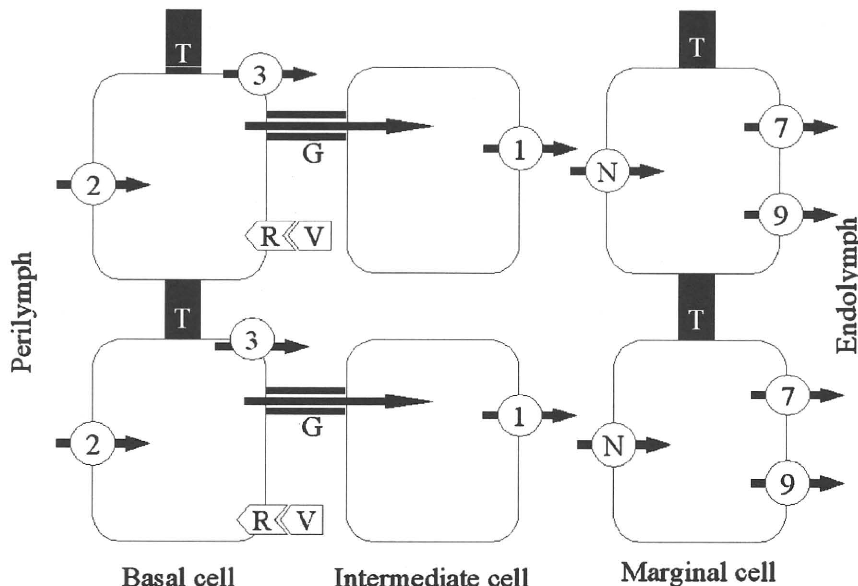

Figure 6. A model diagram of the water transport system in the stria vascularis from the perilymph to the endolymph. 1, AQP1; 2, AQP2; 3, AQP3; 7, AQP7; 9, AQP9; N, NKCC; R, vasopressin type 2 receptor; V, vasopressin; T, tight junction; G, gap junction.

present study VP was administered subcutaneously. It takes a short time for the blood concentration of VP to increase, which may be the reason why enlargement of the intrastrial space showed its peak at 20 min.

It was reported previously that the application of OPC-31260 produced down-regulation of AQP2 mRNA levels and acted on the VP-AQP2 system, resulting in the reduction of endolymphatic hydrops [9,10]. In addition, it was reported previously that V_2-R mediated the action of VP on the endocochlear potential [20]. Thus it was hypothesized in the present study that the administration of OPC-31260 before VP injection may reduce the enlargement of the intrastrial space caused by VP via the blockade of water transport from the perilymph to the the basolateral (perylymph) side of the basal cells, in which AQP2 is expressed. In the present study, the enlargement of the intrastrial space was reduced by the administration of OPC-31260 before VP injection. The present study confirmed the hypothesis.

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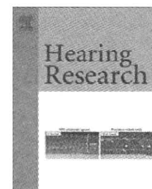

Research paper

Expression of aquaporins and vasopressin type 2 receptor in the stria vascularis of the cochlea

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ABSTRACT

Recently, considerable evidence has been accumulated to support the novel view that water homeostasis in the inner ear is regulated via the vasopressin–aquaporin 2 (VP–AQP2) system in the same fashion as in the kidney. Indeed, multiple subtypes of AQPs including AQP-2 are reported to be expressed in the cochlea. However, the mechanism that underlies VP–AQP-2 mediated water homeostasis remains to be elucidated. In the present study, the localizations of AQP-1, -2, -3, -4, -5, -7, -8, -9, and vasopressin type 2 receptor (V2-R) in the stria vascularis (SV) were molecular biologically and immunohistochemically examined to evaluate the role of the AQP water channel system in water homeostasis of the SV. A RT-PCR study revealed that AQPs and V2-R mRNA are expressed in the cochlea. As for their immunohistochemical localization, the AQP-2 protein is expressed on the basal side of the basal cells of the SV, and proteins of AQP-3 and V2-R are expressed on the apical side of the basal cells. AQP-7 and -9 proteins are expressed on the apical side of marginal cells. AQP-4, -5, and -8 protein expressions could not be detected in the lateral wall of the cochlea. From the present results, water flux in the SV is thought to be regulated at the level of the basal cells by vasopressin. Furthermore, such a distribution of AQP-2, -3, and V2-R suggests that VP–AQP-2 mediated water transport might work actively in the basal cells from perilymph towards endolymph containing AQP-1, -7 and -9.

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1. Introduction

The homeostasis of water in the inner ear is essential for maintaining the functions of hearing and equilibrium. Since the discovery of aquaporin (AQP) water channels (Agre et al., 1993), it has become clear that these channels play a crucial role in water homeostasis not only in the kidney but also in the inner ear. Indeed, expressions of proteins and/or mRNAs of AQPs in the inner ear have already been reported including negative reports (AQP-1: Stankovic et al., 1995; Sawada et al., 2003; AQP2: Kumagami et al., 1998; Beitz et al., 1999; Merves et al., 2000; Mhatre et al., 2002; Sawada et al., 2002; Fukushima et al., 2005; Zhong and Liu, 2003; AQP-3: Beitz et al., 1999; AQP-4: Takumi et al., 1998; Minami et al., 1998; AQP-5: Beitz et al., 1999; Mhatre et al., 1999; Löwenheim and Hirt, 2004; AQP-6: Fukushima et al., 2002; Lopez et al., 2007; Taguchi et al., 2008; AQP-7: Beitz et al., 1999; Huang et al., 2002; AQP-8: Beitz et al., 1999; Huang et al., 2002; and AQP-9: Huang et al., 2002). However, previous reports have had many contradictions. Some data show that AQP-2 mRNA is present in rat cochlea (Mhatre et al., 2002; Sawada et al., 2002; Fukushima et al., 2004). In immunohistochemistry, Merves et al. (2000) showed AQP-2 protein in the inner ear during mouse development, and Mhatre et al. (2002) also showed not in the SV but in the structures bordering the cochlea of both rat and mouse including Reissner's membrane, the organ of Corti, inner and outer sulcus cells and the spiral limbus. However, other studies found AQP-2 protein only in Reissner's membrane (Zhong and Liu, 2003) or did not find it at all (Kumagami et al., 1998; Beitz et al., 1999). Focusing on the stria vascularis (SV), the expressions of AQP-1, -2, -3, -6, and -7 have been histochemically confirmed (Mhatre et al., 2002; Sawada et al., 2003; Fukushima et al., 2005; Zhong and Liu, 2003; Huang et al., 2002; Taguchi et al., 2008; Nishimura

Abbreviations: AQP, aquaporin; BC, basal cell; immune EM, immunoelectron microscopy; IC, intermediate cell; MC, marginal cell; SV, stria vascularis; VP, vasopressin; V2-R, vasopressin type 2 receptor; VP–AQP-2 system, vasopressin–aquaporin-2 system

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et al., 2008). It should be noted that the expression of AQP-2 mRNA is up-regulated by vasopressin and down-regulated by V2-antagonists (Sawada et al., 2002; Takeda et al., 2003). These results indicate that water homeostasis of the inner ear is regulated in part via the vasopressin–aquaporin2 (VP–AQP2) system in the same fashion as in the kidney. In the cochlea, the main site of VP–AQP-2 mediated water homeostasis may be the SV. However, the detailed localization of AQPs and vasopressin type 2 receptor (V2-R) in the SV and the role of AQPs water channels in inner ear water homeostasis remain obscure. In this report, we examine the detailed localization of AQP-1, -2, -3, -4, -5, -7, -8, -9, and V2-R in the SV. First, the mRNA expressions of AQPs and V2-R in the cochlea were checked with RT-PCR, and then their localization in the SV was observed using immunoelectron microscopy (immune EM) in addition to normal immunohistochemical observations. Then, their role in water homeostasis of the SV is discussed.

2. Materials and methods

2.1. Experimental animals

The animals used were young Wistar rats (200–280 g). Molecular, biological, and immunohistochemical studies were performed. The animals were deeply anesthetized with sodium pentobarbital (200 mg/kg, IP injection), perfused from the left ventricle with phosphate-buffered saline (PBS, 10 mM phosphate buffer salts, 137 mM NaCl, 2.7 mM KCl, pH 7.4), and sacrificed.

The care and use of these animals was approved by the Kochi Medical School Animal Care and Use Committee, which conforms to The Animal Welfare Act and the guiding principles for animal care of the Ministry of Education, Culture, Sports, and Technology of Japan.

2.2. RT-PCR

The mRNA expressions of AQP-1, -2, -3, -4, -5, -7, -8, -9, and V2-R were investigated. After the rats had been sacrificed, the cochlea was extirpated, and the lateral wall of the cochlea was prepared under a stereomicroscope. The prepared tissue samples consisted of the spiral ligament, the SV, and the organ of Corti except the modiolus. Total RNA was extracted from two cochleae using the RNeasy Mini kit (Qiagen, Hilden, Germany). cDNA was synthesized from total RNA using the SuperScript II kit (Invitrogen Corp., Carlsbad, CA, USA). A negative control was obtained by excluding reverse transcription. Primers for the detection of AQP and V2-R were designed on the basis of the published sequences from rats: AQP-1 (GenBank X67948), AQP-2 (GenBank D13906), AQP-3 (GenBank D17695), AQP-4 (GenBank U14007), AQP-5 (GenBank U16245), AQP-7 (GenBank AB000507), AQP-8 (GenBank AB005547), AQP-9 (GenBank AF016406), and V2-R (GenBank NM019136). The sequences of the primers and the expected band sizes are shown in Table 1. RT-PCR was performed using HotStar Taq (QIAGEN, Hilden, Germany) and carried out using a thermal Cycler (TP3000, Takara, Ohtsu, Shiga, Japan). The procedure was performed as follows: an initial denaturing period of 15 min at 95 °C, followed by 35 cycles at 94 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min and a final extension period of 10 min at 72 °C. PCR products were electrophoresed on a 3% agarose gel and stained with ethidium bromide, before being directly sequenced using ABI PRISM 310 (PE Applied Biosystems, Foster City, CA, USA) with the Big Dye® terminator v3.1 cycle sequencing kit (PE Applied Biosystems).

