgrowth*

Statistical analysis, using a one-way analysis of variance (ANOVA) followed by a Tukey least significant difference post hoc test was performed, including a correction for the use of multiple post hoc tests (Statview 5.0).

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総　説

乳幼児難聴の聴覚医学的問題「治療における問題点」

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要旨：乳幼児難聴では早期発見・早期支援が重要であり，新生児聴覚スクリーニングを広く行うことに大きな意義があるが普及率は高くない。スクリーニング未施行例や進行性難聴例では介入が遅れる傾向にある。高度難聴のみでなく軽度から中等度難聴でも早期発見・早期介入が重要であり，看過された場合はコミュニケーションに支障をきたし，言語発達，情緒，社会性の発達などに影響が生じる。補聴効果に限界があると予想される高度難聴の場合はコミュニケーションモードの選択を視野に入れた対応が求められ，療育上人工内耳が選択肢と考えられる場合には速やかに人工内耳医療を専門とする医療施設に紹介することが重要である。小児における人工内耳の術後成績には手術年齢，難聴の原因，重複障害の有無，コミュニケーションモードなど多くの因子が影響する。手術適応決定にはこれらの因子を含め考慮すべき多くの因子があり，多職種によるチーム医療での対応が求められる。乳幼児難聴の臨床上の特徴は患児のみならず保護者も対象とし，その経過が長期にわたる事とダイナミックな発達的変化を含む事である。聴力検査一つをとっても高い専門性が求められ，児の生活上の困難や保護者のニーズを把握するには聴覚医学だけでなく発達医学や心理学の知識も必要である。適切な時期の適切な判断が児の将来の発達に影響することを念頭に置いて治療にあたることが肝要である。

ーキーワードー
新生児聴覚スクリーニング，補聴器，人工内耳，重複障害

はじめに

乳幼児の聴覚障害に対する治療における問題は多岐に渡り，例えば難聴発見の遅れ，不正確な診断または診断の遅れ，診断後の対応の遅れや誤り，不適切な治療の選択などが挙げられる。乳幼児の聴覚障害が適切に対応されない場合，コミュニケーションに支障をきたし，言語発達が遅れ，情緒や社会性の発達にも影響が生じうる。難聴は重度であれば1歳前後までに気付かれることが多いが，軽・中等度の場合は言語発達の遅れにより2歳以降に発見されて診断や療育の開始が3歳以降になることもしばしば見られる。

難聴発見の遅れは新生児聴覚スクリーニング（newborn hearing screening：NHS）が普及したためかなり減少してきているが，NHSの施行率およびその後の対応には地域差も多く，いまだに大きな問題である。難聴の検査や診断における問題点については当誌に総説が掲載されている[1,2]が，難聴の不正確な診断または診断の遅れがその後の治療に影響する事は言うまでもなく，諸検査の限界と意義の十分な理解が重要である。聴覚障害については，聴性脳幹反応（auditory brainstem response：ABR），聴性定常反応（auditory steady-state response：ASSR），耳音響放射（otoacoustic emissions：OAE）などの他覚的検査のほか，年齢に応じて聴性行動反

応聴力検査（behavioral observation audiometry：BOA），条件詮索反応聴力検査（conditioned orientation response audiometry：COR），遊戯聴力検査を行うことが重要であり，診断後は裸耳の他補聴器（hearing aid：HA）装用下での評価も重要となる。

難聴診断後の治療法の選択・対応は難聴児の聴取能，言語力の発達に大きく影響する。しかしながら個々の症例に対する具体的な対応について十分に理解している耳鼻咽喉科医師が多くない現状にある。NHSや人工内耳（cochlear implant：CI）の導入以前に，耳鼻咽喉科医師・医療機関で難聴の診断が確定した難聴児の療育やHA調整を地域の療育施設に依存する体制が（一部の地域を除いて）構築されてしまった経緯があるため，診断において十分な知識を有する医師においても，難聴児の治療や療育について経験・知識が乏しく，実際の対応に関する理解が不足する傾向が生まれた。小児のCIが広く行われるようになった現在においても小児難聴の専門外来を開設している医育機関は限られ，小児難聴について研修する教育体制が十分に整っていない状態といえる。

