Another reason why it is still so hard for clinicians to cure intractable tinnitus is that the molecular mechanism of tinnitus generation in the auditory pathway has not yet been clarified. We therefore aimed to establish such a molecular marker. The history of TRP channels in hearing and balance is characterized at great length by the hunt for the elusive transduction channel of sensory hair cells. Such pursuit has not resulted in unequivocal identification of the transduction channel, but nevertheless revealed a number of candidates, such as TRPV4, TRPN1, TRPA1, and TRPML3. Based on mutations in the corresponding mouse genes, TRPV4 (Tabuchi et al., 2005; Cuajungco et al., 2007) and TRPML3 (van Aken et al., 2008) are possible candidates for human hearing, and potentially also balance disorders. In the present study, we focused especially on TRPV1, a member of the non-specific cation ion channel receptor family, which responds to various kinds of noxious pain, such as capsaicin, inflammation, heat, low pH and hypo-osmolarity (Caterina et al., 1997; Benham et al., 2003), because it is expressed in the mouse inner ear ganglia and is upregulated by noxious challenges of kanamycin (Kitahara et al., 2005a). Interestingly, tinnitus is the sensation of a sound in the ear without an external source, similar to phantom pain (Bartels et al., 2007).

In the present study, TRPV1 mRNA levels in the SG were significantly upregulated 2 h post-treatment, significantly downregulated 12–24 h post-treatment and had returned to control levels by 72 h post-treatment. The reasons of discrepancy in these molecular results of mRNA and protein level could be explained by the time lag between mRNA and protein synthesis and/or different sensitivity in mRNA and protein experiments. According to the animal behavioral model of tinnitus, salicylate-induced tinnitus was maximal 2 h post-injection and had disappeared by 24 h post-injection. Furthermore, capsazepine, a TRPV1 antagonist, demonstrated a significant suppression of false positive response increase and of TRPV1 mRNA upregulation in the SG. Taken together, these data suggest that tinnitus in salicylate-treated animals could be caused through the activation of the nociceptive receptor, TRPV1 in the SG. Therefore, we hypothesize that tinnitus is a type of phantom pain sensation in the inner ear (Bartels et al., 2007). The mechanism of TRPV1 regulation in the SG has not been clarified yet. However, TRPV1 is auto-regulated via neurotrophic factors in damaged dorsal root ganglia (Acheson et al., 1995; Anand et al., 2006; Szallasi et al., 2006). In the present study, the blockade of salicylate-induced TRPV1 upregulation in the SG by capsazepine suggests that TRPV1 was also auto-regulated via neurotrophic factors in the salicylate-treated SG (Hansen et al., 2001; Zha et al., 2001; Shepherd et al., 2005; Kitahara et al., 2006). Known TRPV1 antagonists (capsazepine, BCTC and thio-BCTC) were also able to block the response of TRPM8 (Behrendt et al., 2004), which shares many functional and pharmacological properties with TRPV1 (Weil et al., 2005). Although TRPM8 has never been reported to be located in the inner ear, the possible role of TRPM8 in hearing and/or tinnitus should be discussed after further studies of TRPM8 in the inner ear.

The following mechanisms of salicylate-induced TRPV1 activation and tinnitus generation are suggested: TRPV1 and 5-lipoxygenase are co-expressed by SG cells in the inner ear (Balaban et al., 2003). Salicylate, an active component of aspirin, inhibits cyclo-oxygenase activity (Christie et al., 1998) and this cyclo-oxygenase inhibition leads to an excess of intracellular arachidonic acid, which is metabolized by 5-lipoxygenase pathways (Fosslien, 1998). These findings suggest that the resultant increase in arachidonic acid products, such as hydroperoxyeicosatetraenoic acid and hydroxyeicosatetraenoic acid, has the potential to depolarize SG cells by activation of TRPV1 (Hwang et al., 2000). This may either lower their threshold for spike generation or increase their sensitivity to suprathreshold activation and mimic the discharge pattern during low level natural stimulation. Actually, a couple of physiological studies of TRPV1 in the cochlea were reported. Zheng et al. revealed that activation of TRPV1 increases the threshold of the cochlear action potential, but decreases both cochlear microphonic and electrically-evoked otoacoustic emissions (Zheng et al., 2003). Zhou et al. demonstrated that perfusion with capsaicin alone produced a dose-dependent increase of the 900 Hz peak ratio (power normalized re the overall spectrum) of the ensemble background activity (Zhou et al., 2006). The capsaicin effect was attenuated during concurrent perfusion with capsazepine. These findings are consistent with the hypothesis that TRPV1 activation increases background activity of SG cells and support a role of TRPV1 in gating spontaneous and evoked auditory nerve excitability.

In contrast, the peak TRPV1 protein levels in the DCN were delayed relative to the peak levels in SG cells. This delay might indicate the mechanism for the alteration from peripheral tinnitus to central tinnitus and/or the mechanism of chronic tinnitus. There is an interesting case of a patient with intractable chronic tinnitus, who underwent temporal bone removal surgery, however, this treatment failed to cure the tinnitus (House, 1964). It is extremely difficult to identify the site of chronic tinnitus, because it may alternate between the periphery and the CNS, like phantom pain sensation. Further studies addressing the mechanisms of central tinnitus and/or chronic tinnitus are needed.

According to the morphological data, salicylate treatment caused no obvious morphological damage to cochlear hair cells or SG cells. Furthermore, from the behavioral study, the active avoidance score remained stable during the whole period of salicylate injections. Together with the ABR data, this suggests that salicylate application caused no obvious functional damage to the auditory system. However, Guitton et al. (2005) reported that administering salicylate led to transient hearing loss by means of compound action potential (CAP) threshold shifts, directional preponderance of otoacoustic emission (DPOAE) recordings and score measurements. This hearing loss was evaluated around 40 dB SPL at 16 kHz (Cazals, 2000; Guitton et al., 2005) and might not be comparable to the results in the present study, which used 60 dB SPL and 16 kHz sound stimuli.

CONCLUSION

In conclusion, we developed a rat behavioral model of salicylate-induced tinnitus and identified a molecular marker of salicylate-induced tinnitus in the rat auditory pathway. These findings could make "phantom tinnitus" clearly observable and easily accessible. We believe that these findings are important for understanding the mechanism of tinnitus generation and for elucidating de novo treatments for intractable tinnitus.

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ORIGINAL ARTICLE

Tinnitus as a prognostic factor of sudden deafness

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Abstract

Conclusions. The 'tinnitus-rare' group had a poorer prognosis for hearing than the 'tinnitus-often' group in all sudden sensorineural hearing loss (SSNHL), although the 'shorter duration' group had better prognosis than the 'longer duration' when restricted to SSNHL accompanied by tinnitus. This indicates that tinnitus itself may not be a sign for poor hearing prognosis but might be an essential sound for the initiation of repair of a damaged auditory system. *Objectives.* We examined the hearing improvement rate (HIR) and tinnitus at the onset of SSNHL to elucidate the prognostic value of tinnitus accompanying SSNHL. *Patients and methods.* Fifty patients with SSNHL were treated with systemic administration of steroids. Hearing recovery was determined by comparing the hearing levels before and after treatment. Tinnitus was subjectively evaluated by the tinnitus scoring questionnaire. The score for the five-step evaluation of the subjective tinnitus feelings 'loudness', 'duration' and 'annoyance' was obtained at the onset. *Results.* In terms of 'duration', when we divided all the cases into 'tinnitus-rare' group and 'tinnitus-often' group, HIR in the 'tinnitus-rare' group was significantly lower than that in 'tinnitus-often' group. When restricted to the 'tinnitus-often' group, HIR for 'shorter duration' was significantly higher than that for 'longer duration'.

Keywords: *Sudden deafness, vertigo, tinnitus, hearing improvement rate, prognostic factor*

Introduction

Sudden sensorineural hearing loss (SSNHL) is defined as a sensorineural hearing loss of 30 dB or worse in three consecutive speech frequencies that has occurred with sudden onset [1,2]. The incidence of SSNHL is estimated to range from 5 to 20 per 100 000 population [2]. Various kinds of causes of SSNHL have been suggested as follows: viral infection of labyrinth or cochlear nerve, vascular insult, intralabyrinthine membrane rupture and perilymphatic fistula [1].

To date, some prognostic factors for SSNHL have often been reported. Vertigo, particularly severe vertigo, has been considered as a negative prognostic factor [1–3]. Delayed start of treatments after the onset has also been considered as a negative prognostic factor [2,4]. Byl Jr also reported that age over 60 years or below 15 years has been considered a negative prognostic factor [2]. Additionally, the severity of the initial hearing level has been considered a negative prognostic factor [1,2,5,6]. As regards tinnitus, although several papers on this topic have already been published, the prognostic value has been controversial. Wilson et al. and Moskowitz et al. reported that tinnitus was a negative prognostic factor for hearing after SSNHL [7,8]. Cadoni et al. also described that hearing recovery was poorer in SSNHL patients with both tinnitus and vertigo than those with only vertigo [5]. While Byl Jr reported that tinnitus had little prognostic value [2], Danino et al. reported that tinnitus was a favourable prognostic manifestation in an analysis of symptoms and recovery rates in 60 patients with SSNHL [9]. Ben-David et al. found tinnitus to be strongly associated with hearing improvement in 67 patients with SSNHL and indicated that tinnitus was a positive prognostic factor for hearing after SSNHL [10].

