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Aquaporins in the Inner Ear: Distribution and Pathophysiological Implications

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Aquaporins (AQPs) are a family of transmembrane proteins that act as highly selective water channels. They are likely to play a significant role in fluid homeostasis of the auditory and vestibular organs. Seven aquaporin subtypes have been identified in the mammalian inner ear. AQP4 is expressed selectively in the supporting epithelial cells in the organ of Corti and has a potential role in the process of potassium recycling. Regarding a possible secretion function AQP5 is expressed in the lateral wall of the cochlear duct with a base to apex gradient in adult rats. The localization of AQP2 in the endolymphatic sac suggests its possible role in reabsorption of endolymph. It is to be expected that lesions in aquaporin genes or acquired dysfunction in aquaporins may cause or contribute to several disease states. There are opportunities for indirect influence of the aquaporin function through receptor dependent modulation.

Key words: aquaporin, inner ear, endolymph, fluid homeostasis.

The precise regulation of water transport across the cell membrane is essential for the normal organ function. Water is known to diffuse through lipid bilayers, but this is not sufficiently rapid for many physiological processes. The discovery of a family of channel proteins has provided a molecular explanation for the fast water transport across biological membranes. These proteins, termed "aquaporins" (AQP) [1] are found in all life forms.

Aquaporins are small integral membrane proteins that function as water transporters. Each 28-kDa subunit in the aquaporin homotetramer contains an individual aqueous pore. It consists of 6 transmembrane helices and 2 shorter helices (hemipores), that fold into the membrane from the opposite sides of the bilayer, thus forming a way through it, where they are surrounded by the other 6 transmembrane helices [11]. Water permeability through aquaporins is driven by osmotic gradients. The family of mammalian aquaporins consists of 11 members (AQP0-10) [4, 5, 6, 7, 9, 10, 29]. These isoforms show significant difference in function, subcellular localization and distribution in different cell types and tissues.

Aquaporins in the inner ear

Aquaporins are distributed in organs with active water metabolism [7]. Recently, the existence of water channels and their function have been elucidated in the inner ear. The fluids of the inner ear, endolymph and perilymph, are involved in mechano-electrical signal transduction in the cochlear and vestibular organs. The cochlea is divided into three compartments by the membranous structures: scala media, scala vestibuli and scala tympani. Scala media, enclosed within Reissner's membrane and the basilar membrane, contains endolymph, a hypertonic fluid with high K^+ concentration. The continuous fluid space represented by scala vestibuli and scala tympani is filled with perilymph, a near-isotonic fluid rich on Na^+. Homeostasis of these fluid spaces is critical for the normal function of the auditory and vestibular organs [2, 23]. Seven aquaporin subtypes have been identified to date in the inner ear. AQP1, AQP2, AQP3, AQP4, AQP5, AQP7 and AQP9 show a somehow specific cellular localization, which means they play a possible role in the inner ear fluid homeostasis. AQP1 is found in a subset of fibrocytes in the spiral ligament near the bony capsule [3, 22]. AQP2 in Reissner's membrane [15], AQP3 in a subset of fibrocytes in the spiral ligament near basilar membrane [3], AQP4 in the basolateral membrane of the supporting cells in the organ of Corti and inner sulcus cells [27], AQP5 in outer sulcus cells and epithelial cells of the spiral prominence [14], AQP7 in Reissner's membrane and stria vascularis [3] and AQP9 in Reissner's membrane and interdental cells of the spiral limbus [3].

It has been proposed that aquaporins compensate for osmotic gradients induced by transcellular K^+ flow through the gap junction system of cells surrounding the cochlear endolymphatic fluid space [3, 13, 16].

AQP4 has a potential role in the process of potassium recycling

AQP4 is expressed selectively in the basolateral membrane of Deiter's cells, Hensen's cells, Claudius cells and in the basal membrane of the inner sulcus cells [13, 27], shown also by our results in the rat cochlea (Fig. 1A). The physiological role of AQP4 is rapid osmotic equation of the potassium current during the mechano-electrical transduction. The sole lack of AQP4 in AQP4-knockout mice can lead to hearing loss depending on the genetic background [13, 16]. The functional loss can be explained with the disturbance of the potassium recirculation through the supporting cells in the cochlea. This is the first direct evidence that an aquaporin plays a role in hearing. Among the water channels, AQP4 has the highest permeability potential and is also expressed in the membranes of cells with high potassium current in the central nervous system [19, 21, 28] and the retina [17]. In the kidney, AQP4 is expressed in the basolateral membrane of the collecting duct cells (Fig. 1B, C).