2.3. Immunohistochemistry

We used two methods for the tissue preparation. As the first method, after PBS perfusion, 4% paraformaldehyde (PFA, pH 7.4) in PBS was intravenously perfused as well. The temporal bones were dissected and immersed in the fixative overnight and then decalcified in 0.12 M ethylenediaminetetraacetic acid (EDTA) in PBS (pH 7.4) for 7 days. The temporal bones were finally soaked in sucrose in PBS (10% 4 h, 15% 4 h, then 20% overnight) and were then embedded in Tissue Tec OCT Compound (Sakura Finetechnical Co. Ltd., Tokyo, Japan). As the second method, the perilymphatic space was immediately perfused with the fixative containing 4% PFA and 0.1% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). The cochlea was removed and immersed in the same fixative for 1 h at 4 °C. Then, the cochlear duct lateral wall of the basal and second turn was dissected in the fixative, and tissue strips were embedded in Tissue Tec OCT Compound.

These tissues obtained as above were cut with a cryostat into slices (HM 505E, MICROM) of 10 μm thickness and collected on slides. The sections were pre-incubated for 1 h in a solution containing 10% normal goat serum to block nonspecific binding sites. The specimens were then exposed overnight at 4 °C to rabbit antibodies specific for AQP-1 (diluted 1:100, # AB3065, Chemicon, Temecula, CA, USA), -2 (diluted 1:100, # AB3274, Chemicon, Temecula, CA, USA), -3 (diluted 1:80, # AB3276, Chemicon, Temecula, CA, USA), -4 (diluted 1:200, # AB3594, Chemicon, Temecula, CA, USA), -5 (diluted 1:100, # AB3069, Chemicon, Temecula, CA, USA), -7 (diluted 1:50, # AB3075, Chemicon, Temecula, CA, USA), -8 (diluted 1:100, # AB3077, Chemicon, Temecula, CA, USA), -9 (diluted 1:80, # AB3079, Chemicon, Temecula, CA, USA), and V2-R (diluted 1:100, # AB1797, Chemicon, Temecula, CA, USA). The sections were incubated for 6 h at room temperature in Alexa 546 goat anti-rabbit IgG (diluted 1:100, # A-11035, Molecular Probes Inc., Oregon, USA). The sections were washed extensively with PBS, before being examined using a Axiovert 200M controlled by Axiovision LE software.

2.4. Immunoelectron microscopy

The tissue obtained using the second immunohistochemical method was embedded in LR white resin (London Resin Company Ltd., London, England). Thin sections were cut perpendicular to the apical surface of the marginal cells (MC) on an Ultracut E ultramicrotome (C. Reichert AG, Vienna, Austria) and used for immuno-

Table 1
The sequences of the primers for RT-PCR.

Band size		Forward primer sequence	Reverse primer sequence
AQP-1	534 bp	TCACATCAGTGGTGCTCACCTCAAC	GTCCACACCTTCATGCGGTCTGTAA
AQP-2	447 bp	TGAGTTCTTGGCCACGCTCCTTT	ATGGAGAGGGCAGGGCTACC
AQP-3	495 bp	GAGCCCACTTGAACCCTGCTGTG	ACCACCAGTTCTGGCCAGTCGTAA
AQP-4	459 bp	GCGAGGCGGTGGGGTAAGTGTG	CCCATGGCCAGCAGTGAGGTTT
AQP-5	360 bp	GGCCACATCAATCCAGCCATTACTC	GTTCATGGAACAGCCGGTGAAGTAG
AQP-7	338 bp	AGTTCCTGGCGGAGTTCCTGAGTAC	CACCAACAGTTCTCCACCTGCATAG
AQP-8	543 bp	GGTGTCTATCGGTCATCGAGAACAG	CAGCCAGTAGATCCAATGGAAGTC
AQP-9	684 bp	TAGCGAAGGAGACACTCTCCGAGTT	CCACGACAGGTATCCACCAGAAGTT
V2-R	464 bp	ATGGTGGGCATGTATGCCTCCTCCTACATG	AGTGTCATCCTCACGGTCTTGGCCA

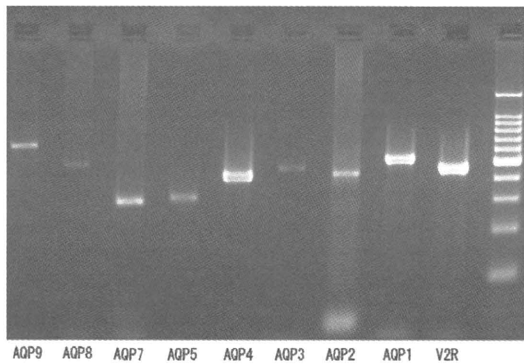

Fig. 1. Expression of AQPs and V2-R mRNAs in the rat inner ear. Expression of AQP-1, -2, -3, -4, -5, -7, -8, -9, and V-2R mRNAs was detected in the lateral wall of the cochlea. The kidney acted as a positive control (not shown).

gold cytochemistry. These sections were treated in a fourfold dilution of Block Ace (Dainippon Sumitomo Pharma, Tokyo, Japan) to block nonspecific binding sites and then were incubated with primary antibodies in a 10-fold dilution of Block Ace containing 0.1% sodium azide for 8 h at room temperature. After incubation with the primary antibody, the sections were washed three times in PBS (0.15 M saline buffered at pH 7.2 with 0.5% BSA and 0.05% Tween-20), and incubated with a 10-fold dilution of goat–anti-rabbit IgG labeled with 10 nm colloidal gold (Sigma, Aldrich) in PBS for 6 h at room temperature. Finally, the sections were counterstained with uranyl acetate and lead citrate. The sections were observed with a Hitachi 7100 electron microscope (Hitachi Co., Tokyo, Japan)

3. Results

3.1. RT-PCR

Fig. 1 shows the RT-PCR products after 35 cycles. AQP-1, -2, -3, -4, -5, -7, -8, -9, and V2-R mRNAs were expressed in the rat cochlea. The PCR products were of the expected size as shown in Table 1. The nucleotide sequences of the bands agreed completely with the known sequences of rat AQPs and V2-R. No PCR product was obtained when RT was omitted (negative controls).

3.2. Immunohistochemistry

V2-R was expressed in the basal cells (BC) of the SV (Fig. 2a-1, original fluorescent image; a-2, pseudocoloured fluorescent image that was superimposed on differential interference contrast image using the Axiovision software. AQP-2, and -3 proteins were expressed in the basal cells (BC) of the SV (Fig. 2c and d). AQP-1 was clearly expressed in type 3 fibrocytes and diffusely expressed in the SV (Fig. 2b). AQP-4, -5, and -8 proteins were not expressed in the SV (Fig. 2e and f). Concerning AQP-4, -5, and -8, the results of RT-PCR and immunohistochemistry were incompatible. AQP-4 protein was expressed in Hensen's cells, Claudius cells, and the inner sulcus cells (Data not shown). AQP-5 was observed in the external sulcus (ES) cells and cells of the spiral prominence (Data not shown). Therefore, PCR products of AQP-4 and -5 seem to reflect the expressions of these proteins in cochlear tissues other than the stria vascularis. AQP-8, however, was not detected anywhere. AQP-7 protein expression was observed on the apical side of the MC and BC of the SV (Fig. 2g). AQP-9 protein expression was observed on the apical side of the MC of the SV (Fig. 2i). Moreover we performed the pre-absorption test on AQP2. There was no expression on the basal cell of the SV (Data not shown).

3.3. Immunoelectron microscopy

SV contains three cell types, MC, intermediate cells (IC) and BC. MC is constructed from one superficial layer and are closely combined by tight junctions. BC consist of a few layers, are combined closely by tight and gap junctions, and have radiating processes that interdigitate with the processes of IC and MC. The BC also have ascending processes that surround and partially isolate the basal processes of the MC.

The plasma membrane of BC in the SV was labeled with immunogold against AQP-2, -3, and V2-R antibodies. AQP-2 was detected on the basal membrane of the BC and the vesicles in the BC, but not on the apical membrane (Fig. 3a–c). AQP-3 was detected on the apical side of the radiating processes of the BC (Fig. 4a–c), but not on the basal membrane. V2-R was observed on the apical side of the radiating processes, but not on the basal side of the BC (Fig. 5a–c).

The plasma membrane of the IC was labeled with immunogold against AQP-1 (Fig. 6a–c), as shown in our previous report (Sawada et al., 2003).

Both AQP-7 and AQP-9 were labeled in the apical region of the MC (Figs. 7a, b and 8a, b). AQP-7 labeling was frequently observed in the vesicles immediately beneath the apical membrane, not on the membrane. AQP-7 was also diffusely detected in the BC, not on the membrane (Fig. 7c).

Meanwhile, immunogold AQP-9 labeling was observed on the plasma membrane of the apical side or on the vesicles beneath the plasma membrane.