In the present study, to elucidate the relationship between hearing prognosis and tinnitus at the onset

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of SSNHL, we examined changes in hearing and tinnitus of patients with SSNHL using pure-tone audiometry (PTA) and the tinnitus scoring questionnaire [11].

Patients and methods

Charts of 50 consecutive patients with the diagnosis of SSNHL accompanying vertigo (25 females and 25 males, mean 48.6 years, range 11–80 years) attending the Dizziness & Vertigo Section of the Department of Otolaryngology in three hospitals (Osaka Rosai Hospital, Osaka University Hospital and Tondabayashi Hospital) from 1998 to 2007 were reviewed in this retrospective study. Patients with Meniere's disease were carefully excluded.

All the patients underwent PTA, electronystagmography (ENG) and MRI for the purpose of excluding possible retrocochlear lesions, including demyelinating diseases, at the first visit. All the treatments started within 7 days after the onset (2.8 ± 1.7 days), including bed rest and intravenous applications of hydrocortisone sodium succinate (from 500 mg/day with dose reductions of 200 mg every 3 days to zero) and lasted for 1–2 weeks at most.

Hearing recovery was determined by comparing the audiometric results at the first visit (2.8 ± 1.7 days: pretreatment) and the last visit approximately 6 months later, when hearing function was assumed to be fixed completely (6.6 ± 1.3 months: post-treatment). The hearing improvement rate (HIR) was used as a credible parameter for hearing recovery after SSNHL [12]. Hearing gain was an absolute value of changes in averaged hearing levels of 250, 500, 1000, 2000 and 4000 Hz from pretreatment to post-treatment. HIR was defined as a result of hearing gain divided by differences between averaged initial hearing levels in the affected and unaffected ear, multiplied by 100.

Tinnitus was subjectively evaluated by the tinnitus scoring questionnaire according to the Tinnitus Research Group of Japan Audiological Society (TRGJ) in 1993 (Table I) [11]. The score of five-step evaluation from 1 to 5 in the three items of subjective tinnitus feelings – 'loudness', 'duration' and 'annoyance' – was obtained from patients at the onset of SSNHL.

For neuro-otologists, it is very important to tell patients with SSNHL their hearing prognosis at the first visit. Therefore, we examined the relationship between tinnitus score at the onset and HIR.

Statistical analysis was performed based on the Mann-Whitney test and Spearman correlation test. All reported p values were two-sided and those under 0.05 were considered to be significant.

Results

The number of patients for each item of the pretreatment tinnitus score, 'loudness', 'duration' and 'annoyance', is summarized in Figure 1.

(i) Tinnitus score pretreatment and HIR

None of three items in the pretreatment tinnitus score, 'loudness', 'duration' and 'annoyance', was significantly related to HIR according to the Spearman correlation test. As no one selected score 3 for 'duration' (Figure 1), we divided the pretreatment tinnitus scores for 'duration' into two groups. We defined scores 1 and 2 as 'tinnitus-rare' and scores 4 and 5 as 'tinnitus-often'. HIR for the 'tinnitus-rare' group ($n=8$; $16.3 \pm 34.0\%$, range -5.0 to 100.0%) was significantly lower than that for the 'tinnitus-often' group ($n=42$: $53.2 \pm 35.4\%$, range -19.0 to 119.0%) (Mann-Whitney: $U=72.0$, $p=0.011 < 0.05$) (Figure 2A). When restricted to the 'tinnitus-often' group ($n=42$), we compared the HIR for score 4, 'shorter duration' and score 5, 'longer duration'. HIR with 'shorter duration' of tinnitus ($n=9$: $99.5 \pm 9.5\%$, range 88.0–119.0%) was significantly higher than that for 'longer duration' of tinnitus ($n=33$: $40.6 \pm 29.3\%$, range -19.0 to 100.0%) (Mann-Whitney: $U=5.0$, $p=1.17\text{E-}05 < 0.001$) (Figure 2B).

(ii) Tinnitus score pretreatment and hearing level pretreatment

None of three items in the pretreatment tinnitus score was significantly related to pretreatment hearing level

Table I. Tinnitus scoring questionnaire: Tinnitus Research Group of Japan Audiological Society, 1993.

	Score				
Parameter	1	2	3	4	5
Loudness	Very quiet	Quiet	Medium	Loud	Very loud
Annoyance	Not at all	Slightly	Frequent	Always	Very much
Duration	Rare	Less often	Often	Very often	Constant

The nature and severity of tinnitus were evaluated in 5 steps from 1 to 5 in the three different categories of subjective tinnitus feelings – 'loudness', 'duration' and 'annoyance'.

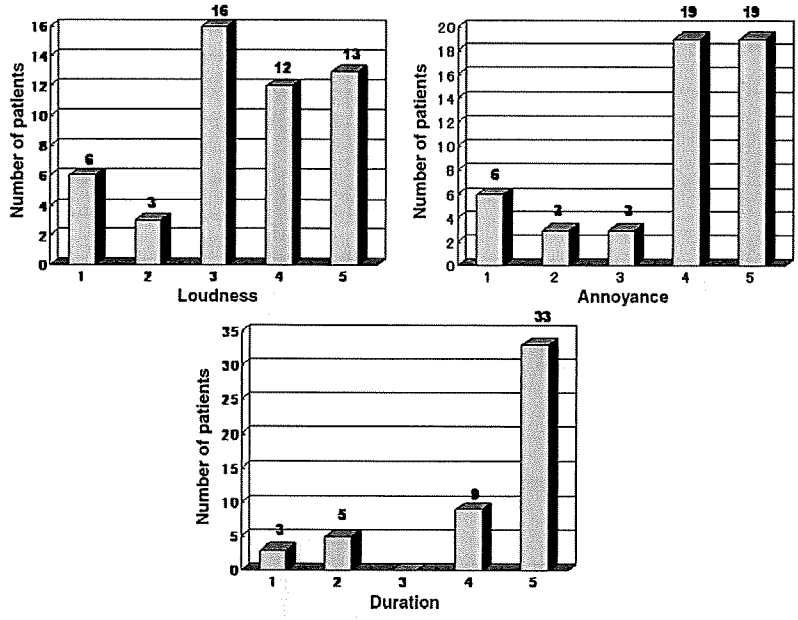

Figure 1. Results of pretreatment tinnitus score. The population in each item of the pretreatment tinnitus score, 'loudness', 'duration' and 'annoyance' was summarized.

according to the Spearman correlation test. When we divided pretreatment tinnitus scores of 'duration' into 'tinnitus-rare' and 'tinnitus-often' groups as above, the pretreatment hearing level in the 'tinnitus-rare' group ($n=8$: 86.0 ± 23.5 dB, range 49.0–115.0 dB) was not different from that in the 'tinnitus-often' group ($n=42$: 74.5 ± 24.4 dB, range 40.0–115.0 dB) (Mann-Whitney: $U=120.0$, $p=0.208$) (Figure 3A). When restricted to the 'tinnitus-often' group ($n=42$), the pretreatment hearing level for score 4, 'shorter duration' of tinnitus ($n=9$: 55.0 ± 11.1 dB, range 35.0–69.0 dB) was significantly better than that for score 5, 'longer duration' of tinnitus ($n=33$: 79.8 ± 24.4 dB, range 40.0–115.0 dB) (Mann-Whitney: $U=63.0$, $p=0.009<0.01$) (Figure 3B).

(iii) Tinnitus score pretreatment and age of patients

When we divided pretreatment tinnitus scores of 'duration' into 'rare' and 'often' groups as in (i), the age of patients in the 'tinnitus-rare' group ($n=8$: 46.4 ± 22.3 years, range 11–70 years) was not different from that in the 'tinnitus-often' group ($n=42$: 49.1 ± 16.4 years, range 15–80 years) (Mann-Whitney: $U=164.5$, $p=0.937$). When restricted to the 'tinnitus-often' group ($n=42$), the age of patients with score 4, 'shorter duration' of tinnitus ($n=9$: 37.6 ± 8.8 years, range 25–52 years) was significantly younger than that with score 5, 'longer duration' of tinnitus ($n=33$: 52.2 ± 16.7 years, range 15–80 years) (Mann-Whitney: $U=59.5$, $p=0.007<0.01$).

Discussion

According to previous studies, tinnitus was reported to accompany SSNHL in 74–87% of patients [2,6,13]. In the present study, 88% of SSNHL patients with vertigo complained of tinnitus. In Japan, tinnitus is usually evaluated by the tinnitus scoring questionnaire according to the TRGJ (1993) (Table I) [11]. This questionnaire is easy to handle but does not really provide an objective evaluation. However, among the three items of subjective tinnitus feelings, only 'duration' was significantly correlated with HIR (cf. (i) in Results). This finding suggests that 'duration' could be the most reliable item for tinnitus evaluation of patients with SSNHL. As tinnitus is a quite subjective symptom, the most reliable way of evaluation in the present study could change the status of tinnitus from a non-evaluable complaint of patients to an important prognostic factor for SSNHL.