早期に十分な教育を行う施設の問題も挙げられる。現在我が国において難聴児を指導する主な施設は，文部科学省管轄の聴覚特別支援学校，厚生労働省管轄の難聴幼児通園施設や医療機関の言語訓練部門などが挙げられる。いずれも0歳から療育を行っているが，その介入の密度には大きな差がある。積極的に支援を行っている施設では，例えば0－1歳児に対して，個人指導，母親指導，グループ指導をそれぞれ週1回以上行い，ビデオ指導を月1回以上，発達チェック表による家庭指導を月1回，両親講座，家族参観，家庭訪問などを年複数回，同時に聴覚の評価を週1回以上，HAの評価を月1回以上，達成度評価，発達評価を年数回行うなど，細やかな指導がなされている。一方，概ね週1回程度の指導（親子ふれあい遊び，歌とリズム，絵本，屋外遊びなどの活動を通してコミュニケーションの実際を学ぶ，など）と適宜施行する聴力検査とHAフィッティング，家庭訪問などに留まる施設もある。

さらに難聴児の療育を担当する施設・言語聴覚士などの療育担当者の間で教育方針が大きく異なることも問題を複雑にしている。例えばCIが一般的な医療になりつつあるとはいえ，CIにかなり否定的な意見を持つ施設や療育担当者も存在し，CIの積極的な適応にあると考えられる児の家族に対しても十分な情報を与えず，十分な効果を受けられる機会が奪われていることもしばしば経験する。難聴児療育の一つの大きな目標は言語力を高めることであり，聴覚入力，視覚入力のいずれの療育方法であっても濃密な教育と症例に応じたコミュニケーションモードの判断が求められるはずである。CIでは難聴児の聴覚が健聴児と同じレベルにまで獲得されるわけではないが，補聴効果の十分でない場合にはより多くの聴覚情報が得られる可能性が高い。療育施設として長期的な療育目標の中からコミュニケーションモードの選択とそれに伴う聴覚入力手段の選択を適宜検討し，保護者に対し積極的な情報提供をすべきであるが，それが十分なされない状況がまだ残っている。

難聴発見時期・療育開始時期の影響

1）NHSの影響

新生児の聴覚障害の約半数は，極低出生体重児，重症仮死，高ビリルビン血症（交換輸血施行例），子宮内感染（風疹，サイトメガロウィルス（CMV）など），家族性，先天異常症候群などのハイリスク児であるが，残りの半数は出生時に異常を示さない児であり，通常の健診等では聴覚障害の早期発見が難しいことがある。早期に支援を開始するためには早期発見が必須であり，そのためには全新生児を対象としたNHSを行うことが重要となる。

米国では2000年に，生後入院中に最初のNHSを行って生後1か月までにはNHSの過程を終え，生後3か月までに精密診断を実施し，生後6か月までに療育を開始する（1-3-6ルール）という聴覚障害の早期発見・早期療育ガイドラインを出した[3]。これは生後6カ月までの難聴発見・聴覚補償教育開始の重要性を指摘したYoshinaga-Itanoらの研究[4]に大きな影響を受けている。本邦では平成12年度より年間5万人規模の新生児聴覚検査モデル事業が予算化され，平成13年度より岡山県など4県で開始，平成16年度までに17都道府県・政令都市で実施された。このモデル事業は平成16年度で終了となり，「新生児聴覚検査事業」は平成17年度から創設され

た「母子保健医療対策等総合支援事業」の対象事業として実施された（平成19年度からは対象事業ではなくなった）。日本産婦人科医会による平成17年度の調査では分娩取り扱い施設の約60％が新生児聴覚検査を行っている。また難聴幼児通園施設および聾学校教育相談における0～1歳児の60％以上がNHSにより発見された児であり，平成18年においては全出生児の約60％以上がNHSを受けたと推定されている。ただし県別の検査施行率には県間で大きな差が見られる（新生児聴覚スクリーニングマニュアルHP；http://www.jaog.or.jp/japanese/jigyo/JYOSEI/shinseiji_html/shi-top.html)。この事業によりNHSの重要性は広く認識されるようになり一定の普及をしてきているが，NHSの浸透はまだ十分ではなく，いまだ難聴発見が大幅に遅れる症例も散見される。最近我々が経験した，就学時まで難聴が発見されなかった特異な一例について示す。