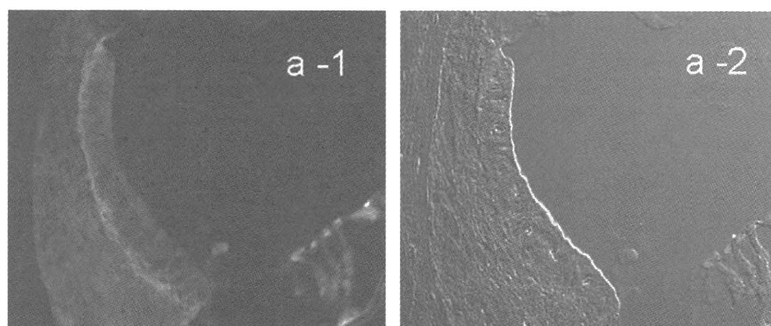

Fig. 2. (1) Localization of V2-R in the rat SV with immunohistochemistry. Fluorescent images were overlaid with differential interference contrast images using Axiovision software. (a-1) Labeling of V2-R antibodies (Fluorescent image): V2-R was positive in the BC of the SV. (a-2) Labeling of V2-R antibodies (overlaid image): V2-R was positive in the BC of the SV. (2) Localization of AQPs in the rat SV with immunohistochemistry. (b) Labeling of AQP-1 antibodies: AQP-1 is diffusely positive in the SV, as well as in the type III fibrocytes. (c) Labeling of AQP-2 antibodies: AQP-2 is positive in the BC of the SV. (d) Labeling of AQP-3 antibodies: AQP-3 is positive in the BC of the SV. (e) AQP-4 was not expressed in the lateral wall of the cochlea. (f) AQP-5 was not expressed in the lateral wall of the cochlea. (g) Labeling of AQP-7 antibodies: AQP-7 is positive in the MC and BC of the SV. (h) AQP-8 was not expressed in the lateral wall of the cochlea. (i) Labeling of AQP-9 antibodies: AQP-9 is positive in the MC of the SV. Negative control (first antibody omitted) showing no labeling in the SV (not shown).

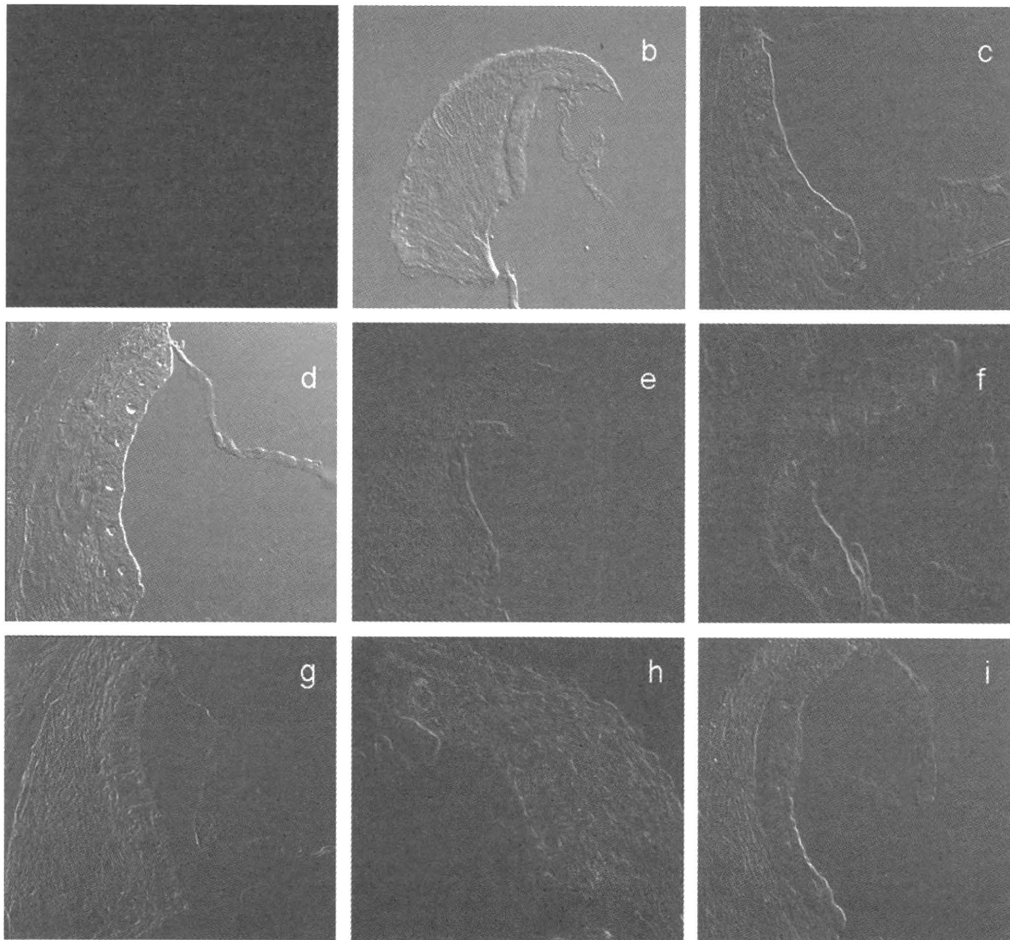

Fig. 2 (*continued*)

4. Discussion

The ultrastructural localizations of AQPs in the inner ear have been already reported for AQP-1 and -6. Takumi showed by immunocytochemical studies including immunogold analysis that AQP-1 is expressed along the plasma membranes of type III fibrocytes in the spiral ligament and of sub-basilar tympanic cells. However, their light microscopic immunocytochemistry detected no cells that were labeled for AQP-1 in the SV. On the other hand, our previous study reported that AQP-1 protein is expressed not only in the spiral ligament but also in the SV (Sawada et al., 2003). Our previous immunoelectron microscopic study revealed that immunogold particles were found along the plasma membranes of the IC. In the present study, these results were reconfirmed. As for the expression of AQP-6 in the SV, it was located in the membrane of the intracellular vesicles of the SV. From the lack of AQP-6 expression on the plasma membrane, AQP-6 was thought not to have a direct role in water flux via the plasma membrane (Taguchi et al., 2008). In addition to the expression of AQP-1 in SV, the present light microscopic immunofluorescence studies revealed that AQP-2, -3, -7, -9, and V2-R are clearly expressed in the SV. Since SV is thought to be the main site of active ion and water transport in the endolymph (Sterkers et al., 1988), it seems to be reasonable that multiple subtypes of aquaporins are expressed there. Specifically, it has been noted that the expression of AQP-2, -3, and V2-R was localized in the BC, at the boundary between the perilymphatic system and the endolymphatic system. Such a distribution of AQP-2, -3, and V2-R suggests that VP–AQP-2 mediated water transport might work actively in the BC.

The present study is the first report to define the ultrastructural localization of AQP-2, -3, -7, -9, and V2-R in the SV. Their distribution on or near the plasma membrane is shown in Fig. 9. AQP-3 and V2-R were localized at the apical side of the BC, while AQP-2 was located along the basal membrane of the BC. In renal collecting duct principal cells, AQP-2 is known to be located on the opposite side to V2-R, and AQP-3 on the same side as V2-R (Nielsen et al., 2002; Robben et al., 2004). Although this distribution pattern is not always confined to the SV, the present study supports that AQP-2, -3, and V2-R are distributed in the same manner as in the kidney. According to Sziklai et al. (1987), VP may exert its effects via the perilymphatic side of the SV. If so, V2-R might be localized at the lateral side of the BC. However, V2-R is located at the apical side. Therefore, VP is thought to act on the BCs via the intrastrial artery.

It is known that VP exerts its effects on the target organ by binding to its receptor, V2-R. The present results suggest that the BC is the target organ of VP and that VP–AQP-2 mediated water transport occurs actively in the BC toward the endolymphatic system. Since the driving force of AQP water channels is the osmotic gradient and the osmolarity of the endolymph is significantly higher than that of the perilymph (Konishi et al., 1984; Sterkers et al., 1984), the water in the perilymphatic compartment might enter into the intracellular space of the BC via AQP-2 water channels, and then the water in the intracellular space of the BC might exit into the extracellular space of the SV (intrastrial space) via AQP-3.

The other possible water pathway is water flow via gap junctions. Orce et al. reported that a gap junction inhibitor significantly reduced osmotic water flow (Orce et al., 2004). In the SV, the BC

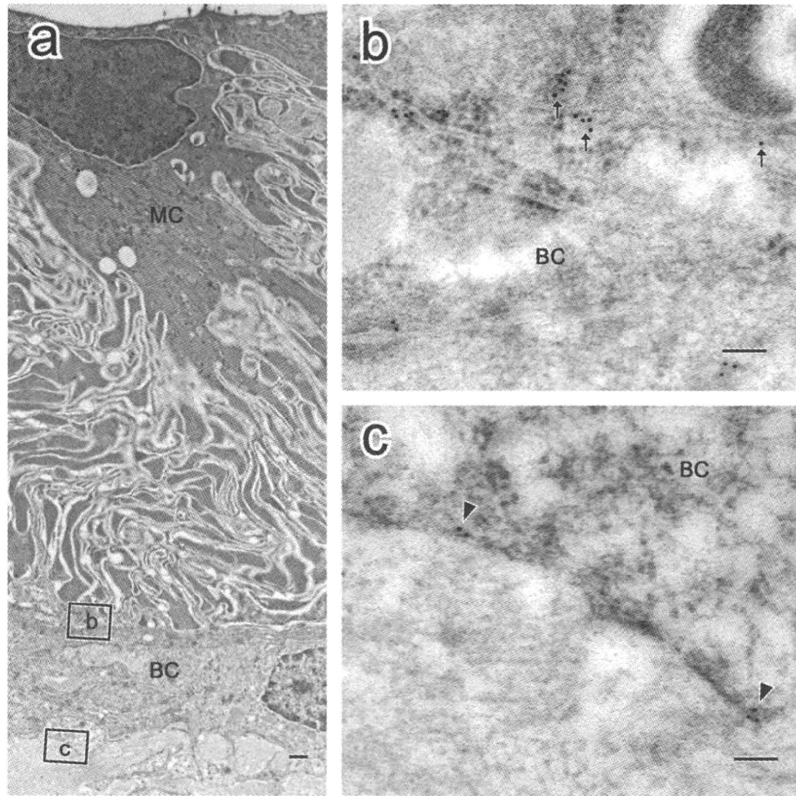

Fig. 3. AQP-2 labeled with immunogold in the SV. AQP-2 labeled with immunogold was observed in the BC, as seen in the higher magnification views (b and c) of the areas enclosed by rectangles in the lower magnification view (a). AQP-2 is positive within the cytoplasm of the BC (b, arrows) and at the basement membrane (c, arrowheads), but not on the apical (intrastrial) side of the plasma membrane. Scale bar: 0.5 μm in (a), 0.1 μm in (b) and (c) (MC, marginal cell; BC, basal cell; Cap., capillary; V, vesicle).