As summarized in the Introduction, the prognostic value of tinnitus in SSNHL has been controversial until now [2,5,7–10]. In the present study, 'shorter duration' of tinnitus was a positive prognostic factor for hearing after SSNHL, as reported by Cadoni et al. [5], Wilson et al. [7] and Moskowitz et al. [8]. Our data also suggested that 'tinnitus-rare' was a negative prognostic sign for results of treatments of SSNHL, as described by Danino et al. [9] and Ben-David et al. [10]. Taking all these facts together, we assume that tinnitus may lose its prognostic value absolutely, as in the paper by Byl Jr [2], when 'shorter duration' and 'tinnitus-rare' are completely mixed up.

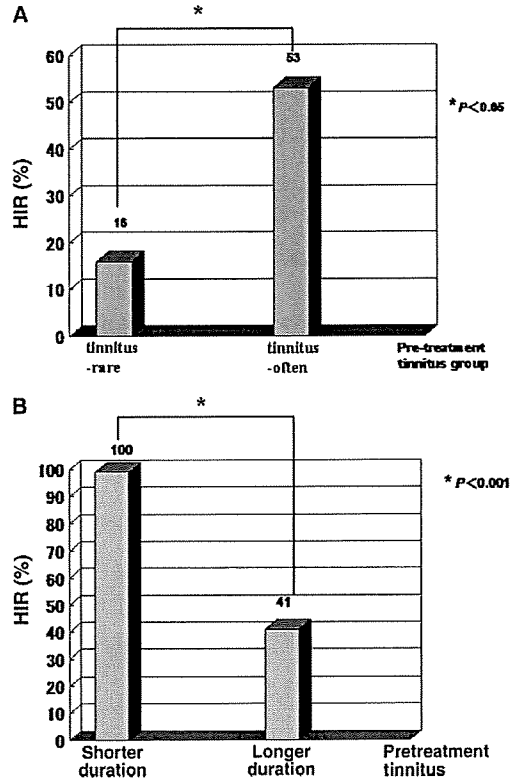

Figure 2. (A) Tinnitus score pretreatment and hearing improvement rate (HIR). The HIR for the 'tinnitus-rare' group (score 1–2) ($n=8$: 16.3±34.0%, range −5.0 to 100.0%) was significantly lower than that for the 'tinnitus-often' group (score 4–5) ($n=42$: 53.2±35.4%, range −19.0 to 119.0%) (Mann-Whitney: $U=72.0$, $p=0.011<0.05$). (B) When restricted to the 'tinnitus-often' group ($n=42$), the HIR for 'shorter duration' of tinnitus (score 4) ($n=9$: 99.5±9.5%, range 88.0–119.0%) was significantly higher than that for 'longer duration' of tinnitus (score 5) ($n=33$: 40.6±29.3%, range −19.0–100.0%) (Mann-Whitney: $U=5.0$, $p=1.17E\text{-}05<0.001$).

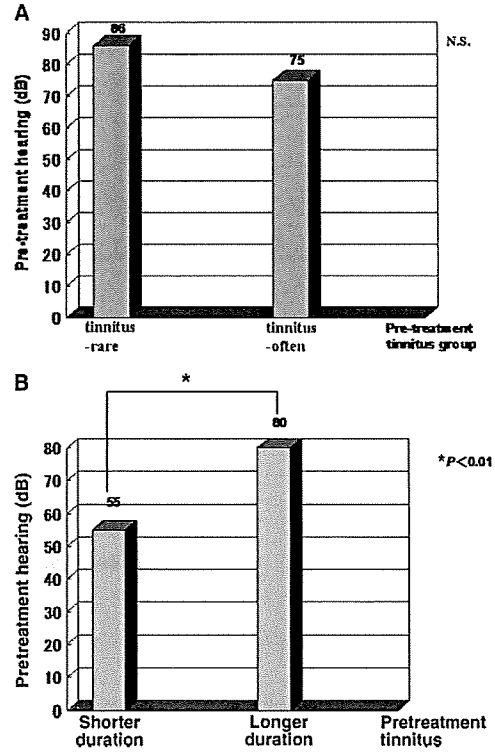

Figure 3. Tinnitus score and hearing level pretreatment. (A) The pretreatment hearing level for the 'tinnitus-rare' group (score 1–2) ($n=8$: 86.0±23.5 dB, range 49.0–115.0 dB) was not significantly different from that of the 'tinnitus-often' group (score 4–5) ($n=42$: 74.5±24.4 dB, range 40.0–115.0 dB) (Mann-Whitney: $U=120.0$, $p=0.208$). (B) When restricted to the 'tinnitus-often' group ($n=42$), the pretreatment hearing level for 'shorter duration' of tinnitus (score 4) ($n=9$: 55.0±11.1 dB, range 35.0–69.0 dB) was significantly better than that for 'longer duration' of tinnitus (score 5) ($n=33$: 79.8±24.4 dB, range 40.0–115.0 dB) (Mann-Whitney: $U=63.0$, $p=0.009<0.01$).

When tinnitus was present at the onset of SSNHL, the 'shorter duration' group had a better HIR than the 'longer duration' group (cf. (i) in Results). This may come from the fact that tinnitus duration was significantly shorter in better hearing (cf. (ii) in Results) and/or younger (cf. (iii) in Results) patients at the onset. These findings suggest that the duration of tinnitus and the amount of damage in the inner ear could have a positive relationship in patients with SSNHL accompanied by tinnitus, resulting in a negative relationship with HIR.

On the other hand, the 'tinnitus-rare' group had a poorer HIR than the 'tinnitus-often' group in all the patients with SSNHL (cf: (i) in Results). This may indicate that tinnitus itself may not be a sign for poor hearing prognosis but might be an essential sound for cell survival. Actually, the tinnitus research group of Kitahara and Balaban [14,15] demonstrated that high doses of salicylate could up-regulate a neurotrophic factor, brain-derived neurotrophic factor (BDNF), in the inner ear for cell survival and lead subsequent transcription of a nociceptive cation ion channel receptor, transient receptor potential cation channel superfamily V type 1 (TRPV1) in the inner ear for tinnitus generation. These findings suggest the hypothesis that tinnitus might be a switch-on signal for inner ear cell survival. According to this hypothesis, it could be speculated that causes and/or sites of lesion in SSNHL without tinnitus are absolutely different from those in SSNHL with tinnitus, which is one of the reasons why the prognostic value of tinnitus in SSNHL has been controversial until now [2,5,7–10].

Conclusion

In conclusion, tinnitus at the onset of SSNHL is important as a prognostic factor for hearing. 'Tinnitus-often' is a positive prognostic sign for

hearing recovery, but 'longer duration' predicts poor results in hearing improvement. Further detailed investigation of tinnitus at the time of SSNHL may elucidate mechanisms of tinnitus generation and may lead to development of effective treatments for tinnitus.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Plasma Vasopressin and V2 Receptor in the Endolymphatic Sac in Patients With Delayed Endolymphatic Hydrops

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Objective: There are some kinds of sicknesses provoked by inadequate adaptation to physical and/or psychogenic stress in daily life. Delayed endolymphatic hydrops (DEH) is an inner ear disease like Ménière's disease (MD) characterized by episodic vertigo in the setting of preexisting unilateral deafness that especially occurs in civilized people with a stressful lifestyle. Its otopathologic finding was demonstrated to be inner ear endolymphatic hydrops through a temporal bone study in 1976, as in the case with MD in 1938. To elucidate the relationship between stress and the inner ear, we examined the plasma antidiuretic stress hormone vasopressin (pAVP) and its type 2 receptor (V2R) expression in the endolymphatic sac in patients with DEH.
Study Design: A prospective molecular biological study.
Methods: Between 1998 and 2007, we enrolled 20 patients with ipsilateral DEH to examine their pAVP during remission from vertigo attacks. Plasma vasopressin was also examined in 87 patients with unilateral MD and 30 control patients with chronic otitis media. Using the real-time polymerase chain reaction method with tissue samples obtained during surgery, we examined V2R mRNA expression in the endolymphatic sac in 6 patients with ipsilateral DEH, 9 patients with unilateral MD, and 6 control patients with acoustic neuroma.
Results: Plasma vasopressin (1.5 times versus controls; unpaired t test, $p = 0.140$) and V2R mRNA expression in the endolymphatic sac (35.8 times versus controls; unpaired t test, $p = 0.002$) were higher in patients with DEH compared with those with acoustic neuroma. There were no significant differences in pAVP or V2R expression in the endolymphatic sac between DEH and MD. Patients with DEH showed a significantly negative correlation between pAVP and V2R (Pearson test, $r = -0.92$, $p = 0.009$) as in those with MD (Pearson test, $r = -0.68$, $p = 0.043$).
Conclusion: Civilized people are frequently exposed to stress in their daily life, and pAVP can easily become elevated at any time. Therefore, a negative feedback system between pAVP and V2R in the endolymphatic sac may function for inner ear fluid homeostasis against stress-induced increases in pAVP. For the pathogenesis of endolymphatic hydrops resulting in vertigo attacks in patients with DEH as well as MD, pAVP may represent a matter of consequence, but V2R overexpression in the endolymphatic sac could be much more essential as a basis for these diseases. **Key Words:** Delayed endolymphatic hydrops—Endolymphatic sac—Ménière's disease—Stress—V2 receptor.

