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Onset of aquaporin-4 expression in the developing mouse brain

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ABSTRACT

The main water channel in the brain, aquaporin-4 (AQP4) is involved in maintaining homeostasis and water exchange in the brain. In adult mammalian brains, it is expressed in astrocytes, mainly, and in high densities in the membranes of perivascular and subpial endfeet. Here, we addressed the question how this polarized expression is established during development. We used immunocytochemistry against AQP4, zonula occludens protein-1, glial fibrillary acidic protein, and β-dystroglycan to follow astrocyte development in E15 to P3 NMRI mouse brains, and expression of AQP4. In addition we used freeze-fracture electron microscopy to detect AQP4 in the form of orthogonal arrays of particles (OAPs) on the ultrastructural level.

We analyzed ventral, lateral, and dorsal regions in forebrain sections and found AQP4 immunoreactivity to emerge at E16 ventrally before lateral (E17) and dorsal (E18) areas. AQP4 staining was spread over cell processes including radial glial cells in developing cortical areas and became restricted to astroglial endfeet at P1–P3. This was confirmed by double labeling with GFAP. In freeze-fracture replicas OAPs were found with a slight time delay but with a similar ventral to dorsal gradient. Thus, AQP4 is expressed in the embryonic mouse brain starting at E16, earlier than previously reported. However a polarized expression necessary for homeostatic function and water balance emerges at later stages around and after birth.

1. Introduction

During development of the mammalian central nervous system, neurons are generated from neuroepithelial cells which in later phases go through a stage of radial glia (Götz and Huttner, 2005). One well studied function of radial glia cells is to guide newborn neurons to migrate from the ventricular zone to the appropriate arising layers, like in the cerebral cortex (Rakic, 1981; Hatten, 1999; Campbell and Götz, 2002). Some of the radial glial cells detach from the pial and ventricular surface and differentiate into astrocytes. These in turn become polarized in the sense that they face the superficial or perivascular glia limitans on one end, and neuropil including synapses and neuronal cell bodies on the other, expressing different proteins in either membrane domain. For example, the potassium channel Kir4.1, and the water channel aquaporin-4 (AQP4) occur in high densities in perivascular and superficial membrane domains; this is thought to facilitate potassium siphoning and is crucial to maintain homeostasis (Rash, 2010).

Before and during the time of transformation from radial to astroglial cells, blood vessels grow into the brain parenchyma. They are then surrounded by astroglial endfeet involved in inducing the blood–brain barrier (Risau and Wolburg, 1990; Engelhardt, 2003). This marks the polarization of astroglial membrane domains and the onset of regulation of ionic homeostasis in the brain that provides the environment for neuronal signaling. However, it is not clear whether the expression and distribution of AQP4 is directly influenced by this process.

In this study, we have set out to investigate the onset of expression and distribution of aquaporin-4 during mouse brain

Abbreviations: AQP4, aquaporin-4; OAP, orthogonal arrays of particles; GFAP, glial fibrillary acidic protein; ZO-1, zonula occludens protein-1.

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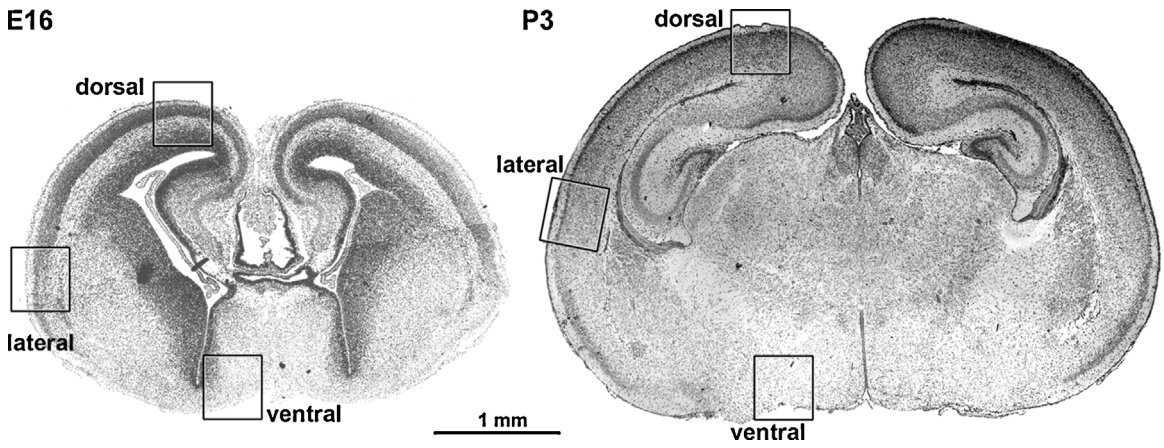

Fig. 1. Methylene blue stained section through the brain of a mouse at E16 (left), and P3 (right). The areas indicated by the squares represent the approximate regions analyzed in this study from E16 to P3.

development using immunocytochemistry and freeze fracture electron microscopy. The results show that anti-AQP4 immunoreactivity is detectable on astrocytic processes prior to its polarized occurrence on astrocytic endfeet. In freeze-fracture electron microscopy, astroglial membranes can be identified by orthogonal arrays of particles (OAPs) found in high densities on subpial and perivascular endfeet in the adult brain. OAPs have been shown to consist at least in part of AQP4 (Rash et al., 1998; Wolburg et al., 2011). We detected OAPs in low densities earlier than previously reported yet consistent with the immunocytochemical data appearing at different developing times depending on location.