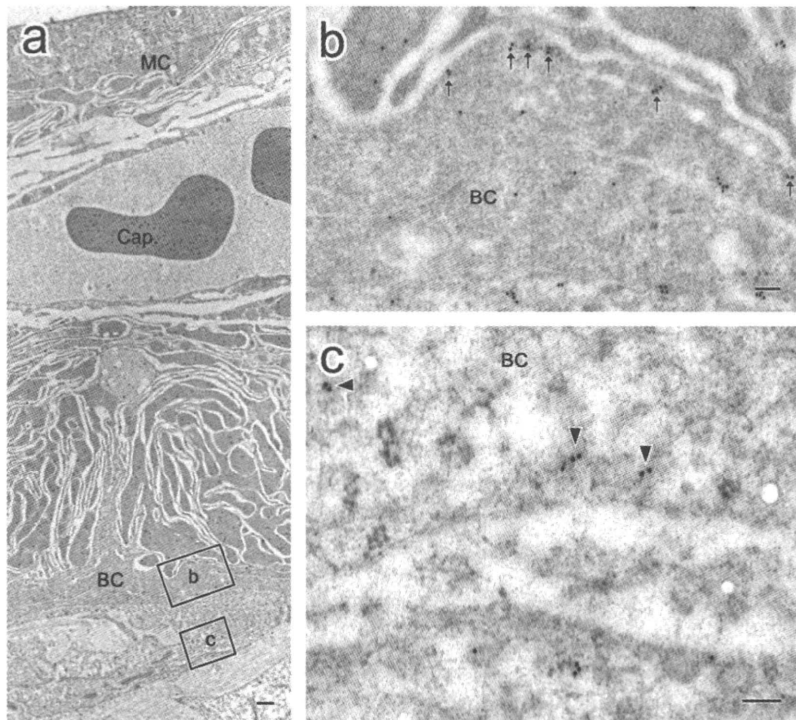

Fig. 4. AQP-3 labeled with immunogold in the SV AQP-3 labeled with immunogold was observed in the BCs, as seen in (b) and (c) (higher magnification views of the areas enclosed by rectangles in the low magnification view of SV (a)). AQP-3 is positive at the apical membrane in the radiating processes (b, arrows), but not on the basement membrane (c). Scale bar: 0.5 μm in (a), 0.1 μm in (b) and (c).

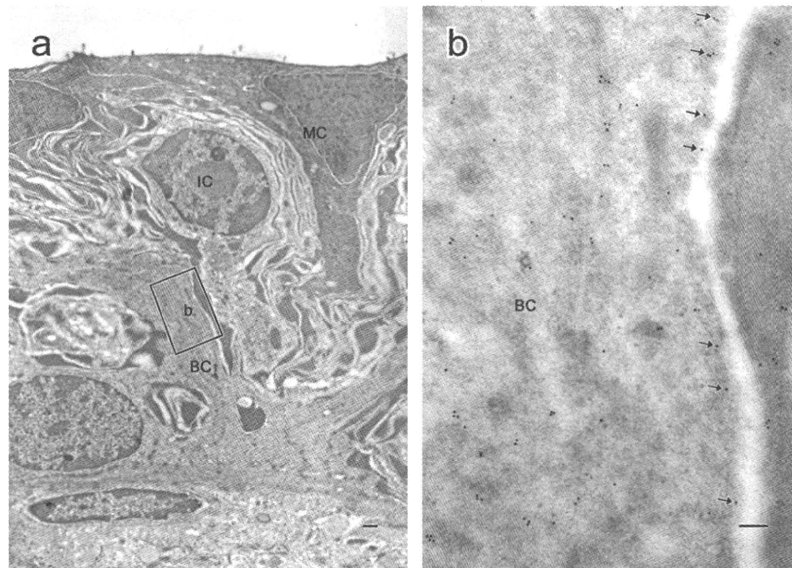

Fig. 5. V2-R labeled with immunogold in the SV. Immunogold V2-R labelings were observed at the apical membrane in the radiating processes (b, arrows), but not on the basement membrane (c, arrows), as seen in the higher magnification views of the area enclosed by a rectangle in the low magnification view of SV (a). Scale bar: 0.5 μm in (a), 0.1 μm in (b).

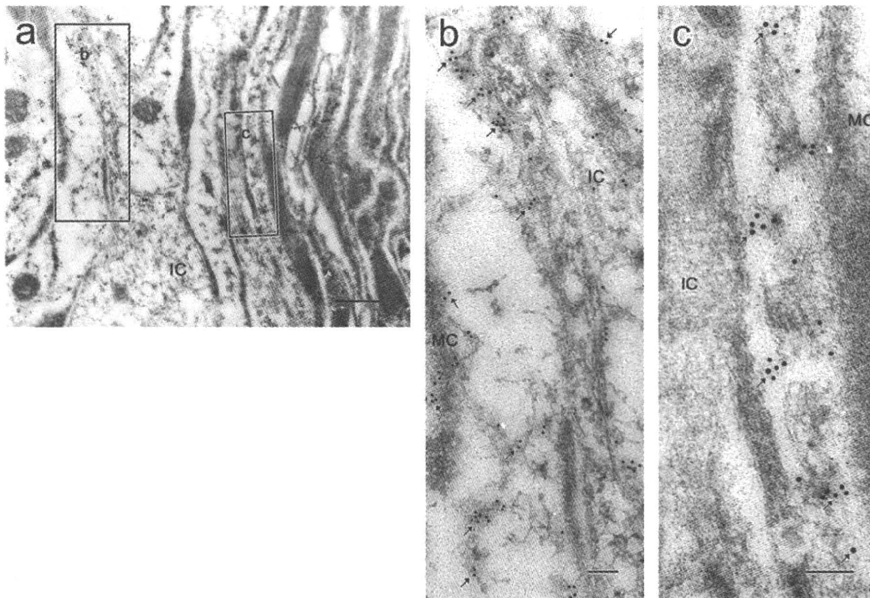

Fig. 6. AQP-1 labeled with immunogold in the SV. Immunogold AQP-1 labelings were observed in the plasma membrane of the IC, as shown in the higher magnification views (b and c) of the areas enclosed by rectangles in the lower magnification view (a). Scale bar: 0.5 μm in (a), 0.1 μm in (b) and (c).

and IC are coupled by the gap junction system. Gap junction channels have a pore with a diameter of 1.0–1.5 nm, permitting intercellular passage of particles with a molecular weight of less than 1000, for example, ions, nucleotides, siRNAs, dyes, and inositol phosphates (Zhao et al., 2006). If gap junction channels permit water passage (Verselis and Brink, 1986), water may flow into the intrastrial spaces from the IC via AQP-1 since AQP-1 is expressed in the IC (Sawada et al., 2003, present study). Furthermore, the BC connect the fibrocytes of the spiral ligament via the gap junction system. This connection permits water influx into the BC via AQP-1 water channels on type III fibrocytes of the spiral ligament. Therefore, the syncytium, coupled with fibrocytes, BCs, and ICs via the gap junction system, is thought to function as the first barrier to water flow from the perilymphatic to endolymphatic compartments. More studies are required to elucidate these mechanisms.

Here, one question arises: via what pathway does water flow out from the extracellular space and into the scala media? The most probable candidate is a route through the MC. Actually, two subtypes of water channels, AQP-7 and AQP-9 are expressed in the apical region of the MC, as shown in Figs. 7b and 8b. The localization of AQP-7 is compatible with Huang's results (Huang et al., 2002). However, AQP-7 labeling was detected on the vesicles immediately beneath the apical membrane in the MC, but not on the membrane in the immune EM. Recent reports have shown that AQPs are present in cytoplasmic vesicles in human parotid aciner cells (Smith et al., 1999) and the mouse liver (Ferri et al., 2003), and intracellular vesicles in the rat kidney (Yasui et al., 1999).

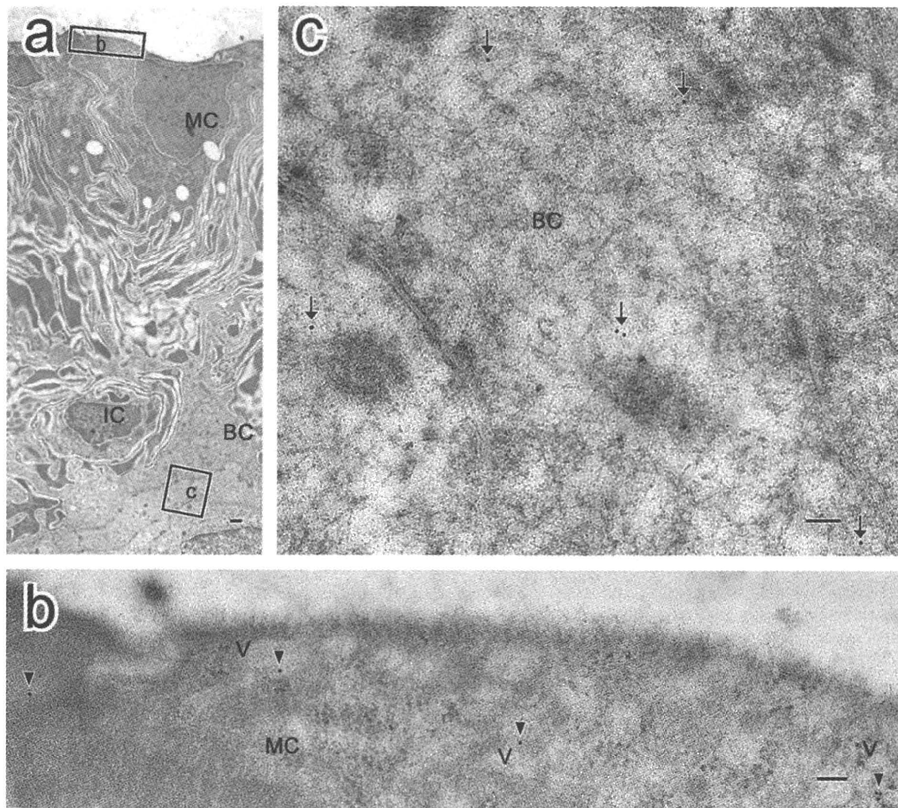

Fig. 7. AQP-7 labeled with immunogold in the SV. Immunogold AQP-7 labelings were observed in the MC and BC, as seen in the higher magnification views (b and c) of the areas enclosed by rectangles in the lower magnification view (a). In the MC, AQP-7 was positive on the vesicles immediately beneath the plasma membrane of the apical membrane (b, arrowheads), not on the membrane. In the BC, AQP-7 was positive in the cytoplasm (c, arrows). Scale bar: 0.5 μm in (a), 0.1 μm in (b) and (c).