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There are some kinds of sicknesses provoked by inadequate adaptation to physical and/or psychogenic stress in daily life. Vertigo attacks of Ménière's disease (MD) due to the inner ear abnormality represent a common example. Delayed endolymphatic hydrops (DEH) due to inner ear abnormality similar to MD is characterized by episodic vertigo in the setting of preexisting unilateral deafness and occurs in people with a stressful lifestyle (1). However, it is very difficult to prove a significant relationship between stress and inner ear abnormality because the definition of stress is too obscure for a scientific analysis of these aspects.

Since the otopathologic finding in DEH was demonstrated to be inner ear endolymphatic hydrops by a temporal bone study in 1976 (2), as in cases with MD in 1938 (3,4), it has gradually become understood that inner ear end organs, including the endolymphatic sac, regulate the fluid homeostatic system via water metabolism–related molecules such as vasopressin and aquaporin (5). Subsequently, it was proposed that the pathogenesis in DEH as well as MD could be inner ear endolymphatic hydrops due to a disorder of water metabolism–related molecules.

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In the present study, to elucidate the relationship between stress and the inner ear abnormality, we tested the hypothesis that the plasma antidiuretic stress hormone vasopressin (pAVP) and its type 2 receptor (V2R) in the endolymphatic sac will be increased in patients with DEH compared with controls.

MATERIALS AND METHODS

The use of all the human materials in the present study was approved by the Ethics Committee of Osaka University, School of Medicine (certificate no. 0424).

DIAGNOSIS AND ENROLLMENT

Patients were eligible for enrollment if they had received a clinical diagnosis of DEH or MD according to the 1995 American Academy of Otolaryngology—Head and Neck Surgery criteria (6). These criteria can be briefly described as follows: 1) Repeated attacks of vertigo: a definitive spell is spontaneous vertigo lasting at least 20 minutes. A mixed type of spontaneous nystagmus is observed during attacks. 2) Fluctuating cochlear symptoms: the hearing test usually reveals profound sensorineural hearing loss or deafness in the affected ear (ipsilateral type of DEH) or marked fluctuation of the threshold in the low and middle tone range contralateral to the affected ear (contralateral type of DEH). 3) Exclusion of other causes: to exclude other disorders, a thorough history, neurological, neurotological, and magnetic resonance imaging examinations were performed. Intractable DEH was designated in cases where various forms of medical and psychological management failed for at least 6 months. Medical management included diuretics, β-histine, diphenidol, dimenhydrinate, and diazepam, which were thought to be effective for persistent symptoms in DEH (7).

Patients designated as having intractable DEH had endolymphatic sac drainage, if there was no reason for declination of surgery. The technical details of this surgery were described before (8–10).

LABORATORY EXAMINATION FOR PLASMA VASOPRESSIN

Patients and Controls

Between 1998 and 2007, we enrolled 20 patients at Osaka University Hospital with ipsilateral type of DEH to examine their pAVP level. We also enrolled 87 patients with unilateral MD and 30 patients with chronic otitis media (OM) without any direct inner ear damage. Before collecting blood samples, we were given permission from all the patients with DEH, MD, and OM. Blood samples in all 3 groups were collected in the early morning of the day of surgery. Endolymphatic sac drainage was performed as an inner ear surgery for DEH and MD, and tympanoplasty was performed as a middle ear surgery for OM. There were no significant differences in patients' background (sex and age) among DEH (M/F = 10:10, 36.0 ± 2.5 yr), MD (M/F = 38:49, 47.2 ± 1.4 yr), and OM (M/F = 19:11, 45.4 ± 2.5 yr) except for age (patients with DEH were the youngest of all).

Patients with MD did not have any vertigo attacks and did not take any medicine for endolymphatic hydrops after hospitalization. Patients in all 3 groups took the same kind of nonrestricted meals before surgery and had no water from the morning on the day of surgery. Patients' conditions of medication, meals, and water intake at the collection of blood samples were almost the same in all 3 groups and were thought to have no influence on the pAVP level.

Procedures

The blood for a pAVP assay was transferred into an ethylenediaminetetraacetic acid tube and centrifuged at 4°C, and the separated plasma was stored at −80°C. The pAVP was determined by radioimmunoassay (arginine vasopressin radioimmunoassay kit; Mitsubishi, Tokyo, Japan). The normal pAVP level ranged from 0.3 to 4.2 pg/ml (mean, 2.25 pg/ml) based on the data acquired by blood samples collected at 8:00 to 10:00 AM from 105 healthy subjects with their informed consent (61 men, 44 women) who had no history of vestibular or cochlear disease (11).

MOLECULAR EXAMINATION FOR VASOPRESSIN RECEPTOR

Patients and Controls

Before surgery, we obtained permission for collection of the endolymphatic sac tissue during surgery from 6 of 20 patients with ipsilateral DEH and from 9 of 87 patients with unilateral MD mentioned. We also prepared 6 patients with acoustic neuroma (AN) without any direct endolymphatic sac damage as controls. Tissue samples from a part of the endolymphatic sac in groups, DEH, MD, and AN, were collected during surgery (endolymphatic sac drainage for DEH and MD groups and acoustic neuroma removal surgery for the AN group). There were no significant differences in patients' background (sex and age) among DEH (M/F = 3:3, 34.8 ± 4.2 yr), MD (M/F = 4:5, 47.9 ± 4.9 yr), and AN (M/F = 3:3, 53.0 ± 6.5 yr) except for age (patients with DEH were the youngest of all).

Tissue Preparation

For real-time polymerase chain reaction (PCR; DEH = 1–6, unilateral MDs = 1–9, ANs = 1–6) and Western blotting (DEH = 4–6, MDs = 7–9, ANs = 4–6), tissues were obtained from the endolymphatic sac during endolymphatic sac drainage for DEH and MD groups or from AN removal surgery for the AN group, placed immediately in chilled phosphate-buffered saline (PBS; pH 7.3) and frozen with dry ice powder.

Real-Time PCR

Total RNA Extraction

Total RNA was extracted from dissected frozen tissues using Trizol reagents (Gibco/BRL, Alameda, CA, USA).

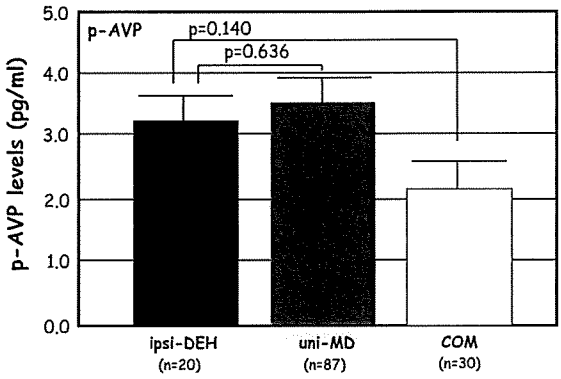

FIG. 1. Plasma vasopressin levels in patients with DEH compared with control patients. Plasma vasopressin was 1.5 times higher in patients with ipsilateral DEH (ipsi-DEH; n = 20; 3.10 ± 0.58 pg/ml) than in control chronic otitis media (COM) patients (n = 30; 2.11 ± 0.38 pg/ml) during the early morning of the day of surgery, although this difference was not statistically significant (unpaired *t* test, $p = 0.140$). There were no significant differences between the pAVP levels in patients with ipsi-DEH and unilateral MD (uni-MD; n = 87; 3.47 ± 0.35 pg/ml; unpaired *t* test, $p = 0.636$).

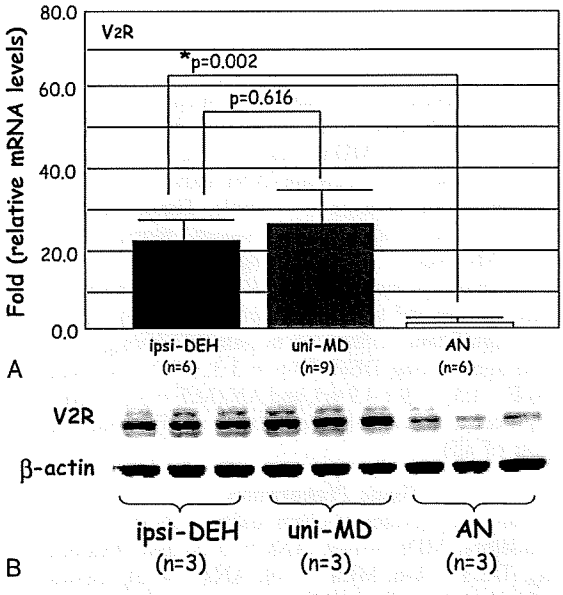

FIG. 2. V2 receptor mRNA and protein expression levels in the endolymphatic sac in patients with DEH compared with control patients. *A*, V2 receptor mRNA expression in the endolymphatic sac was 35.8 times significantly higher in patients with ipsilateral DEH (ipsi-DEH; n = 6; 22.21 ± 5.28-fold) than in control AN (AN) patients (n = 6; 0.62 ± 0.10-fold) as evaluated by real-time PCR (unpaired *t* test, $*p = 0.002$). There were no significant differences between the V2R mRNA expression levels in patients with ipsi-DEH and unilateral MD (uni-MD; n = 9; 27.86 ± 8.19-fold; unpaired *t* test, $p = 0.616$). *B*, V2R protein expression in the endolymphatic sac was also higher in patients with DEH and MD than in control AN patients as evaluated by Western blotting.