この症例は初診時6歳8ヶ月の男児で3人兄弟の末っ子である。妊娠・分娩時に異常は無く，NHSは未施行であった。喃語様の発声はあったものの有意味語の表出はなかったにも拘らず，乳幼児健診で聴覚障害などの異常を指摘されなかった。就学時健診ではじめて言語発達遅滞を指摘され，近医総合病院耳鼻咽喉科を受診した。聴力検査では両側聾であり，ABRで両側無反応のため，当科を紹介受診した。初診時，外耳・鼓膜は正常で，遊戯聴力検査にて右耳は聾，左耳に残聴を認めた。WISC-III知能検査では動作性IQは正常域，言語性IQは測定不能であった。画像診断では内耳奇形（蝸牛は低形成で，前庭は嚢胞性）と内耳道狭窄を認めた。左耳へのHAの仮装用では利得40dB程度での装用が可能であり（図1a），フィッティングが可能と判断し，療育先を紹介した。9歳3カ月の時点では，HA装用は常用にいたっているが，聴取能は極めて不良である（図1b）。コミュニケーションモードは視覚中心で言語発達は緩慢であり，語彙はいくつかあるものの文字として入っているものはわずかで語彙検査では3歳未満に相当した。11歳時点でのコミュニケーション能力はジェスチャーとキュードスピーチでわずかに可能な程度である。本児に対しても0歳時からの早期療育を開始していれば，少なくとも視覚入力を併用して，より早期での言語獲得は可能であったと思われる。就学時まで高度難聴が見逃される事は極めて稀ではあるが，このような不幸な事例がいまだ存在する事は注意が必要である。

2）軽度・中等度難聴の影響

難聴支援や療育の開始時期については，難聴が軽度～中等度であっても高度であっても早期ほど良いと考えられる。軽度から中等度難聴児は一見聴こえも発育も悪くなく見えるために発見が遅れやすい傾向にある。しかし部分的な聴覚の感覚遮断状態にあるため，放置されると言語発達に深刻な影響が予想

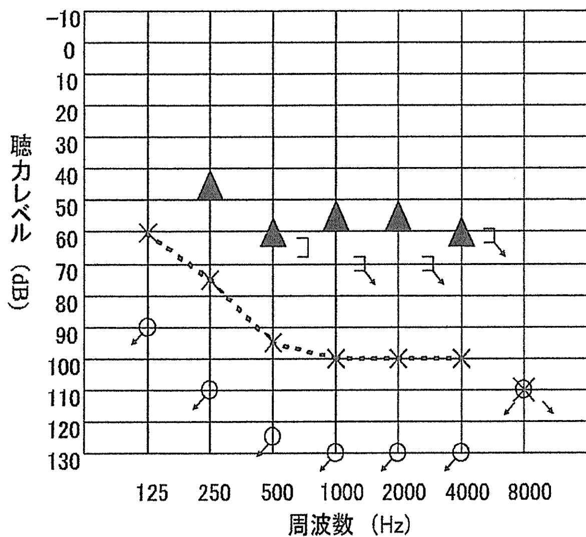

図1a　初診時オージオグラム（遊戯聴力検査）▲は補聴器装用時閾値を示す。

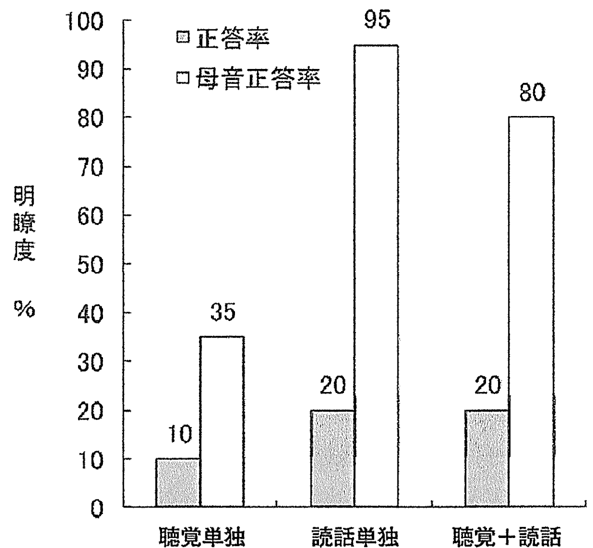

図1b　音節明瞭度（67-S語表：肉声）