2. Materials and methods

2.1. Animals

Brains from NMRI mice were prepared in the laboratory of Dr. H.C. Bauer, Salzburg University, Austria. The procedures were in accordance with the Austrian

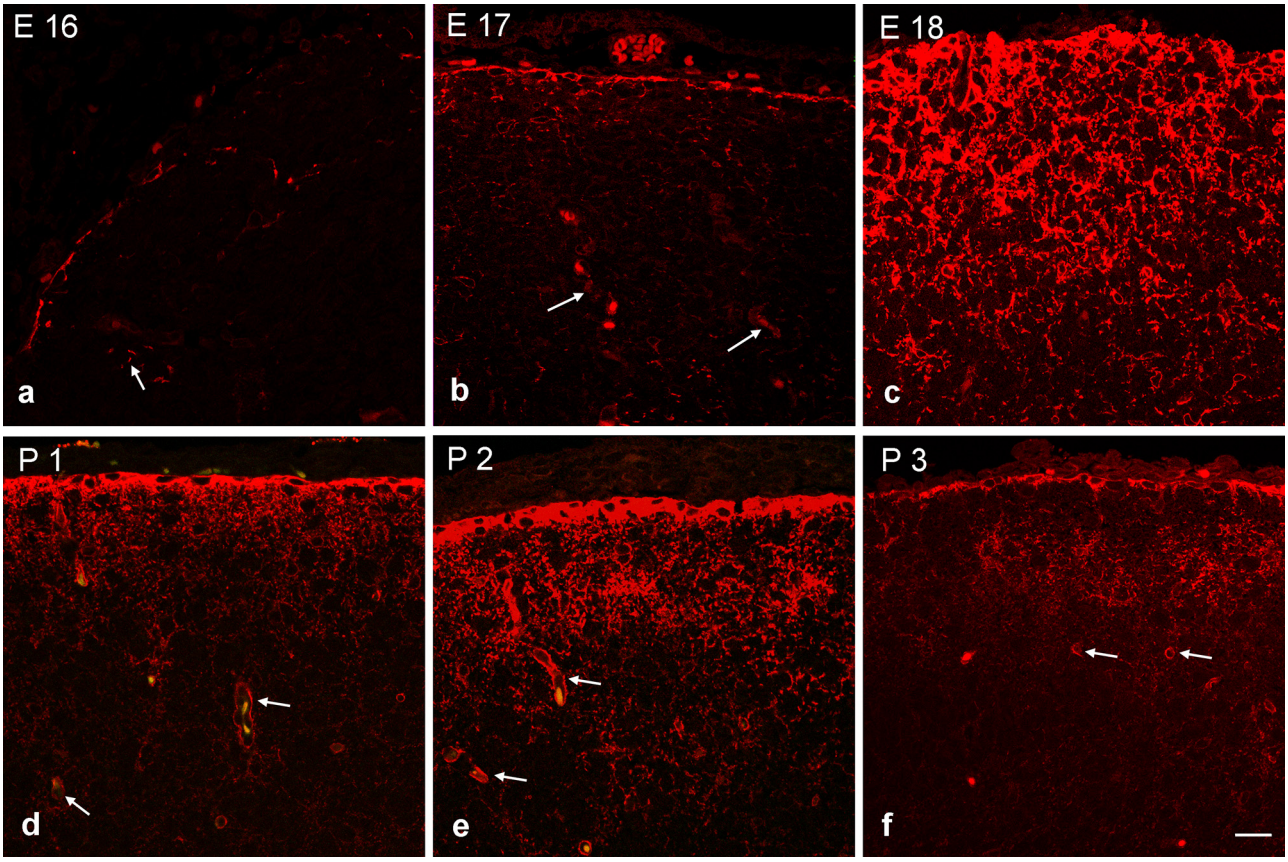

Fig. 2. Reactivity of AQP4 antibodies (red) in the ventral (basal) forebrain area/preoptic regions of coronal sections through the developing mouse brain at stages E16 to P3 (a–f) as indicated. Pial surface is up in all panels. At E16 (a), few positively stained processes were found often close to the pia mater which increased dramatically between E 17 and E 18 (b and c). From P1 to P3, AQP4 immunoreactivity distribution became increasingly restricted to subpial and perivascular endfeet (arrows). Note autofluorescent blood cells in the capillaries appear yellow. Scale bars = 20 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)