Briefly, samples were homogenized in 0.8 ml of Trizol reagent. Chloroform was then added, and the mixture was centrifuged to separate the RNA phase from the DNA phase. The RNA phase was used for RNA precipitation using isopropyl alcohol. The RNA samples were rinsed with ethanol and dissolved with RNase-free water. Finally, the RNA samples were treated with RNase-free Dnase I (Roche, Nutley, NJ, USA) to remove contaminated genomic DNAs before reverse transcription.

Reverse Transcription of RNA

The reverse transcription mixture included 10 μl of 10× PCR Taq Gold buffer II (Applied Biosystems, Inc., Foster City, CA, USA), 30 μl of 25 mmol/L $MgCl_2$, 4 μl of 25 mmol/L of each deoxynucleotide triphosphate, 5 μl of 100 μmol/L of random primers (Gibco/BRL), 2 μl of RNasin (Applied Biosystems), 1.25 μl of Super-Script II (Applied Biosystems), and 5 μl (250 ng) of DNA-free total RNA in a final volume of 100 μl. The mixture was incubated at 25°C for 10 minutes, 48°C for 30 minutes, and 95°C for 5 minutes in a 9600 Thermocycler (Applied Biosystems).

Reverse Transcription–PCR

Samples with reverse transcriptase were forwarded for PCR (95°C for 12 min and, 35 cycles at 95°C for 15 s, and 60°C for 1 min) and electrophoresed on 1.5% agarose gel to check the results of reverse transcription–PCR. Samples without reverse transcription were also forwarded for PCR as negative controls to make sure of no genomic DNA contamination.

TABLE 1. *Raw data for 6 patients with DEH (A) and 9 patients with MD (B)*

	pAVP, pg/ml	V2R mRNA, fold	V freq, per mo	H level, dB	Duration, mo
(A)					
Ipsi-DEH 1	1.0	40.74	2.0	115.0	36
Ipsi-DEH 2	1.4	25.11	1.0	100.0	60
Ipsi-DEH 3	1.7	28.54	1.3	101.3	18
Ipsi-DEH 4	4.7	18.78	4.3	112.5	45
Ipsi-DEH 5	6.9	18.33	4.0	115.0	30
Ipsi-DEH 6	11.6	1.76	1.0	115.0	60
(B)					
Uni-MD 1	0.5	64.78	1.0	30.0	34
Uni-MD 2	0.8	69.28	1.0	45.0	60
Uni-MD 3	1.3	13.72	1.3	66.3	84
Uni-MD 4	2.0	20.18	3.3	60.8	98
Uni-MD 5	2.7	1.90	1.7	70.0	48
Uni-MD 6	2.7	32.38	7.3	57.5	18
Uni-MD 7	3.5	29.52	8.0	66.5	30
Uni-MD 8	4.2	17.32	4.0	60.0	48
Uni-MD 9	6.0	1.70	2.0	58.5	60

The raw data for 6 patients with ipsilateral DEH (ipsi-DEH) and 9 patients with unilateral MD (uni-MD) include the pAVP level, V2R mRNA expression level in the endolymphatic sac, vertigo frequency (V freq), hearing level (H level), and duration of disease (Duration) before surgery. In both ipsi-DEH (*Pearson test, $r = -0.92$, $p = 0.009$) and uni-MD patients (**Pearson test, $r = -0.68$, $p = 0.043$), there were significantly negative correlations between pAVP and V2R mRNA expression in the endolymphatic sac.

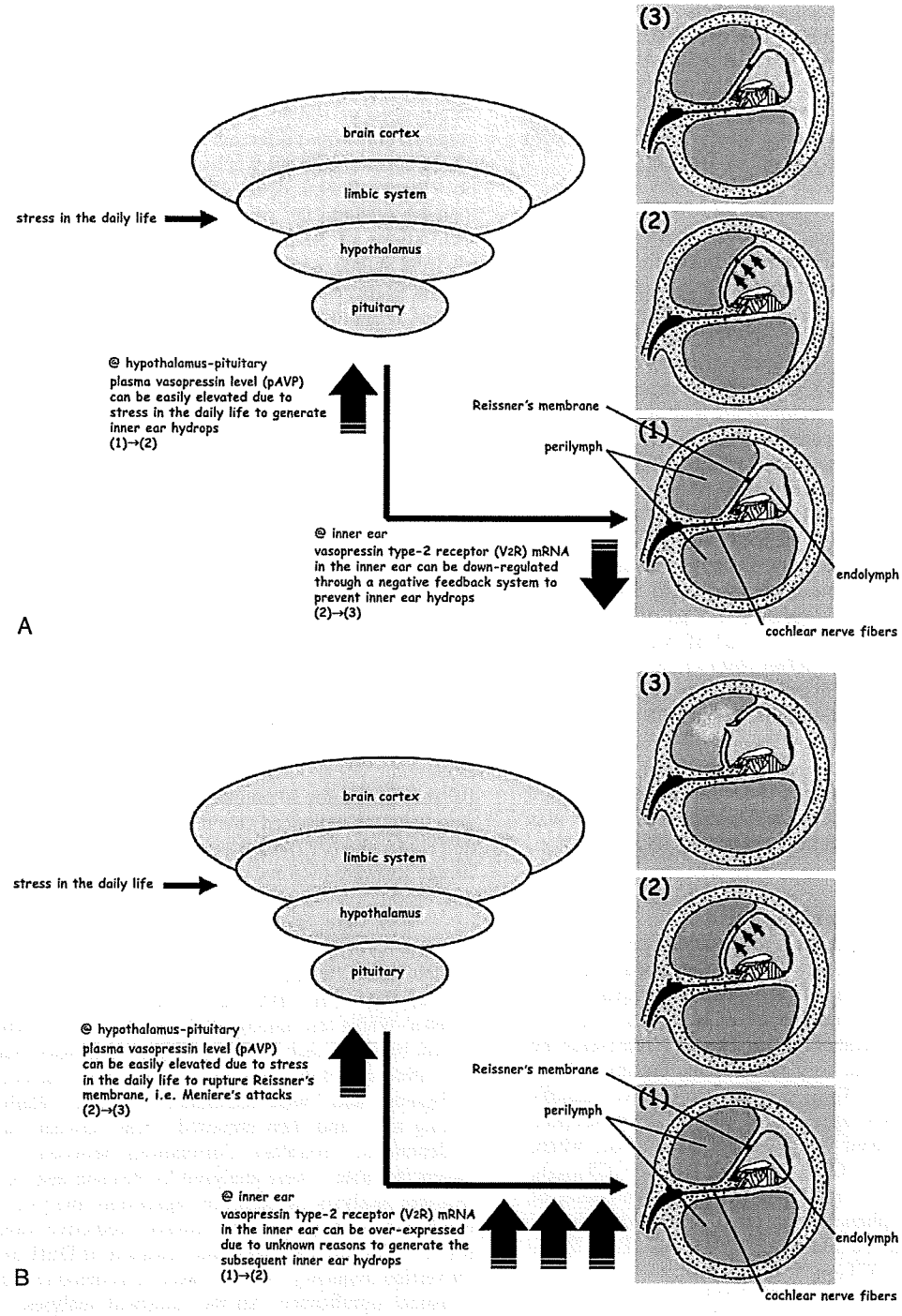

FIG. 3. Schematic representation of a hypothetical mechanism for endolymphatic hydrops generation and subsequent attacks of DEH and MD via pAVP and V2R. *A*, Healthy subjects: civilized people are frequently exposed to stress in their daily life, and pAVP can easily become elevated at any time (1)→(2). Therefore, a negative feedback system between pAVP and V2R in the inner ear may function for inner ear fluid homeostasis against stress-induced increases in pAVP (2)→(3). *B*, Patients with MD: autoimmune responses and/or virus infections in early childhood can lead V2R overexpression and hypersensitivity of cyclic AMP–linked signaling in the inner ear (1). After years, endolymphatic hydrops can gradually be generated (2). In the V2R-overexpressing and cyclic AMP–hypersensitized inner ear, the Reissner membrane can rupture after even a small elevation of pAVP due to stress, thereby resulting in attacks of MD (3). Upward arrows indicate upregulation; downward arrows, downregulation; number of arrows, strength of regulation.