AQP5 and AQP2 play a potential role in inner ear fluid homeostasis

The endolymphatic hydrops is the most well-known volume regulation disorder of the inner ear. It is described histopathologically in different inner ear diseases, especially Morbus Menière. The occurrence of endolymphatic hydrops can be explained with an overproduction or a reduced absorption of endolymph. Along with this theory, a potential

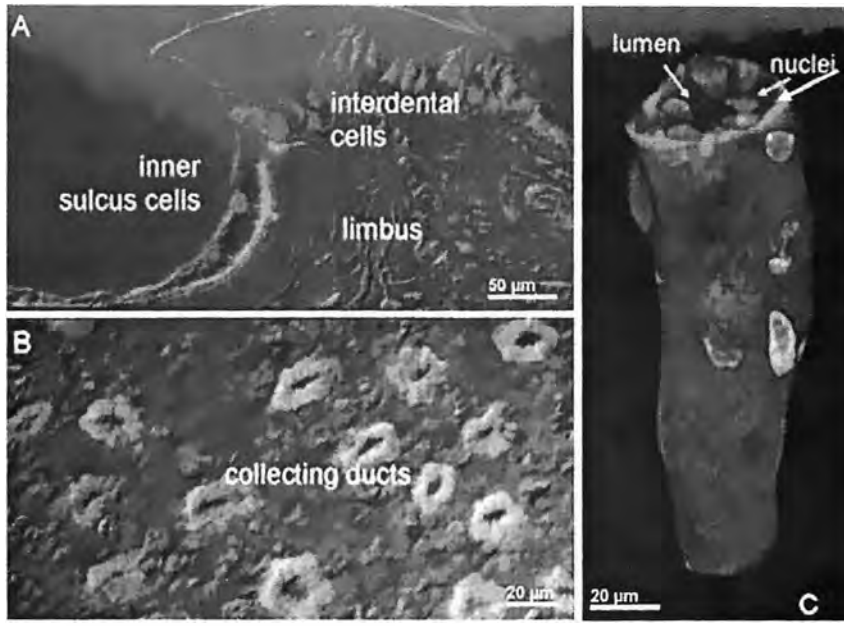

Fig. 1. Immunolocalization of AQP4 (red)
A — AQP4 in the basal membrane of the inner sulcus cells in the rat cochlea; B — AQP4 in the basolateral membrane of the kidney collecting duct cells; C — AQP4 immunolabelling in microdissected kidney collecting duct — basolateral expression

role of AQP5 and AQP2 in the pathogenesis of the endolymphatic hydrops is also discussed.

AQP5 has been originally cloned from the rat submandibular gland [20] and later described also in the other salivary glands, like parotid glands (Fig. 2), lacrimal gland, cornea, lower airway epithelium and the submucosal glands of the upper airway [18, 20]. This tissue distribution has implied a secretory role for AQP5.

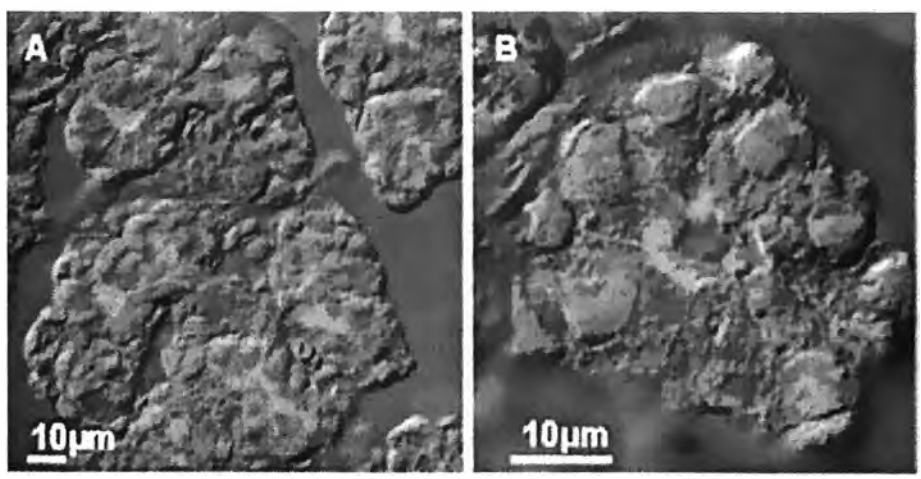

Fig. 2. Immunolabelling of AQP5 in glandula parotis of a rat
A — AQP5 positive labelled acini; B — subcellular localization of AQP5 in the apical plasma membrane. AQP5-red, DAPI (nuclear staining)-blue

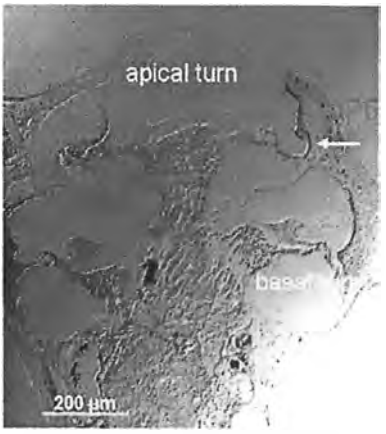

Fig. 3. Immunolocalization of AQP5 (red) in the rat cochlea — a sagital cryosection. Note that the AQP5-signal is only in the apical turns of the cochlea, while the basal turns are AQP5-negative

Regarding a possible secretion function, AQP5 is expressed in the lateral wall of ductus cochlearis, in the outer sulcus cells and the cells of the spiral prominence just below the *stria vascularis* [14]. Interestingly, in adult rats AQP5 is found only in the apical turn of the cochlea, but not in the basal (Fig. 3).