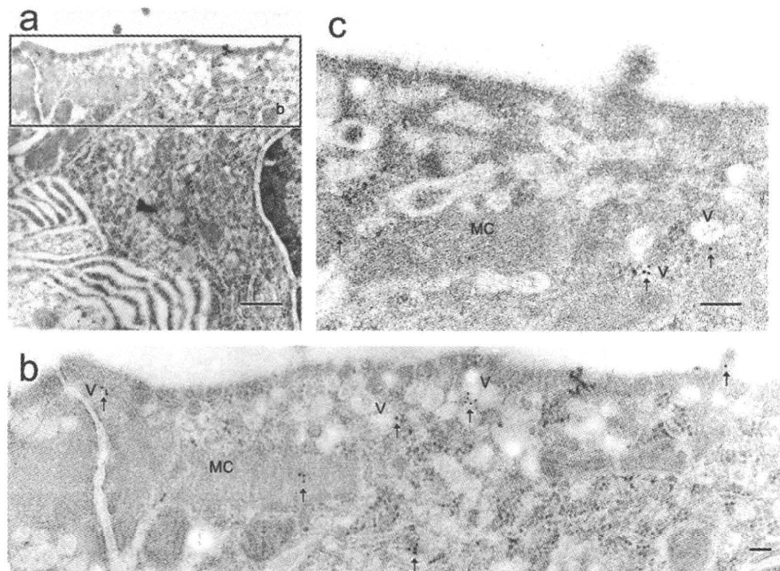

Fig. 8. AQP-9 labeled with immunogold in the SV. Immunogold AQP-9 labelings were observed in the MC, as seen in the higher magnification views (b) of the area enclosed by a rectangle in the lower magnification view (a). AQP-9 was positive in the apical region of the MCs (b and c, arrows). (c) Another place of the MC. Scale bar: 0.5 μm in (a), 0.1 μm in (b) and (c).

Transport vesicles are in part involved in endocytosis and exocytosis (Burgoyne and Morgan, 2003). Indeed, an application of VP produces an increase in vesicle formation in MC (Nishimura et al., 2008). These results indicate that the AQP-7 observed on the vesicle immediately beneath the apical membrane is related to exocytosis as a water transport route under VP regulation. Recently, AQP-7 has been reported to be permeable not only to water but also to glycerol and so-called aquaglyceroporins (AQP-3, -7 and

Fig. 9. Localization of AQPs in the SV. AQP2, localized on the basement membrane of the basal cell; AQP-3 and V2-R, localized on the apical membrane of the basal cell; AQP-1, localized n the apical membrane of the intermediate cell; AQP-7, localized at the apical side of the marginal cell; AQP-9, localized on the apical membrane of the marginal cell (TJ, tight junction; GJ, gap junction; V, vesicle). AVP binding to V2-R causes AQP-2 trafficking to the plasma membrane (basement membrane) from intracellular vesicles via cAMP activation.

-9) (Agre et al., 2002). The role of AQP-7 in the MC still remains obscure. However, the outward water flux across the apical membrane is explicable by AQP-9 water channels.

The question is whether there is any route of water passage across the basolateral MC membrane. The basolateral membrane of the MC is reported to abundantly express multiple types of ion channels (Wangemann and Schacht, 1996), but not to express any type of AQP. However, certain volume sensitive ion transport systems were recently suggested to play multiple roles, for example, in volume regulation, trans-epithelial salt and water movement, and intracellular pH control (Strange, 2004). The Na^+–K^+–$2Cl^-$ cotransporter is one of the ion channels to be implicated in cell volume regulation and ion–water coupling transport by secretory epithelial tissue (MacAulay et al., 2004). This cotransporter may be essential for the secretory function of epithelial tissues that produce extracellular fluids rich in K^+, such as the Sertoli cells of the testis and the salivary epithelium (Pace et al., 2000). Although high K^+ levels are extremely rare in fluid secretion by epithelial tissue, the Na^+–K^+–$2Cl^-$ cotransporter is reported to be abundantly expressed in the basolateral membrane of strial MC, which is thought to produce the endolymph, which has a high K^+ concentration (Crouch et al., 1997; Mizuta et al., 1997; Sakaguchi et al., 1998). Since water can flow uphill against the osmotic gradient via the Na^+–K^+–$2Cl^-$ cotransporter (Zeuthen and MacAulay, 2002), it is likely that the fluid of K^+ ion–water coupling enters into the MC through the Na^+–K^+–$2Cl^-$ cotransporters located on the basolateral membrane.

Another possible mechanism underlying water passage through the MC is water flux via AQP-6 water channels. AQP-6 is abundantly expressed in MC as well as in IC and BC. The AQP-6 water channel is a potential candidate of water and ion cotransport. Among many members of water channel family, AQP-6 is the only water channel known to function as an ion channel, and allows cation and anion permeation with marked specificity for nitrate. However, AQP-6 is localized to the membranes of intracellular vesicles, which do not shuttle to the plasma membrane. In our previous study (Taguchi et al., 2008), AQP-6 was found to be diffusely expressed in the cytoplasm. The primary role of AQP-6 is cellular regulation rather than trans-epithelial fluid flux. Therefore, it is unlikely that water flows across the plasma membrane of the MC through the AQP-6 water channel.

In view of the inference described above, the following routes of water flux with two barriers can be proposed on the basis of the assumption that osmolarity increases gradually in the direction from the perilymphatic compartment to the endolymphatic compartment, although the osmotic gradient within the SV remains to be elucidated. The first barrier between the perilymphatic and endolymphatic compartments is composed of the syncytium of the fibrocytes, BC, and IC, coupled with the gap junction system. According to the osmotic gradient, water flows into the syncytium through the AQP-1 channels on the type III fibrocytes (Fig. 9, line 1) or AQP-2 water channels on the BC (Fig. 9, line 2), and flows out into the intrastrial space through AQP-3 water channels on the BC (Fig. 9, line 3) or AQP-1 on the IC (Fig. 9, line 4). Generally, the driving force of AQP water channels is generated by the combination of trans-epithelial hydrostatic and osmotic pressure. Therefore, the amount of water moving per unit of time and surface is dependent on the osmotic or hydrostatic gradient and the AQP concentration on the epithelium (Parisi et al., 1997). AQP-1, -2, and -3 expression in the kidney is known to be directly and secondarily mediated by vasopressin and corticosteroids (Nielsen et al., 1999, 2002). In the inner ear, AQP-1 and -2 expression is mediated by corticosteroid and VP, respectively (Fukushima et al., 2002; Sawada et al., 2002). The water inlets and outlets of the syncytium are likely to be under hormonal regulation.

The second barrier is the MC. Water, which flows into the intrastrial space from the syncytium, is pumped up into the MC coupled with K^+ ions through the Na–K–Cl cotransporter, which is abundantly expressed on the basolateral side of the MC (Fig. 9, line 5), and then, the water in the MC flows out into the endolymphatic space through AQP-9 water channels (Fig. 9, line 6) and/or AQP-7 water channels (Fig. 9, line 7).

The above-proposed water flux mechanism is based on two hypotheses (1) the osmolarity within the SV increases gradually in the direction from the perilymphatic compartment to the endolymphatic compartment and (2) the cotransporter works as a water pump (Loo et al., 1996; Zeuthen and MacAulay, 2002; MacAulay et al., 2004). Furthermore, expression of AQP-10, -11, and -12 in the SV was not examined in the present study because of the lack of the commercially available antibodies with adequate sensitivity. We cannot categorically deny that these AQPs are expressed in the SV, especially, in the basolateral (intrastrial) side of the MC, or that they play a role in water flux in the SV; however, the experimental findings that application of VP or loop diuretic induces enlargement of the intrastrial space (Nishimura et al., 2008; Higashiyama et al., 2003) seems to support the present proposed water flux mechanism. Further study is required to establish the water flux mechanism of the lateral wall of the cochlea.

Acknowledgments

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Somatosensory input influences the vestibulo-ocular reflex

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ABSTRACT

To evaluate the influence of somatosensory input on the vestibulo-ocular reflex (VOR), we used sinusoidal rotation tests in 19 young, healthy volunteers. For the control condition, subjects were sinusoidally rotated in complete darkness and with eyes opened at a frequency of 0.2 Hz with a maximum angular velocity of 30°/s for 30 s, and at frequencies of 0.4 and 0.8 Hz with a maximum angular velocity of 60°/s for 30 s. Sinusoidal tests were performed at earth vertical axis rotation (EVAR). For the experimental condition, we introduced somatosensory stimulation as subjects were sinusoidally rotated at the control parameters. Subjects were told to grasp an earth-fixed metallic bar with their right hands. Thus, their right arms continued to move as the rotating chair apparatus moved. We observed a significant increment (34%) in VOR gain change only at 0.2 Hz EVAR when subjects held the bar compared to that of the controls, who did not hold the bar. Gain change did not differ significantly across the other conditions. We hypothesize that arthrokinetic input (i.e., arm movement) had an additive effect on VOR in this study. This input might relate to a low-frequency component that strongly enhances the velocity storage system. Our findings have applications to types of vestibular rehabilitation regimens that implement somatosensory input.

