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Expession of Aquaporin-2 in the Endolymphatic Sac of the Rat

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Aquaporins (AQPs) are water-specific membrane channel proteins, which have also been identified in the inner ear. AQP2 is a vasopressin-regulated member of the aquaporin gene family. Although the role of AQP2 in the kidney has been well defined, its role in hearing remains to be determined. In this study, we examined the AQP2 expression pattern in the postnatal rat endolymphatic sac (ES) using fluorescent microscopical immunostaining. AQP2 has been detected in wholemount preparations of the *saccus endolymphaticus* at cellular and subcellular level. The presence of AQP2 in the endolymphatic sac strongly suggests its functional role in the endolymph volume regulation. Future experiments may have direct implications on the pharmacological treatment of inner ear diseases associated with idiopathic endolymphatic hydrops.

Key words: aquaporin-2, endolymphatic sac, vasopressin, endolymph volume regulation, endolymphatic hydrops.

Introduction

Aquaporins (AQPs) act as water-specific membrane channel proteins. They have been identified in organs with active water metabolism. The rapid movement of water across cell membranes is fundamental for the normal inner ear function. The inner ear labyrinth is divided into two distinct fluid compartments designated as perilymphatic and endolymphatic fluid spaces. The complex tubular system of the endolymphatic fluid space runs along the cochlear and vestibular parts of the inner ear. Various aquaporins have been described to be localized in the epithelia and connective tissues surrounding the endolymphatic fluid space and are implicated in the endolymph volume regulation in various anatomical sites of the inner ear [1, 2].

Of the eleven mammalian aquaporins seven have been shown to be expressed in the inner ear. These aquaporins include AQP1, AQP2, AQP3, AQP4, AQP5, AQP7 and AQP9 and demonstrate predominantly nonoverlapping cellular and subcellular distributions. All the seven aquaporins are described in the cochlea. AQP1 is found in a subset of fibrocytes in the spiral ligament near the bony capsule [2, 7], AQP2 in Reissner's membrane [5], AQP3 in a subset of fibrocytes in the spiral ligament near the basilar

membrane [2], AQP4 in the basolateral membrane of the supporting cells in the organ of Corti and inner sulcus cells [10], AQP5 in outer sulcus cells and epithelial cells of the spiral prominence [4, 6], AQP7 in Reissner's membrane and the *stria vascularis* [2] and AQP9 in Reissner's membrane and interdental cells of the spiral limbus [2].

Although the role of AQP2 in the kidney has been well defined, its role in hearing remains to be determined. AQP2, the vasopressin-regulated water channel, was initially considered to be expressed exclusively in the renal collecting duct. AQP2 has since been identified in the adult rat cochlea [5] and also reported to be expressed in the epithelium of the endolymphatic sac (ES), considered to be important in regulating the volume of the endolymph [3].

This study reports the cellular and subcellular localization of AQP2 in the endolymphatic sac of a postnatal rat.

Materials and Methods

Tissue preparations

Wistar rats (postnatal day 4 (P4)) from the same litter were anaesthetized and then decapitated. Temporal bones were removed immediately and placed in cold (4°C) HHBSS (HEPES-buffered saline with Hank's balanced salt solution). *Dura mater* was gently removed from the ES and the specimen were fixed in ice-cold 4% paraformaldehyde for 30 min over ice and washed in phosphate buffered saline (PBS). The endolymphatic sacs were then removed and placed in 24-well plate with PBS.

Immunofluorescence study

The wholemount preparations were permeabilized in 0.5% Triton X-100 in PBS for 10 min, followed by 30 min preincubation in 4% NGS (Normal Goat Serum), 0,1% Triton X-100 in PBS. The samples were then incubated with a polyclonal rabbit anti-aquaporin-2 antibody (Chemicon) diluted 1:200 in 0,1% NGS, 0,1% Triton X-100 in PBS overnight at 4°C. Negative controls were incubated in the dilution buffer in the absence of AQP2 antibody. The reaction was visualized by fluorescein-conjugated goat anti-rabbit secondary antibody (Alexa Fluor 488, Molecular Probes), diluted 1:400 in the same dilution buffer for 60 min in the dark at room temperature. After washing with PBS the samples were counterstained with F-actin marker Alexa Fluor®568 phalloidin (Molecular Probes). The nuclei were stained with DAPI (4′-,6-diamidino-2-phenylindol, 1μg DAPI/ml PBS) for 5 min in the dark at room temperature. The wholemounts were covered with FluorSave™Reagent (Calbiochem). Microscopic analyses were made using a Zeiss Axioplan 2 epifluorescence microscope or a Zeiss 510 Meta laser scanning microscope

Results

Immunofluorescence staining of wholemount preparations of the rat endolymphatic sac using an anti-AQP2 antibody demonstrated the presence of this water channel in the epithelial cells of the ES (Fig. 1). We used F-actin counterstaining in order to detect the cell borders.