Biochemical studies reveal base to apex gradients in the composition of endolymph possibly reflecting different requirements in high versus low frequency hearing [23]. Among the cochlear aquaporins it is only AQP5 that has demonstrated such a base to apex gradient possibly reflecting a molecular basis for the observed biochemical differences [14]. The restricted expression of AQP5 suggests its potential role in low frequency hearing. This may be pathophysiologically related to the low tone hearing loss, observed in patients with endolymphatic hydrops. Nevertheless, auditory brain stem response thresholds in AQP5-knockout mice do not differ significantly from these in wild-type mice [13]. Taking in consideration the cholinergic effect of AQP5 in the salivary glands, it can be hypothesized that the secretion ratios in the apex of the cochlea are also cholinergically regulated. This has to be more closely investigated in future.

The localization of AQP2 in the endolymphatic sac (ES) [12] confirmed by our ongoing experiments (Fig. 4A, B) and in the cochlea [15] suggests its possible role in reabsorption of endolymph. In the kidney, AQP2 expression is regulated by the antidiuretic hormone (ADH), which mediates AQP2 trafficking from intracellular vesicles to the apical plasma membrane in the kidney collecting duct through vasopressin type 2 receptor (V_2-receptor). This receptor is also expressed in the endolymphatic sac [12].

This is indirect evidence for an ADH-dependent regulation of the AQP2 function. Malregulation of this ADH-AQP2 system results in endolymphatic hydrops, the morphological feature of Menière's disease. This assumption is supported by clinical and experimental evidence. Plasma levels of ADH are higher in patients with Menière's disease [24]. On the other hand, acute and chronic application of ADH produces endolymphatic hydrops in guinea pigs and rats [8, 12, 25]. Thus, the effect of ADH in the inner ear contrasts with that in the kidney and leads to a decreased fluid reabsorption [12]. Such an experimentally produced hydrops can be reduced by applying pharmacological V_2-receptor antagonists; respectively V_2-receptor antagonists can be used in the treatment of diseases

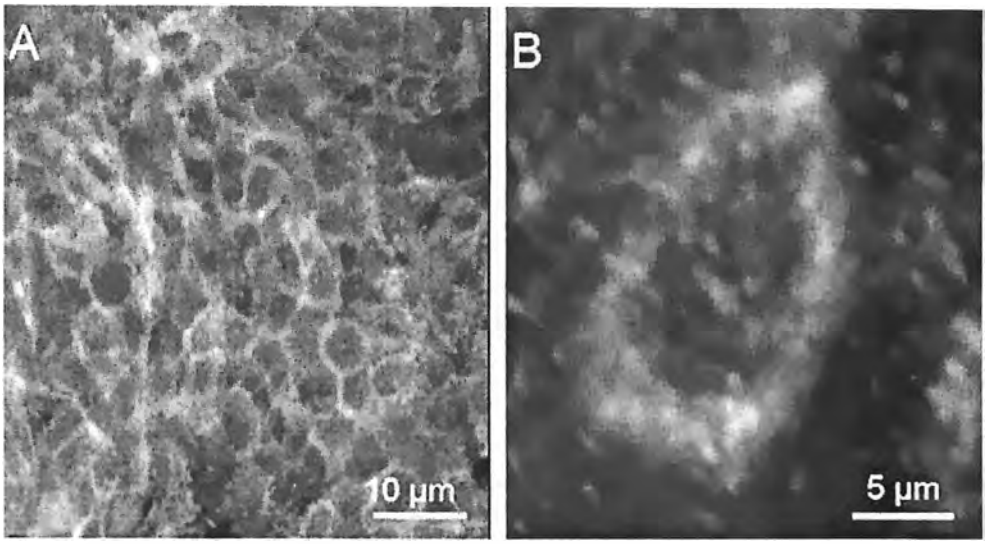

Fig. 4. Immunolocalization of AQP2 (green) in the endolymphatic sac. A — Cellular localization of AQP2 in the endolymphatic sac; B — Subcellular distribution of AQP2 (green) in the cell membrane and submembranous localization. The labelling of F-actin (red) shows the cell border. The nuclei are labelled with DAPI (blue)

with endolymphatic hydrops, including Morbus Menière [26]. Recently, various kinds of V_2-receptor antagonists have been developed. These substances (aquaretica) are already under clinical investigation.

Implications

Aquaporins are suspected in numerous disorders involving fluid transport. Taking in consideration the significance of the proper regulated water transport in the inner ear, it is to be expected that lesions in aquaporin genes or acquired dysfunction in aquaporins may cause or contribute to several disease states. There are opportunities for indirect influence of the aquaporin function through receptor dependent modulation - in the case of AQP5 through the cholinergic receptors of the parasympathic nervous system, and for AQP2 through the specific V_2-receptor subtype for the ADH. Further investigations on aquaporins may lead to the development of new therapeutics through rational drug design.

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