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Vestibulo-spinal reflexes contribute to the maintenance of upright stance and body balance, while the vestibulo-ocular reflex (VOR) stabilizes the visual image on the retina during movement. Information from visual, vestibular, and proprioceptive stimuli is also important for maintaining body balance [5,11,17]. The contribution of the proprioceptive system concerns mostly neck proprioception [14,17,20,23,24]. Patients with vestibular disorders often suffer from dizziness or unstableness, which greatly affects their quality of life. The typical treatment for these patients is vestibular rehabilitation, which employs visual inputs or physical vestibular habituation training [5,13,21].

The influence of somatosensory inputs to the vestibular system, apart from neck input, are not negligible. Previous reports have demonstrated that touching a subject's index finger contributes to the stabilization of postural sway [6,15,16,19]. Lackner et al. observed that, when situated in a dark environment, subjects with vestibular loss who lightly touched a stationary object with their fingers were significantly more stable than normal subjects who did not touch the object [18]. Another study, in which compensatory nystagmus was induced in human subjects who walked a circular path inside a stationary, darkened optokinetic drum, found that somatosensory information from muscle and joint receptors sufficiently induced an illusion of self-motion and caused nystagmus [2]. This phenomenon was also observed in similar studies using monkeys [25,26]. However, few studies exist that have evaluated the influence of somatosensory input with rotation nystagmus tests.

In the present study, we used earth vertical rotation (EVAR), a stimulation method used in evaluating semicircular canal function [10,27,28], to examine the influence of somatosensory input on VOR. Specifically, we investigated whether differences in VOR exist between subjects who received somatosensory input versus those who did not.

Nineteen young, healthy volunteers (14 males and 5 females, aged 22–36 years old; mean age 26.5 ± 4.53 years) without any history of vertigo or disequilibrium participated in the study. This study was conducted in accordance with the Declaration of Helsinki, and all procedures were carried out with the adequate understanding and written consent of the subjects. The review board of the St. Marianna University School of Medicine approved the study protocol.

Rotation testing was performed using a device designed and built at our institute. Subjects were seated on a computer-controlled rotating chair (FVV-3000A, Daiichi Medical) (Fig. 1), which consisted of a chair affixed to a turntable attached to a stand. Rotation rate and angle of tilt were controlled with a digital computer. Each subject was fitted with goggles attached to a charge-coupled device (CCD) camera. The subject's body was supported by 5-point belts, and the subject's head was secured to the chair with a band. The following control conditions were used.

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Fig. 1. Rotation chair device for somatosensory stimulation testing. A fixed metallic bar is attached to the ceiling and the floor of the rotation testing device. During earth vertical axis rotation (EVAR), the subject grasps the bar with his/her right hand at about eye level. The bar itself remains stationary as the chair rotates.

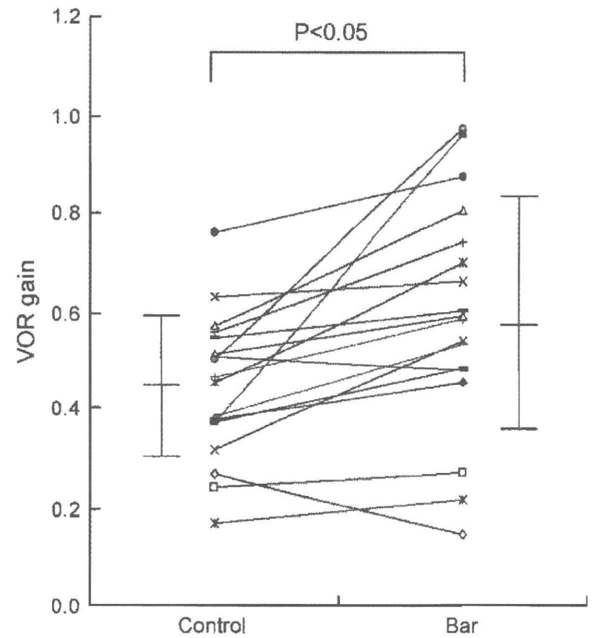

Fig. 2. VOR gain at 0.2 Hz EVAR in subjects who did and did not (control) grasp a fixed bar. VOR gain measured from subjects who grasped the bar was significantly larger than that from controls.

In complete darkness and with eyes opened, subjects were sinusoidally rotated at a frequency of 0.2 Hz with a maximum angular velocity of 30°/s for 30 s, and at frequencies of 0.4 and 0.8 Hz with a maximum angular velocity of 60°/s for 30 s [27,28]. Sinusoidal trials were performed using EVAR [27,28]. For the experimental condition, we introduced somatosensory stimulation as subjects partook in the rotation tests at the parameters set for the control conditions. Briefly, each subject was instructed to grasp an earth-fixed metallic bar with his/her right hand. Thus, the subject's right hand constantly moved as the rotating chair apparatus moved (Fig. 1). All stimuli were randomized to avoid order effects. Subjects participated in one trial per condition. To remain alert during rotation, subjects were asked to perform mental arithmetic during the whole procedure. Eye movements were recorded with an infrared CCD camera trained on the subject's right eye, and the center of the pupil was traced. Before each sinusoidal rotation test, we calibrated the electro-oculography signal by asking each subject to gaze at targets placed 10° up, right, down, and left from his/her center of gaze. Horizontal eye position data were digitally differentiated, and the fast components of nystagmus identified and removed automatically by computer (2D VOG-Video-Oculography, version 3.2, SensoMotric Instruments). The resulting slow component velocity records were smoothed using a 7-point moving-average technique. Subsequently, we used Fourier transform to calculate the VOR gain (eye velocity cf. head velocity) [7,27,28]. The VOR gain was adjusted according to input frequency by decreasing the gain by 50% at 0.2 Hz and by increasing the gain by 200% at 0.8 Hz. We used the Mann–Whitney *U* test for the statistical analyses; $P < 0.05$ was considered significant.

The total number of subjects participating in the study was 19, but only 18 successfully partook in the rotation test implementing the bar condition. We encountered a problem recording eye movements in one subject.

Table 1 shows VOR gain and gain changes of controls and subjects exposed to somatosensory input (bar condition). There was a significant increase ($P = 0.03$) in VOR gain change at 0.2 Hz EVAR in subjects who held the bar (Fig. 2). Gain change did not differ significantly across the other conditions. VOR gain values for the controls ($n = 19$) were 0.44 ± 0.15 (mean ± SD) at 0.2 Hz, 0.66 ± 0.28 at 0.4 Hz, and 0.58 ± 0.26 at 0.8 Hz. VOR gain values for the subjects who held the bar ($n = 18$) were 0.59 ± 0.23 at 0.2 Hz, 0.69 ± 0.31 at 0.4 Hz, and 0.71 ± 0.66 at 0.8 Hz. Gain changes at 0.2 Hz in subjects who held the bar were 0.34 ± 0.43 in 0.2 Hz, 0.16 ± 0.78 in 0.4 Hz, and 0.61 ± 1.63 in 0.8 Hz. Surprisingly, we observed a significant increment (34%) in VOR gain change at 0.2 Hz EVAR in subjects who grasped a stationary bar as they underwent the rotation test (Table 1, Fig. 2). After the tests, most subjects stated that grasping the bar gave them sta-

Table 1
VOR gain changes in controls and subjects exposed to somatosensory input.

	VOR gain (gain change)		
	0.2 Hz	0.4 Hz	0.8 Hz
Controls = 19	0.44 ± 0.15	0.66 ± 0.28	0.58 ± 0.26
Bar = 18	0.59 ± 0.23 (0.34 ± 0.43)*	0.69 ± 0.31 (0.16 ± 0.78)	0.71 ± 0.66 (0.61 ± 1.63)

Values within parentheses show changes in VOR gain: (bar − control)/control. We calculated the gain changes by averaging the gain changes of each subject. The gain change of subjects exposed to somatosensory input (i.e., grasping a fix bar) during EVAR at 0.2 Hz was significantly larger than that of controls. Gain changes did not differ significantly across the other conditions.

* $P < 0.05$.

bility and relief, because it enabled them to know which position they were in.

Brandt et al. reported arthrokinetic nystagmus and self-motion sensation [3]. They presented a compelling illusion of body rotation and nystagmus can be induced when the horizontally extended arm of a stationary subject is passively rotated about a vertical axis in the shoulder joint. Lateral nystagmus with the fast phase beating in the opposite direction to the arm movement was found consistently, and the nystagmus continued after the cessation of stimulation. They indicated a convergence of vestibular and somatosensory afferents from joint receptors, and information about joint movements plays an important role within the multisensory process of self-motion perception [3]. de Graaf et al. studied the gain of smooth ocular tracking for visual, vestibular, and arthrokinetic cues and demonstrated that arthrokinetic and vestibular information significantly increased smooth ocular tracking gain [8]. They concluded that information from the limbs about linear (self-)movement has analogous characteristics and generates analogous responses to the information about angular (self-)movement [8]. The findings of these previous reports support our results. In the present study, we similarly concluded that the constant arm movement experienced by our subjects during the rotation test represented arthrokinetic input, which had an additive effect on VOR.

In our study, we observed a significant increment in VOR gain in subjects who grasped a stationary bar as they underwent EVAR at 0.2 Hz but not at 0.4 or 0.8 Hz. This result showed that arthrokinetic somatognosic proprioceptive input was effective specifically at a low frequency. Normally, VOR can deliver high-level performance only for brief head movements. In other words, VOR can compensate for high-frequency head movements but not for low-frequency head movements [13]. A phenomenon called velocity storage can partially improve VOR performance at low frequencies [9,22]. The velocity storage mechanism stores information about an individual's current head velocity, which is derived from several kinds of motion sensors [1,22]. The vestibular system uses somatosensory to drive the velocity storage mechanism [12]. In a study assessing nystagmus in monkeys that ran around the perimeter of a circular platform, Cohen et al. found that after-nystagmus and optokinetic after-nystagmus were similar, suggesting that somatosensory inputs or efference copies related to angular walking activated velocity storage [4,25,26]. They proposed that velocity storage might support compensatory ocular nystagmus during locomotion and minimize inappropriate after-nystagmus when movement stops [25,26]. Brandt et al. observed a similar phenomenon called arthrokinetic after-nystagmus, in which nystagmus continued even after arthrokinetic stimulation stopped [3]. Taken together, these findings led us to hypothesize that somatosensory input, including arthrokinetic information, may be related to a low-frequency component that strongly enhances the velocity storage system [1,9,22].