Polymerase chain reaction products were electrophoresed on 3% Seakem GTG agarose gel (FMC Bioproducts, Rockland, ME, USA) and purified using QIA quick Gel Extraction Kit (Qiagen, Valencia, CA, USA). Sequencing was accomplished by means of ABI Prism dRhodamine Terminator Cycle Sequencing Ready Reaction Kit with ABI 310 DNA sequencer (Applied Biosystems).

Real-Time Quantitative PCR

Polymerase chain reactions were performed in the presence of the oligonucleotide primers for V2R (NM: 000054; forward: actgtgaggatgacgctagtgattg; reverse: ggacacgctgctgctgaaag) (Takara, Kyoto, Japan) and β_2-microglobulin (*B2M*) (NM: 004048; forward: cgggcatt cctgaagctga; reverse: ggatggatgaaacccagacacatag) (Takara) and quantified by SYBR Green PCR reagents (Applied Biosystems). *B2M*, an endogenous housekeeping gene, was used as an internal control for this method. Each sample determination was performed in triplicate.

The PCR mixture included 5 μl of 10× SYBR PCR buffer, 6 μl of 25 mmol/L $MgCl_2$, 4 μl of each deoxynucleotide triphosphate (blended with 2.5 mmol/L of deoxyadenosine triphosphate, deoxyguanosine triphosphate, and deoxycytidine triphosphate and 5 mmol/L of 2′-deoxyuridine 5′-triphosphate), 2.5 μl of each gene-specific primer (5 μmol/L), 0.5 μl of AmpErase UNG (0.5 U), 0.25 μl of AmpliTaq Gold (1.25 U), and 5 μl (250 ng) of complementary DNA in a final volume of 50 μl. The conditions for the real-time PCR were as follows: 50°C for 2 minutes, 95°C for 12 minutes, and 35 cycles at 95°C for 15 seconds and 60°C for 1 minute in ABI PRISM 7700 Sequence Detection System (Applied Biosystems). 7700 Sequence Detection software was used for instrument control, automated data collection, and data analysis.

Data Analysis

The number of PCR cycles was recorded until the fluorescence intensity exceeded the predetermined threshold. The quantification of the initial amounts of template molecules relied on this number of PCR cycles, which is termed the cycle threshold (CT). The dCT represents the CT of the target gene normalized to the human endogenous *B2M* ($dCT = CT_{target} - CT_{B2M}$). Relative quantification of the mRNA expression levels of target genes (fold range) was calculated using the 2^{-ddCT} method, where $ddCT = (CT_{target} - CT_{B2M})_A - (CT_{target} - CT_{B2M})_B$ (12). For example, changes in the gene expression of V2R in endolymphatic sac in DEH compared with AN were quantified as the fold range: 2^{-ddCT} ($ddCT = [CT_{V2R} - CT_{B2M}]_{DEH} - [CT_{V2R} - CT_{B2M}]_{AN}$).

Western Blotting

Samples from the endolymphatic sac were homogenized on ice with a polytron homogenizer (PCU-11; Kinematica, Bohemia, NY, USA) in 20 mmol/L HEPES (pH 7.2), 25 mmol/L NaCl, 2 mmol/L EGTA, 50 mmol/L NaF, 1 mmol/L Na_3VO_4, 25 mmol/L β-glycerophosphate, 0.2 mmol/L dithiothreitol, 1 mmol/L phenylmethanesulfonyl fluoride, 60 μg/ml aprotinin, 2 μg/ml leupeptin, and 0.1% Triton X-100). After incubating at 4°C for 30 minutes, homogenates were sonicated (Sonifier 250; Branson Ultrasonics, Danbury, CT, USA) on ice for 1 minute and centrifuged at 10,000 × *g* at 4°C for 30 minutes. The supernatant was collected. Protein concentrations of these supernatants were measured with a protein assay kit (Pierce, Rockford, IL, USA). Gel samples were prepared by adding the sample buffer containing final concentrations of 50 mmol/L Tris (pH 6.7), 2% sodium dodecyl sulfate, and 2% mercaptoethanol. Twenty micrograms of protein extracts was boiled for 10 minutes, cooled to room temperature, and loaded on 10% sodium dodecyl sulfate–polyacrylamide gels. Equal amounts of protein in each sample were further checked by immunoblotting with β-actin monoclonal antibody (diluted to 1:500; Oncogene Research Products, Cambridge, MA, USA).

Proteins were transferred to Hybond–polyvinylidene difluoride membranes (Amersham, Piscataway, NJ, USA) by using standard electroblotting procedures. Membranes were incubated sequentially in the following solutions at 4°C: 2% nonfat dry milk, 1% bovine serum albumin (BSA) and normal goat serum (NGS) in 0.3% Triton X-100 in PBS for 3 hours; antisera against V2R (diluted to 1:500; sc-18100-R; Santa Cruz Biotechnology, Santa Cruz, CA, USA) in 1% BSA and NGS in 0.3% Triton X-100 in PBS for 24 hours; 0.1 mol/L PBS for 30 minutes; horseradish peroxidase–conjugated secondary antibody (Dako, Carpinteria, CA, USA) in 1% BSA and NGS in 0.3% Triton X-100 in PBS for 3 hours; 0.1 mol/L PBS for 30 minutes. Protein bands were visualized using an enhanced chemiluminescence detection kit and Hyperfilm MP (Amersham) and analyzed using Scion Image software (Scion Corp., Frederick, MD, USA).

STATISTICAL ANALYSIS

Statistical differences of patients' backgrounds (sex and age) among DEH, MD, and controls were examined by Kruskal-Wallis test among all 3 and then Mann-Whitney *U* test between each 2 (independent variables). Statistical differences of the data among DEH, MD, and controls in Figures 1 and 2 were determined by Kruskal-Wallis test among all 3 and then unpaired *t* test between each 2 (independent variables). Correlations between 2 parameters in Table 1 were analyzed by Pearson test. A multivariate analysis (a multiple regression analysis) was conducted to determine the relative predictive value of pAVP, V2R mRNA levels, and duration of DEH or MD for vertigo frequency. $p < 0.05$ were considered to indicate statistical significance. All the statistical analyses in the present study were performed using SPSS version 14.0 (SPSS, Inc., Chicago, IL, USA).

RESULTS

The raw data for 6 patients with ipsilateral DEH and 9 patients with unilateral MD, including their pAVP level,

V2R mRNA expression level in the endolymphatic sac, vertigo frequency (number of attacks per month), hearing level (four-tone average of 0.25, 0.5, 1, and 2 kHz) and duration of disease before surgery, are shown in Table 1.

The pAVP level was 1.5 times higher in patients with DEH (n = 20; 3.10 ± 0.58 pg/ml) than in control OM patients (n = 30; 2.11 ± 0.38 pg/ml), although this difference was not statistically significant (unpaired *t* test, $p = 0.140$; Fig. 1). There were no significant differences between the pAVP levels in patients with DEH and MD (n = 87; 3.47 ± 0.35 pg/ml; unpaired *t* test, $p = 0.636$).

The V2R mRNA expression level in the endolymphatic sac was 35.8 times higher in DEH patients (n = 6; 22.21 ± 0.58-fold) than in control AN patients (n = 6; 0.62 ± 0.10-fold), and this difference was statistically significant (unpaired *t* test, $p = 0.002$; Fig. 2A). These results were confirmed at the protein expression level by Western blotting (Fig. 2B). There were no significant differences between the V2R mRNA expression levels in patients with DEH and MD (n = 9; 27.86 ± 8.19-fold; unpaired *t* test, $p = 0.616$).

In both groups of patients with DEH (Pearson test, $r = -0.92$, $p = 0.009$) and MD (Pearson test, $r = -0.68$, $p = 0.043$), there were significantly negative correlations between pAVP and V2R mRNA expression in the endolymphatic sac (Table 1). A multivariate analysis (a multiple regression analysis) showed no significant predictive value of pAVP (DEH, $p = 0.87$; MD, $p = 0.78$), V2R mRNA levels (DEH, $p = 0.97$; MD, $p = 0.81$), or duration of DEH or MD (DEH, $p = 0.69$; MD, $p = 0.26$) for vertigo frequency. However, both pAVP (>2.5 pg/ml) and V2R mRNA expression in the endolymphatic sac (>10.00-fold) were relatively higher in Patients 4 and 5 with DEH and in Patients 6 to 8 with MD who experienced relatively more frequent vertigo attacks (≥4.0 attacks/mo).

DISCUSSION

For more than 10 years, it has been pointed out that the pAVP levels in patients with endolymphatic hydrops, including MD and DEH, during remission (13) as well as attacks (11), were significantly higher than those in patients with vertigo because of nonendolymphatic hydrops such as benign paroxysmal positional vertigo and vestibular neuronitis. It was also revealed that systemic injection of vasopressin induced bilateral endolymphatic hydrops and hearing deterioration in guinea pigs (14). These findings led us to the hypothesis that a high level of pAVP is one of the causes of inner ear endolymphatic hydrops in patients with MD and DEH. In contrast, however, it was reported that the pAVP levels in patients with unilateral MD did not differ significantly from those in healthy volunteers (15). Furthermore, the hypothesis of a high level of pAVP, which should have equal effects on both ears, contradicts the fact that 70% to 80% of patients with MD are unilateral (16). In fact, there were no significant differences between the pAVP levels in patients with unilateral and bilateral MD in our recent study (17). Therefore, the above hypothesis is insufficient for explaining the pathogenesis of endolymphatic hydrops, which has remained unsolved since endolymphatic hydrops was demonstrated in patients with MD in 1938 (3,4) and in patients with DEH in 1976 (2).