At subcellular level AQP2 was localized in the cytoplasm and in the basolateral membrane of the endolymphatic sac cells (Fig. 2), which can be seen well on the AQP2-phalloidin merged image (Fig. 2C).

AQP2 immunoreactivity was absent in the *stria vascularis*, Reissner's membrane and the organ of Corti (data not shown).

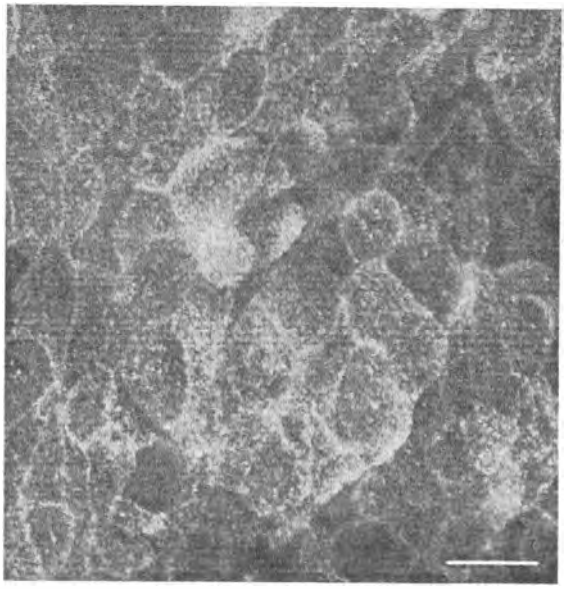

Fig. 1. Epifluorescence microscopic analysis of the AQP2 (green) expression in wholemount preparations of postnatal rat endolymphatic sac. Counterstaining of F-actin (red) serves as a marker for cellular outlines. The nuclei are labeled with DAPI (blue). Scale bar = 10 μm

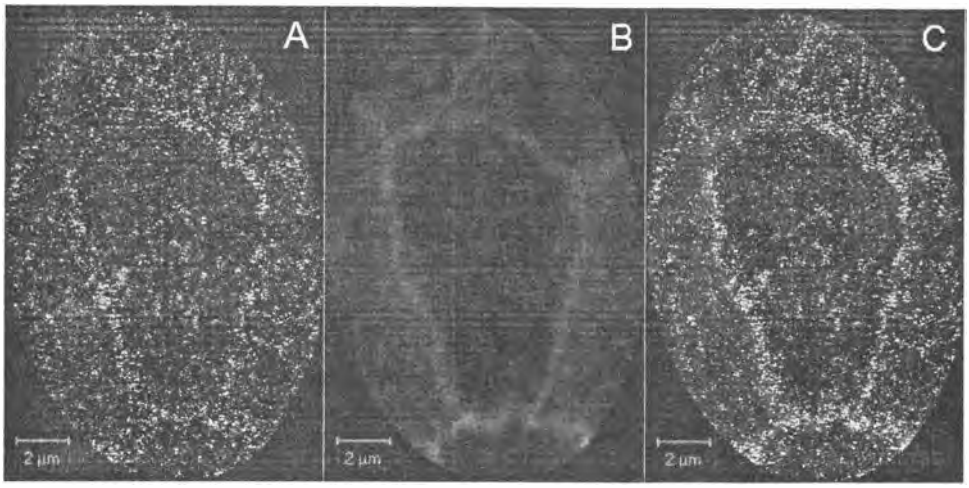

Fig. 2. Laser scanning microscopic analysis of the AQP2 (green) expression in wholemount preparations of postnatal rat endolymphatic sac. At subcellular level AQP2 (A) is localized in the cytoplasm and the cell membrane as shown in the merged image (C) of AQP2 (A) and F-actin counterstaining (B). Scale bar = 2 μm

