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Original Contribution

LOCALIZATION OF AQUAPORIN-5 IN THE COCHLEA

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ABSTRACT

Aquaporins (AQPs) are a family of small membrane proteins that function as water transporters. They are distributed in organs with active water metabolism such as the inner ear. Several aquaporin subtypes have been identified in the cochlea and have been proposed to play a role in hearing. In this study, to examine the tissue distribution and cellular localization of aquaporin-5 (AQP5) in the postnatal rat cochlea, we performed fluorescent microscopical immunostaining for AQP5 at different postnatal stages. We found AQP5 in the outer sulcus cells (OSC) and the cells of the spiral prominence along the entire length of the lateral wall for the early postnatal stages. AQP5 immunolabeling in adult rats was limited to the apical turn of the cochlear duct. Our results suggest a potential role of AQP5 in the fluid homeostasis of the developing and mature inner ear and a likely role in the low frequency hearing.

Key words: aquaporin-5, inner ear, ductus cochlearis, outer sulcus cells, endolymph;

INTRODUCTION

Water is the most abundant molecule in the human body and its proper distribution is required to maintain the fluid balance within different anatomical compartments. The discovery of a family of channel proteins, aquaporins (AQPs), has provided a molecular explanation for the fast water transport across biological membranes. Aquaporins form membrane pores selectively permeable for water. To date 13 members (AQP0-AQP12) of the growing family of mammalian water channels have been identified. They are divided into aquaporins (AQPO, AQP1, AQP2, AQP4, AQP5, AQP6 и AQP8), aquaglyceroporins (AQP3, AQP7, AQP9 и AQP10) and superaquaporins (AQP11 и AQP12). Several aquaporin subtypes have been localized in the mammalian inner ear and have been proposed to play a role in hearing [2, 5, 8, 9, 10, 14, 15, 17, 20, 22, 23].

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Aquaporin-5 (AQP5) was first cloned from the rat submandibular gland [19]. Subcellular localization of AQP5 in the salivary glands is described in the apical plasma membrane of acinar cells in rat [11, 18] and human [3]. Structurally, AQP5 is a homotetramer and each 28 kDa subunit consists of six transmembrane domains and two shorter helices, which enter the membrane from the opposite sides, thus forming a water-selective pore in the bilayer [6]. Regarding a possible secretion function, AQP5 was also demonstrated in the cochlea with a difference in tissue distribution and cellular localization in rat and mouse [12, 13].

The goal of this study was to examine the tissue distribution and cellular localization of AQP5 in postnatal and adult rat cochlea.

MATERIAL AND METHODS

Experimental animals

Wistar rats used in this study were obtained from Charles River and maintained in an animal facility with the permission of the Committee for Animal Experiments of the Regional Council of Tuebingen.

Tissue preparations

Wistar rats from the same litter were euthanized by $C0_2$ at postnatal day 4 (P4) and 32 (P32). The cochleae were dissected out of the temporal bone and perfused with an ice-cold 4% paraformaldehyde in phosphate-buffered saline (PBS) through the oval and round windows and a small fenestra in the apex of the cochlear bony capsule. They were then postfixed in the same solution for 30 min over ice and washed in PBS. For cryoprotection the cochleae were immersed in 30% sucrose and embedded in Tissue-Tek (Tissue-Tek Compound, Sakura). Midmodiolar cryosections were made with a Microm cryostat. Submandibular salivary glands were taken from the same animals as positive controls and processed in parallel.

Immunofluorescence study

Cryosections from the P4 and P32 cochleae and submandibular glands were permeabilized in 0.5% Triton X-100 in PBS for 10 min, followed by 30 min preincubation in 4%NGS, 0.1% Triton X-100 in PBS. The samples were then incubated with a polyclonal rabbit anti-aquaporin-5 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 AQP5 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. The nuclei were stained with DAPI (4'-,6- diamidino-2-phenylindol, lpg DAPI/ml PBS) for 5 min in the dark at room temperature. The cryosections were covered with FluorSave™Reagent (Calbiochem). Microscopic analyses were made using a Zeiss Axioplan 2 epifluorescence microscope.

RESULTS

Immunofluorescence staining of cryosections from the rat cochlea using an anti-AQP5 antibody demonstrated the presence of this water channel in the lateral wall of the cochlear duct (Fig. 1).