Regarding vasopressin receptors, V2R molecules have been detected in rat (18,19) and human (18,20) inner ear end organs. V2R was clearly distributed together with a V2R-linked water channel molecule, aquaporin 2, in the luminal epithelium of the human endolymphatic sac (20). Interestingly, the physiological interactions between pAVP and V2R in the endolymphatic sac attenuated the membranous turnover via cyclic AMP–dependent signaling in a contrasting manner with the kidney in rats (18), and then these interactions also translocated aquaporin 2 from the luminal side to the basolateral side in a contrasting manner with the kidney in human (our unpublished observations). These previous findings indicate that V2R and cyclic AMP–linked signaling could suppress the endolymphatic fluid absorption in the endolymphatic sac.

In the present study, we first detected 35.8-fold higher V2R mRNA expression in the endolymphatic sac in patients with ipsilateral DEH compared with controls, whereas the pAVP level was only elevated by 1.5 times. There were no significant differences in the pAVP or V2R expression in the endolymphatic sac between ipsilateral DEH and unilateral MD. These findings in the present study suggest that V2R overexpression in the endolymphatic sac in ipsilateral DEH as well as unilateral MD could attenuate the membrane turnover and cause the endolymphatic fluid overflow into the endolymphatic space after even a small increase in pAVP. In other words, patients with unilateral V2R overexpression in the endolymphatic sac could develop unilateral endolymphatic hydrops, resulting in attacks of vertigo in the overexpressing ear after even a small elevation in pAVP due to stress in their daily life.

Second, we detected a significantly negative correlation between pAVP and V2R expression in the endolymphatic sac in DEH as well as MD, consistent with the previous study on intact rats (19). These findings suggest that a negative feedback system between pAVP and its receptor in the endolymphatic sac could function for inner ear fluid homeostasis against stress-induced increases in pAVP (Fig. 3A). A negative feedback system between the hypothalamus-pituitary and inner ear has not been clarified yet. However, the direct neuroanatomic interactions between the hypothalamus-pituitary and inner ear were demonstrated electrophysiologically (21,22) and morphologically (23). Inner ear volume and/or pressure changes modified the pAVP secretion (24–26), which indicates that the negative feedback system is really working. Anyway, this negative correlation may explain why the previous studies of pAVP in MD and DEH produced a large variety of results and have been quite controversial (10, 11,13,15).

Finally, we would like to speculate about the possible causes for attacks associated with inner ear abnormality in

MD and DEH. It has been reported that MD is usually triggered by immune, infectious, traumatic, or other insults to the inner ear in association with a small misplaced malfunctioning endolymphatic sac (27–29). Among these insults, immune-mediated responses in the inner ear end organs, such as the endolymphatic sac, stria vascularis, and spiral ligament, are thought to be the main bases for the fluid homeostatic disorder in MD (30,31). Certain virus infections, such as varicella-zoster, Epstein-Barr, and adenovirus infections, of the endolymphatic sac in early childhood represent other bases for the dysfunction of endolymph absorption (32,33). Taken together with the present data, it is suggested that autoimmune responses and/or virus infections could modify the V2R regulatory system in the endolymphatic sac, resulting in V2R overexpression there (Fig. 3B-1). Years after such insults to the inner ear, hardworking people tend to become frequently exposed to stress in their daily life (1). In the V2R-overexpressing inner ear, endolymphatic hydrops could gradually be generated (Fig. 3B-2), and the Reissner membrane could rupture (2) after even a small elevation in pAVP due to stress, thereby resulting in attacks of MD and DEH (Fig. 3B-3).

CONCLUSION

Civilized people are frequently exposed to stress in their daily life, and pAVP can easily become elevated at any time. Therefore, a negative feedback system between pAVP and its receptor, V2R, in the endolymphatic sac may function for inner ear fluid homeostasis against stress-induced increases in pAVP. For the pathogenesis of endolymphatic hydrops resulting in vertigo attacks in MD and DEH, pAVP may represent a matter of consequence, but V2R overexpression in the endolymphatic sac could be much more essential as a basis for these diseases.

The results of the present study encourage us to continue our investigations and ascertain ideal treatments for the inner ear in patients with DEH as well as MD, ranging from psychotherapy for leading a stressless life to gene therapy for the stress hormone receptor V2R regulation in the inner ear.

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JOHNS
特集●メニエール病を理解する●

メニエール病に対する薬物療法
—内服・点滴，高浸透圧脱水療法と水分摂取療法—

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● Key Words ●メニエール病，水分摂取療法，Vasopressin●

はじめに

メニエール病に対して行う治療として急性期（めまい発作時）の治療と発作間欠期の治療があり，前者の目的はめまい発作の軽減と嘔気，嘔吐などの自律神経症状の軽減であり，後者のそれは発作再発の予防，聴覚・前庭機能の改善，維持，メニエール病の進行を防ぐことである。

Ⅰ．急性期の治療

めまい発作には，患者を安静にさせ，鎮暈薬，鎮嘔薬を使用し，点滴補液をして嘔吐による電解質バランスの補正と脱水を防ぐことに重点を置く。一般に嘔吐のために経口摂取は困難であるので，薬剤の投与は非経口で行う。7%炭酸水素ナトリウム（メイロン®），10%グリセリン液（グリセオール®）の静注や点滴静注を行う。

鎮静薬としては，ジアゼパム（セルシン®，ホリゾン®），フェノバルビタール（フェノバール®）など，鎮暈薬としてサリチル酸ジフェンヒドラミン，ジプロフィリン（トラベルミン®），塩酸ジフェニドール（セファドール®），ジメンヒドリナート（ドラマミン®），テオクル酸プロメタジン（アボミン®）など，鎮吐薬としては，メトクロプラミド（プリンペラン®），マレイン酸ブロクロルペラジン（ノバミン®）などがそれぞれ用いられる。

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Ⅱ．発作間欠期の治療

1．内耳循環の改善

内耳血流を増加させる目的で，アデノシン三リン酸ナトリウム（アデホス®，トリノシン®），カリジノケナーゼ（カリクレイン®），dl-イソプレナリン塩酸塩（イソメニール®），メシル酸ベタヒスチン（メリスロン®），塩酸ジフェニドール（セファドール®）などが用いられる。また7%炭酸水素ナトリウム（メイロン®）も脳循環改善に伴う内耳循環が期待される。

2．ステロイド薬

聴力の改善を目的に，突発性難聴に準じてステロイド薬が使用される。また，メニエール病も自己免疫疾患の可能性が指摘され，その意味においてもステロイド薬の使用も好まれる。

3．利尿薬

内リンパ水腫の改善の目的で種々の利尿薬が使用される。その例としてイソソルビド（イソバイド®），フロセミド（ラシックス®），尿素，アセタゾラミド（ダイアモックス®），クロルタリドン（ハイグロトン®），トリクロルメチアジド（フルイトラン®）などである。

Ⅲ．高浸透圧脱水療法[1]と水分摂取療法（Hydration Therapy）[2]

近年の分子生物学的研究により水チャネル（Aquaporin：AQP）が同定された。そのうち内耳には AQP1，AQP2，AQP3，AQP4，AQP5，AQP6，AQP7，AQP9 などの存在が報告され，内

リンパ，外リンパ液量およびイオン組成の恒常性に重要な役割を果たしている可能性が示唆されている。特に，Arg-Vasopressin（AVP）調節性のAQP2が内リンパ水腫の形成に重要な役割を演じているという報告がなされている。

AQP2活性（水透過性）は，AVPによりV2-receptorを介して制御されている。血漿AVPの上昇により，内耳膜迷路に存在するV2-receptorにAVPが結合すると，Gsタンパクを介してadenylate cyclaseが活性化され，細胞内cAMPが増加する。このcAMPが主なトリガーとなってAQP2を含む細胞内小胞が細胞膜へ移動し癒合する。その結果，膜迷路を形成する細胞の水透過性が上昇し，内リンパ水腫の形成を促進する可能性が示唆された。

Takedaらのグループは，モルモットを用いた動物実験で腹腔内に大量のAVPを投与することにより組織学的に内リンパ水腫が発生することを報告した[3,4]。一方，われわれはラットを用いた動物実験において，AVPを腹腔内に投与することにより，ABR閾値上昇（聴覚の低下）を認めた。さらに，24時間水分制限ラットでは，等量のAVP腹腔内投与において，通常の水分摂取制限を行わないでAVP腹腔内投与したラットに比して早期にABR閾値が上昇した。

さらに，実際の臨床例においてTakedaらのグループは，メニエール病をはじめとする内リンパ水腫関連疾患では，他のめまい疾患に比して血漿AVP値が上昇したと報告している[5]。