Discussion

This study reports the AQP2 expression pattern in the endolymphatic sac of the rat. The ES epithelium is thought to resorb the endolymph fluid. The strategic localization of AQP2 in cell types that interface with the endolymph supports its potential role in the homeostasis of the inner ear fluids, particularly as it relates to volume regulation of the endolymph. Thus, dysfunction or abnormal downregulation of AQP2 function can directly contribute to the etiology of endolymphatic hydrops, the histopathologic hallmark of Menière's disease. Menière's disease is of unknown origin. It is clinically defined by a triad of symptoms consisting of hearing loss, tinnitus and episodic vertigo. Previous studies of Menière's disease demonstrate that the vasopressin serum concentration is significantly elevated in patients with endolymphatic hydrops [9]. In the kidney vasopressin regulates water retention. This effect is mediated by the V_2-receptor (V_2-R). Upon binding of vasopressin to the receptor, AQP2 is translocated into the apical cell membrane of the collecting duct principal cells. The endolymphatic sac and other inner ear structures have been suggested as further targets for vasopressin, as based on several lines of experimental evidence, from both *in vitro* and *in vivo* studies. First, the V_2-R and AQP2 have been detected by various methods in the inner ear and in particular the ES [3, 5]. Second, binding of radioactive vasopressin could be demonstrated in human ES tissue. Third, vasopressin-dependent membrane turnover could be antagonized by a V_2-R-antagonist in rat endolymphatic sac organotypic culture preparations [3] and forth, endolymphatic hydrops could be induced by exogenous application of vasopressin in the guinea pig [3, 5, 9]. Furthermore, specific V_2-receptor antagonists have been shown to antagonize the effects of vasopressin *in vitro* [3] and surgically induced endolymphatic hydrops *in vivo* [8]. Future experiments may have direct implications on the pharmacological treatment of inner ear diseases associated with idiopathic endolymphatic hydrops such as Meniere's disease.

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References

1. Beitz, E., H. P. Zenner, J. E. Schultz. Aquaporin-mediated fluid regulation in the inner ear. – Cell. Mol. Neurobiol., **23**, 2003, 315-329.
2. Huang, D., P. Chen, S. Chen, M. Nagura, DJ. Lim, X. Lin. Expression patterns of aquaporins in the inner ear: evidence for concerted actions of multiple types of aquaporins to facilitate water transport in the cochlea. – Hear. Res., **165,** 2002, 85-95.
3. Kumagami, H., H. Lowenheim, E. Beitz, K. Wild, H. Schwartz, K. Yamashita, J. Schultz, J. Paysan, H. P. Zenner, J. P. Ruppersberg. The effect of anti-diuretic hormone on the endolymphatic sac of the inner ear. – Pflugers Arch., **436**, 1998, 970-975.
4. Mhatre, A. N., S. Steinbach, H. Kambridge, A.T.M. Shamsul Hoque, A. K. Lalwani. Identification of aquaporin-5 (AQP5) within the cochlea: cDNA cloning and in situ location. – Biochem. Biophys. Res. Comm., 264, 1999, 157-162.
5. Mhatre, A. N., R. E. Stern, J. Li, A.K. Lalwani. Aquaporin-4 in the mammalian inner ear and its role in hearing. – Biochem. Biophys. Res. Comm., **297**, 2002, 987-996.
6. Penkova, Zh., B. Hirt, M. Mueller, H. Loewenheim, P. Dimov, N. Lazarov. Localization of aquaporin-5 in the cochlea. – Trakia J. Sci., **6**, 2008, 28-31.
7. Stankovic, K. M., J. C. Adams, D. Brown. Immunolocalization of aquaporin CHIP in the guinea pig inner ear. – Am. J. Physiol., **269**, 1995, 1450-1456.
8. Takeda, T., S. Sawada, S. Takeda, H. Kitano, M. Suzuki, A. Kakigi, S. Takeuchi. The effects of V2-antagonist (OPC-31260) on endolymphatic hydrops. – Hear. Res., **182**, 2003, 9-18.
9. Takeda, T., S. Takeda, H. Kitano, T. Okada, A. Kakigi. Endolymphatic hydrops induced by chronic administration of vasopressin. – Hear. Res., **40**, 2000, 1-6.
10. Takumi, Y., E. A. Nagelhus, J. Eidet et al. Select types of supporting cell in the inner ear express aquaporin-4 water channel. – Eur. J. Neurosci., **10**, 1998, 3584–3595.