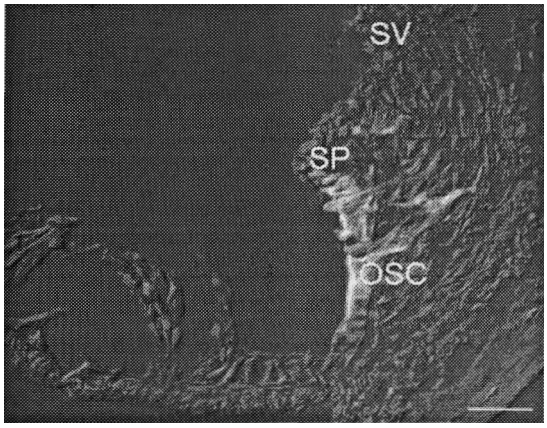

Fig. 1 **Immunofluorescent labeling of AQP5 (green) in the lateral wall of rat P32 cochlear duct (apical turn) in midmodiolar cryosections. The nuclei are labeled with DAPI (blue).**

SV - stria vascularis, SP - spiral prominence, OSC - outer sulcus cells. Scale bar = 20 pm.

AQP5 immunoreactivity was absent in the stria vascularis, Reissner's membrane and the organ of Corti. AQP5 signal was localized in the outer sulcus cells (OSC) and cells of the spiral prominence (SP), between stria vascularis and Claudius cells. Interestingly, AQP5 labeling was found along the entire length of the lateral wall for the early postnatal stages and only in the apical turn of the cochlear duct for the adult animals (Fig. 1), while the basal turn was AQP5 negative (Fig. 2).

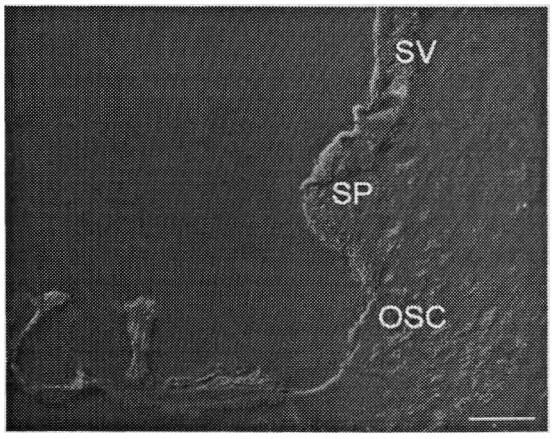

Fig. 2 **Immunofluorescent labeling of AQP5 in the lateral wall of rat P32 cochlear duct. The basal turn of rat P32 cochlear duct is AQP5 negative. The nuclei are labeled with DAPI (blue).**

SV - stria vascularis, SP - spiral prominence, OSC - outer sulcus cells. Scale bar = 20 pm.

DISCUSSION

AQP5 has been previously described in cells responsible for secretion of saliva, tears and pulmonary fluids [11, 18, 19]. We found AQP5 in the lateral wall of the cochlear duct, in the outer sulcus cells and cells of the spiral prominence with a base-to-apex expression gradient in adult rats. The outer sulcus cells are situated between the spiral prominence and the cells of Claudius. In adult rats their apical membrane is covered by Claudius cells [13]. The basal surface of these cells forms root processes in the spiral ligament. The outer sulcus cells are considered to play a role in the process of potassium recycling. They express the ion transporting enzyme **Na^+-K^+-ATPase** which activity decreases from the basal to the apical turns of the cochlea. Such diminution is also known for the osmotic gradients and electric potential gradients in the cochlear duct [1, 7, 16, 21]. These differences are probably a result of the elevated transport of **K^+ and Cl^-** in the basal cochlea. Among the aquaporins in the inner ear it is only AQP5 that shows such a base-to-apex expression gradient. This could be a sign of the molecular changes that lead to the observed biochemical differences [13]. The basal expression of AQP5 in the early postnatal stages and its loss in adult animals probably accompany the need of tightly regulated water transport in the specific functional regions of the cochlea where the increased ion transport generates electrochemical gradients and endocochlear potential in the developing inner ear [12]. AQP5 and the other aquaporins in the cochlea compensate for the osmotic gradients and thus play a potential role in the regulation of fluid homeostasis in the inner ear [4, 9].

The observed gradients in AQP5 expression could be a result of the different requirements in the low versus high frequency hearing regions of the cochlea [21]. According to the place-frequency map of the cochlea the sensory cells at the apical region where AQP5 expression is limited in adult rats are optimally responsive to the low frequency tones. The cells at the basal region are tuned to high frequency [13]. It can be speculated that AQP5 has a potential role in the low frequency hearing. However AQP5-knockout mice show normal inner ear morphology and hearing [9].

CONCLUSION

In this study we localized the tissue distribution and cellular expression of AQP5 in the cochlea. Our results suggest a potential role of this water channel in the fluid homeostasis of the developing and mature inner ear and a likely role in the low frequency hearing.

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