以上のことから，内リンパ水腫の発生，メニエール病の発症に血漿AVPの増加が関連していると推測される。

メニエール病の発作間欠期の治療は，めまい，不安感に対する対症療法は別として，内リンパ水腫の軽減とその再発の予防が目的とされる。つまり水，塩分の摂取制限，浸透圧利尿薬（isosorbideなど）の投与，高浸透圧脱水の状態を維持することにより，内リンパ水腫を軽減し，その発作の再発を予防しようとするものであった（高浸透圧脱水療法）[1]。

血漿AVPの分泌刺激として血漿浸透圧の上昇以外に，出血などによる体液量の低下や精神的・肉体的ストレスの増加などがある。高浸透圧脱水の状態を形成する目的で，現在においても水分摂取を制限する生活指導がなされている場合がある。換言すれば水分摂取の制限などによる高浸透圧脱水と循環血漿量の低下の状態は脳下垂体後葉からAVPの分泌を増加させ，長期的には内耳に内リンパ水腫を誘導してしまう可能性が示唆される。それゆえ，これまでの治療法の基本方針に従えば，一時的には血漿から内リンパへの溶液の移動の低減はあるが，長期的には水分摂取制限・浸透圧利尿薬→循環血漿量の低下・高浸透圧脱水→脳下垂体からのAVP分泌→内耳（血管条）でのAQP2の活性化（水透過性の増加）→内リンパへの水の流入→内リンパ水腫が引き起こされる結果となった。血管条での水透過性亢進という過剰反応としての機能的変化が反復することで，次第に同組織の器質的変化が蓄積するものと推測される。

動物実験において，われわれは比較的少量のAVPの投与で血管条中間細胞に細胞内浮腫と考えられる所見を認めたことを報告した[6]。この変化は初期には可逆的な変化であるが，反復することにより非可逆的な変化になりかねない。また脱水による循環障害も加わることにより蝸牛内リンパ電位（EP）は低下し，その結果内耳有毛細胞の機能・器質的障害に発展し聴力の長期予後は不良であったと推測される。

そこで，われわれはメニエール病に対する治療法として内耳循環の改善と血漿浸透圧を適切に，むしろ低値に保つことを目的として水分の十分な摂取が重要ではないかと仮説を立てた。つまり，水分を十分に摂取することは，循環血漿量を増加させる刺激であり，また血漿浸透圧を低値（正常域内）に維持することに役立つ。その結果，脳下垂体後葉からのAVPの分泌を抑制し，結果的に内リンパ水腫を軽減し，めまいや聴力低下の発症を予防できるのではないかと考えた。水分摂取療法は，これまでのメニエール病に対する治療法の基本方針とはまったく逆の治療方針に従う内リンパ水腫関連疾患に対する治療法である[2]。

本治療法の基本方針は，十分な水分摂取・浸透圧利尿薬の短期投与→循環血漿量を増加させる，血漿浸透圧を正常域下限に保つ→下垂体からの

AVP分泌の最小化→内耳（血管条）でのAQP2の活性の適正化，血管条への循環の改善→内リンパへの水の流入の減少，血管条の機能改善→めまい発作の予防，聴力の改善を行うメカニズムに基づくものである。

本治療法は，患者個別に循環器系および腎臓系の異常がないことを確認した後，体重，年齢，生活習慣を考慮し，1日に必要な水分摂取指導を行うものである。患者の1日に摂取する水分量は，治療期間を通じて35 ml/Kg/dayに維持される。

めまい発作，聴力低下を主訴とするメニエール病の疑いの強い患者に対し，初期から水分摂取治療を開始する。この際，短期[7]のisorbide投与を行うが，長期的には水分摂取とめまい発作や蝸牛症状（聴力低下，耳閉塞感など）の出現時に短期的な浸透圧利尿薬の投与を行う。

水分摂取療法[2]の目的は，血漿AVP濃度を下げること以外に循環血漿量の増加に伴う内耳特に血管条の循環改善にある。そのため検査上血漿AVP値が正常域にあるものもに対しても同様の治療を行う。

また，本療法はV2-receptorの機能適正化に関する効果も期待される。動物実験における外因性のAVPの投与が，V2-receptorの活性を低下させるという報告[8]がある。しかし脱水負荷を与えた場合は内因性のAVPの分泌を増加させるが，この状態でもV2-receptorの活性はおそらく低下せず，むしろ上昇しているものとわれわれは推測する。脱水環境が長く続き，慢性的になった状態では，次第にAVPの分泌は低下し血漿AVP値は低下するが，V2-receptorの活性は上昇したまま継続しているのではないだろうか。慢性的な，また反復するストレス負荷の場合も脱水負荷と同様に血漿AVP値は上昇は次第落ち着くが，V2-receptorの活性は上昇した状態で継続するのではないだろうか。その後新たな大きなストレスが加わったときに再度AVPの分泌が増加し，内リンパ水腫を発生させ発作に至るものと考えられる[9]。水分摂取療法における十分な水分摂取は，この上昇したV2-レセプターの活性を徐々に正常化させているのではないかと推測する。

実際の治療方法とその結果の概略を以下に示す。

患者個別に循環器系および腎臓系の異常がないことを確認した後，35 ml/kg/dayの飲水を開始し，治療中（2年以上）は同摂取量を維持するため患者に繰り返し指導する。また蝸牛症状（聴力低下，耳鳴，耳閉塞感），回転性めまい症状の出現時にイソソルビド（90 ml/day）を短期間（3日間）投与する[7]。ただし，メニエール病発作には，精神的・肉体的ストレスは大きく関与しているものと考えられるため，Takahashiら[10]の提唱している，生活習慣の改善法を指導している。本治療法を行うにあたって，既存のメニエール病の治療方法とその予後，また水分摂取療法を行う理論的背景と予想される病態メカニズムを患者に十分説明し，同意を得ている。

治療結果の検討は，AAO-HNS（1995）の基準を用いて行った。対象は当科めまい外来で治療中のメニエール病確実例で，水分摂取療法を開始後，2年以上経過観察を行えた症例である。

めまいに関しては，約90%で消失し，残りの約10%についても，めまい発作回数は減少している。聴力の長期予後に関してもこれまでの治療法に対して有意に改善していることを報告した[11]。

水分摂取療法中の患者血漿AVP値，血漿浸透圧，尿比重，尿浸透圧の変化を図に示す。血漿AVP値，尿比重，尿浸透圧はhydrationに伴い低下していくが，血漿浸透圧の変化はない。このことは血漿浸透圧を生体内で恒常的に保つために，他の因子が大きく変動していることになる。血漿浸透圧を低下させる方向の負荷が，結果的に血漿AVP値を低下させていることになる。

また，水分摂取療法を行う前に，他院にてイソソルビドを長期間投与されていた症例では，本治療開始後，約3カ月間は症状が安定しない場合もあったが，それ以後めまいは安定し，聴力は改善する場合が多かった。

まとめ

メニエール病の治療においてはまず保存的治療を行うが，一般に使用される薬剤の例をあげた。ただし，これまでいかなる薬物治療においても，長期の聴力予後は不良であった[12]。しかし近年の分子生物学的研究による水チャネルの発見と内耳

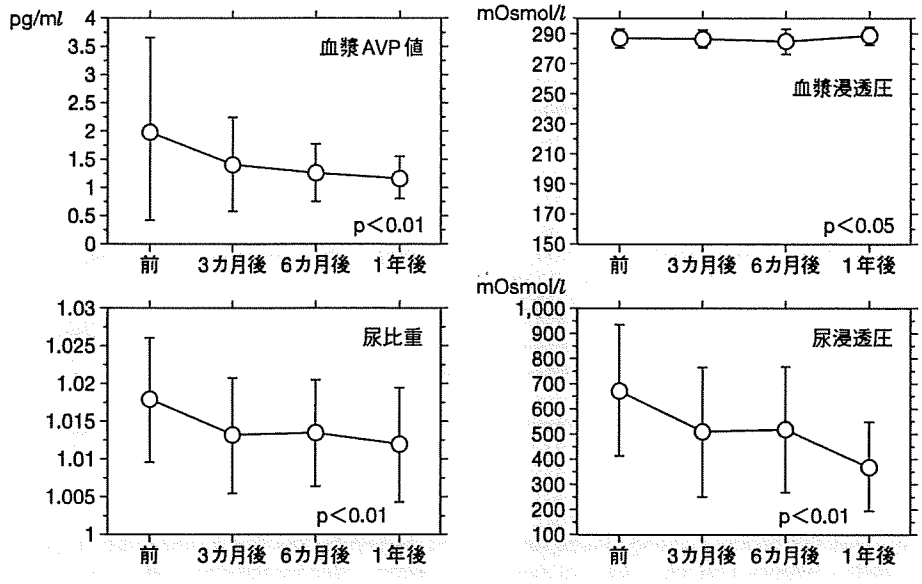

図　Hydration後の変化

での同定に基づくメニエール病の新たな発生機序の検討が，新規の治療法の開発に結びつき始めており，今後の展開が期待される。

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