In the present study, somatosensory input in the form of continuous arm movement had an additive effect on VOR. This finding can be applied to vestibular rehabilitation requiring somatosensory input. For example, walking with a cane may reduce dizziness in patients with vestibular disorders. Further investigations of patients will be needed.

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ORIGINAL ARTICLE

Does vertigo disappear only by rolling over? Rehabilitation for benign paroxysmal positional vertigo

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Abstract

Conclusion. We propose that the rolling-over maneuver (ROM) is as effective as the canalith repositioning maneuver (CRP) for the treatment of benign paroxysmal positional vertigo (BPPV). ROM involves easy movements, with only a small load. This therapy is suitable for most BPPV patients, even for those without an indication for CRP. *Objectives.* BPPV is a common vestibular disorder. CRP is known to be an effective therapy for the treatment of BPPV. Because of its various movements of the head and body, it is impossible to perform CRP in BPPV patients with orthopedic impairments or in the elderly. For these patients, we perform a maneuver called ROM, which involves easy movements. In this study, we compared the efficacy of ROM with that of CRP in patients with posterior semicircular canal-type BPPV. *Patients and methods.* The study included 22 patients with BPPV who were randomized and divided into the following 2 groups: 1) those treated by the modified Epley maneuver as CRP; and 2) those treated by ROM. *Results.* We found no significant difference between the two groups in the number of days from onset to remission of both nystagmus and vertigo.

Keywords: *BPPV, canalith repositioning procedure, Epley maneuver, vestibular rehabilitation, habituation training*

Introduction

Benign paroxysmal positional vertigo (BPPV) is a common vestibular disorder characterized by brief episodes of vertigo triggered by changes in head position, and in most cases physical therapy is effective. Its etiology is thought to be cupulolithiasis or canalolithiasis [1–3]. On the basis of the canalolithiasis hypothesis, free-floating debris in the semicircular canal acts like a plunger, and causes continued movement of the endolymph even after head movement has ceased. This causes bending of the cupula to evoke positional nystagmus and vertigo; therefore, the canalith repositioning maneuver (CRP) is performed as the treatment of BPPV. Recently, the Epley maneuver for the treatment of patients with BPPV of the posterior semicircular canal type has been increasingly performed as a CRP [4–6]. Because CRP requires various movements of the head and body, it is impossible to perform in BPPV patients with movement disability or in the elderly. Banfield et al. [7] reported that habituation training was also effective for BPPV. The Brandt-Daroff maneuver is another therapy for BPPV patients [8] but these maneuvers cannot be performed in patients with neck or spine disorders. For these patients, our group established a therapy called the rolling-over maneuver (ROM), that requires only very simple movements [9,10]. Sato and Koizuka [10] reported that when they performed ROM for BPPV patients, nystagmus and vertigo disappeared within a week in 90% of patients, and all recovered within 2 weeks. In this study, we compared the efficacy of ROM with that of CRP in patients with posterior semicircular canal-type BPPV.

Patients and methods

The present study included 22 patients with posterior semicircular type BPPV as diagnosed according to the following criteria. (1) Absence of an identifiable central nervous system disorder that would

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explain positional vertigo at neurologic examination and in neurophysiologic studies. (2) No spontaneous nystagmus. (3) History of brief episodes of positional vertigo. (4) The patients show direction-changing torsional positional nystagmus triggered by the Dix-Hallpike maneuver.

The eye movements of subjects who showed clear and typical positioning nystagmus under infrared charge-coupled device video Frenzel glasses were analyzed. This was a prospective study with patients randomized and divided into the following two groups. 1) Those treated by the modified Epley maneuver [11] as the CRP. This maneuver was performed at the first visit to the hospital. 2) Those treated by ROM [9,10]. ROM was performed at home every day.

In the modified Epley maneuver, the patient's head was turned 45° towards the affected side in a sitting position. The patient was then moved from a sitting position to a head hanging position. After resolution of the nystagmus, the head was rotated to the opposite side. Next, the head and body were rotated 135° from the supine position until the patient was facing downward. Finally, the patient slowly sat up.

The ROM is shown in Figure 1. This therapy involved moving the patient from a supine and nose-up position to a right ear-down head position (Figure 1A), and maintaining this position for 10 s before subsequently returning the head to a nose-up position, which was maintained for a further 10 s (Figure 1B). The patient was then moved to a left ear-down head position, which was maintained for 10 s (Figure 1C), before returning to the original nose-up position. The right and left ear-down positioning was performed with only head movement by neck torsion or whole body movement without neck torsion, depending on the capabilities of the patients (Figure 1A' and C'). Patients repeated these maneuvers 10 times in 1 set, and 2 sets a day (before getting up in the morning and before sleeping at night).

All patients were required to return to the hospital every week after the initial visit. At every visit, they were interviewed and examined using the Dix-Hallpike positioning nystagmus test. After the disappearance of positional nystagmus, patients were asked to indicate the time at which the symptom of positional vertigo disappeared. The patients' characteristics were statistically analyzed with the Mann-Whitney U test and $p<0.05$ was considered significant. Residual rates of nystagmus and symptoms were calculated after the first visit to our hospital with the Kaplan-Meier method. The differences between the two groups of patients in remission curves were analyzed using the log-rank test, and values of $p<0.05$ were considered significant.

This study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 1983, and all procedures were carried out with the adequate understanding and written consent of the subjects, and approved by the review board of St Marianna University School of Medicine.

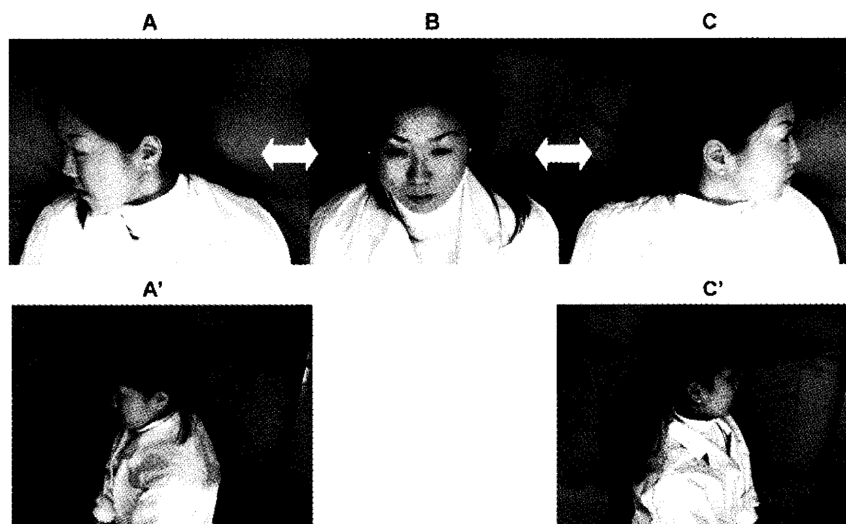

Figure 1. How to perform the rolling-over maneuver (ROM). Patients lying on a bed move to the right ear-down head position for 10 s (A), then maintain the nose-up position again for 10 s (B). Next, patients turn their heads to the left ear-down position for 10 s (C), and finally return to the nose-up position (B). Whole-body movements without neck torsion were suitable for patients with neck movement disabilities, and neck torsion movements in the supine position were suitable for patients with trunk movement disabilities. The former patients can choose (A') instead of (A), and (C') instead of (C). Patients should repeat this maneuver 10 times in 1 set, with 2 sets a day at home. (The person in these photographs gave us written informed consent for publication.)

Results

Twelve patients with BPPV (six males and six females; age range 28–77 years, mean age 53.6 ±17.7 years) were treated with CRP. Most (n =10) were patients with idiopathic BPPV without any fundamental vestibular events. The remaining two patients had a past history of sudden deafness or vestibular neuritis. Three of 12 had recurrent BPPV. Ten patients with BPPV (two males and eight females; age range 54–77 years, mean age 63.6 ±8.5 years) were treated with ROM. In these 10 patients, 1 had a menopausal disorder, which is a known cause of BPPV [12,13]. Two of the 10 had recurrent BPPV. None of the patients in this study had cervical or thoracic spine problems. There was no difference in age distribution between the two groups. The number of days from onset to the remission of nystagmus was 16.1±12.8 in the CRP group and 12.0±6.6 in the ROM group (Figure 2A). The number of days from onset to the remission of vertigo was 13.8±13.4 in the CRP group and 7.6±2.6 in the ROM group (Figure 2B). There was no significant difference between the two groups in the number of days from onset to the remission of both of nystagmus and vertigo (Figure 2 and 3). During the 1-year observation period, BPPV recurrence was noted in only one patient in the CRP group.

Discussion

The recovery period in patients with BPPV treated with both CRP (modified Epley maneuver) and ROM was about 2 weeks in this study. It is known that BPPV recovers spontaneously in about 2–6 weeks [14]. Imai et al. [15] investigated the natural course of vertigo remission in BPPV patients, and reported that the posterior semicircular canal type required 39 days (n =69) on average. Randomized control studies have shown the efficacy of the Epley maneuver [5,6]. Sekine et al. [16] compared the efficacy with the Epley maneuver and the natural course of remission in BPPV patients. They reported the residual rate of positional vertigo in untreated and modified Epley maneuver-treated patients with posterior semicircular canal-type BPPV: 51% of untreated patients and 22% of treated patients at 1 week, and 20% of untreated patients and 9% treated patients at 1 month. After the modified Epley maneuver, the remission of positional vertigo in BPPV patients was significantly quicker than in untreated patients. In the present study, there was no significant difference in efficacy between the CRP and ROM groups, suggesting that ROM is as effective as CRP for the treatment of BPPV.

CRP is a standard treatment for BPPV; however, there are BPPV patients for whom this procedure is not suitable. Because CRP requires many head and body position changes and movements, it is not suitable for patients with motion disability or elderly patients. There are some previous reports of vestibular habituation training as therapy for BPPV [7,8,17]. Norré reported vestibular habituation training and speculated that rehabilitation exercises stimulate the central adapting mechanism [17]. We hypothesized that ROM mechanically promotes loosening of the otolithic debris from the cupula and dispersion of the debris into the canal. The fatigability of vertigo in the patients with BPPV

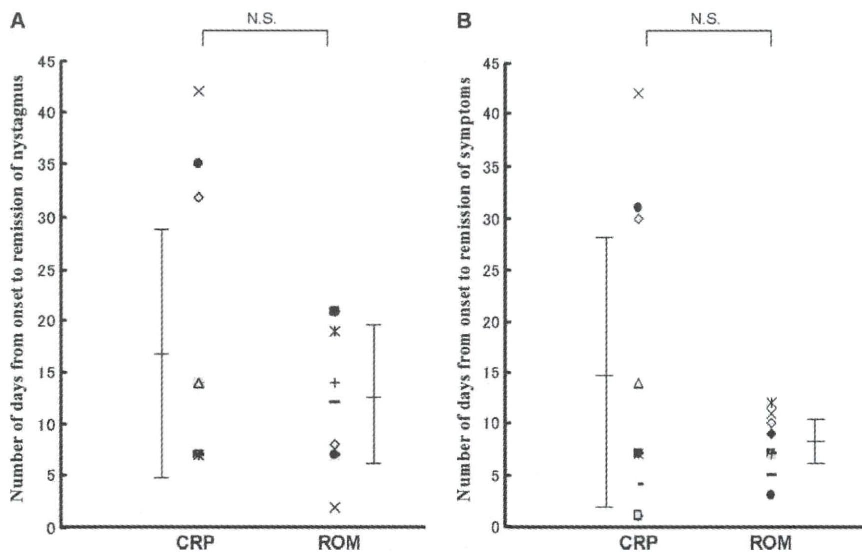

Figure 2. Comparison of number of days from onset to the remission of nystagmus (A) and vertigo (B). There was no significant difference between efficacy in the CRP group and the ROM group. CRP, canalith repositioning procedure; ROM, rolling-over maneuver.

during individual sessions was too rapid for a habituative central mechanism that required hundreds of repetitions and longer term. Using the whole membranous labyrinth model of bull frogs, Otsuka and Suzuki noted that the otoconia are separated into some small pieces within the canal upon changing the position of the model of canalolithiasis. Their findings supported our hypothesis of dispersion of the debris in the canal when the patients changed their head positions [18]. They also observed that the otoconia attached to the base of the crista or the otoconia occlude the canal, which can be the cause of the intractable BPPV. In the Brandt-Daroff maneuver, the patient is instructed to sit on the side of an examining couch and then tilts to lie alternately on their affected and unaffected sides [8]. Brandt and Daroff reported that positional vertigo resolved within 14 days, and considered the mechanism to be a mechanical loosening and dispersion of otolithic debris from the cupula. A prospective comparison of the Epley maneuver with vestibular habituation training involving 60 patients found the 2 methods to have equal efficacy [19]. In contrast, Sato et al. [9] examined BPPV patients with orthopedic disorders, and assessed whether the ROM and Brandt-Daroff maneuver can be used as physical therapy for these patients. They found that the Brandt-Daroff maneuver could not be performed in those patients because of their cervical or thoracic spine problems, although their symptoms and signs improved within 14 days of ROM treatment.

Even patients without movement impairment are sometimes anxious when performing the Brandt-Daroff maneuver at home because of the large movement of the upper body, and those patients tend to stop performing the exercise at home. On the other hand, ROM involves only easy movements, namely rolling over in bed; therefore, compliance is relatively good and patients can continue this exercise every day at home.

The residual rate of nystagmus and vertigo was slightly higher in the patients treated with CRP in the present study (Figure 3). One of the reasons for this may be the frequency of the therapy. That is, the therapist should perform CRP at the hospital, although the patients themselves could perform ROM at home every day. A review found that the Epley maneuver is effective for BPPV but does not result in long-term resolution of symptoms [20]. Sekine et al. [16] reported that the Epley maneuver was effective within a month, but its efficacy gradually decreased. These previous reports support our results. BPPV is recognized as a disorder with a high recurrence rate, although we could not follow the patients for more than a year in this study. Sato

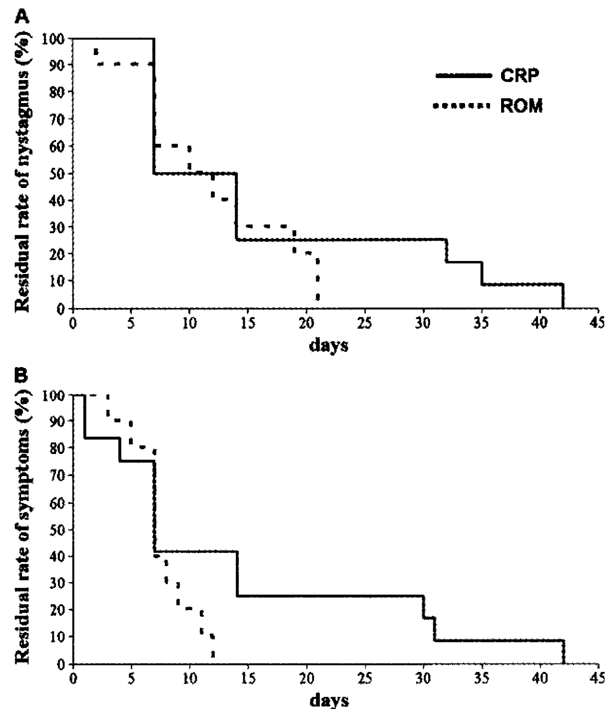

Figure 3. Residual rate of nystagmus (A) and vertigo (B). Solid line, patients treated with CRP; dotted line, patients treated with ROM.

and Koizuka [10] followed 12 patients with BPPV after resolution of the disorder. They reported a recurrence rate of 50%, and all patients with recurrence had voluntarily stopped performing ROM. The remaining half who did not show recurrence for more than a year all continued ROM at home; therefore, we suggest that continuing ROM even after resolution of BPPV may decrease the recurrence rate. Dispersing the otolithic debris when it appears in the canal again and preventing its accumulation by continuing ROM may decrease the recurrence rate of BPPV [18]. In conclusion, we propose that ROM is as effective as CRP for the treatment of BPPV. ROM involves very easy movements with only a small load, compared with other physical therapies. Therefore, this approach is suitable for most BPPV patients, even for those in whom CRP is not indicated, such as patients with orthopedic impairments or in the elderly.

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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ORIGINAL ARTICLE

Polymorphic analysis of the heat-shock protein 70 gene (HSPA1A) in Ménière's disease

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Abstract

Conclusions. The single nucleotide polymorphism (SNP) 190 G/C in *HSP70-1* (HSPA1A) was found to be a factor associated with Ménière's disease (MD). It is suggested that SNP 190 G/C could be scientific evidence to prove the relation between MD and stress as a trigger factor. *Objective.* Although it is widely known that MD might be caused by psychological stress, definitive scientific evidence has not been established. To understand an association between stress and MD, we analyzed the SNP of *HSP70-1* (HSPA1A) in patients with MD. *Subjects and methods.* We enrolled 49 patients given a diagnosis MD, consisting of 20 men and 29 women. Controls were 100 normal subjects. We isolated genomic DNA from peripheral leukocytes with the PAX gene Blood DNA kit (Qiagen®), and determined the SNP of HSPA1A. All subjects were investigated as regards hearing level and vertigo attacks, and were evaluated as to the profile of mood states (POMS) concerning psychological stress. *Results.* A SNP located on position 190 was found within a regulatory region in HSPA1A, and the frequency of having at least one 190C allele was significantly higher in the MD patients than the controls ($p <$ 0.001).

Keywords: *Ménière's disease, psychological stress, gene polymorphism, single nucleotide polymorphisms, heat-shock protein 70, HSP70-1 gene, HSPA1A, POMS*

Introduction

Ménière's disease (MD) is an inner ear disorder with various recurrent symptoms such as hearing loss, tinnitus, and vertigo. It has been suggested that MD is related to a pathologic endolymphatic hydrops of the inner ear, but the etiology is still unknown. Some MD patients have a large number of attacks and progressive hearing impairment. Although the scientific evidence for the cause has not been sufficiently established, it is well known that these patients with severe symptoms often have some mental stress [1–3]. Stress-related hormones have been measured to assess the presence of stress. However, it is difficult to evaluate the stress responses with MD patients by stress-related hormones, because various factors affect the hormonal reaction.

In general, the stress response appears to increase the expression of heat-shock proteins (HSPs) in cells. HSPs act to protect cells against various kinds of stress such as oxidative free radicals and toxic metal ions [4–6]. HSP70 proteins function as molecular chaperones and are involved in cell survival under stress condition [7]. Three genes encoding the HSP70 are located in the human leukocyte antigen (HLA) class III region on the short arm of chromosome 6 [8]. These genes are defined as *HSP70-1* (HSPA1A), *HSP70-2* (HSPA1B), and *HSP70-Hom* (HSPA1L) [9]. HSPA1A and HSPA1B encode an identical protein, which is the major heat-inducible HSP70 [10]. HSPA1L encodes a protein that is not induced by heat shock. The sequences of these three genes have been determined. Eight polymorphisms are identified within these three genes; three each in HSPA1A and HSPA1B, and two in HSPA1L [9]. The polymorphisms are at positions −110, 190, and 438 in the HSPA1A.

The development of biogenetics allows genetic association studies of complex diseases, especially using single nucleotide polymorphisms (SNP). We

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