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DIFFERENTIAL EFFECT OF α-SYNTROPHIN KNOCKOUT ON AQUAPORIN-4 AND Kir4.1 EXPRESSION IN RETINAL MACROGLIAL CELLS IN MICE

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Abstract—Aquaporin-4 water channels and the inwardly rectifying potassium channels Kir4.1 are coexpressed in a highly polarized manner at the perivascular and subvitreal endfeet of retinal Müller cells and astrocytes. The present study was aimed at resolving the anchoring mechanisms responsible for the coexpression of these molecules. Both aquaporin-4 and Kir4.1 contain PDZ-domain binding motifs at their C-termini and it was recently shown that mice with targeted disruption of the dystrophin gene display altered distribution of aquaporin-4 and Kir4.1 in the retina. To test our hypothesis that α-syntrophin (a PDZ-domain containing protein of the dystrophin associated protein complex) is involved in aquaporin-4 and Kir4.1 anchoring in retinal cells, we studied the expression pattern of these molecules in α-syntrophin null mice. Judged by quantitative immunogold cytochemistry, deletion of the α-syntrophin gene causes a partial loss (by 70%) of aquaporin-4 labeling at astrocyte and Müller cell endfeet but no decrease in Kir4.1 labeling at these sites. These findings suggest that α-syntrophin is not involved in the anchoring of Kir4.1 and only partly responsible for the anchoring of aquaporin-4 in retinal endfeet membranes. Furthermore we show that wild type and α-syntrophin null mice exhibit strong β1 syntrophin labeling at perivascular and subvitreal Müller cell endfeet, raising the possibility that β1 syntrophin might be involved in the anchoring of Kir4.1 and the α-syntrophin independent pool of aquaporin-4. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: water channels, inwardly rectifying potassium channels, dystrophin, syntrophins, Müller cells.

Macroglial cells are polarized cells, structurally and functionally (Amiry-Moghaddam and Ottersen, 2003). A fundamental question in glial cell biology is how the polarization of astrocytes is maintained. The importance of this question is underlined by recent findings, pointing to perturbations of brain function when astrocyte polarization is deficient due to experimental manipulations or disease (Amiry-Moghaddam and Ottersen, 2003; Amiry-Moghaddam et al., 2003a,b, 2004; Vajda et al., 2002).

The dystrophin-associated protein complex (DAP) (Eid et al., 2005), known to be crucial for the integrity of the neuromuscular synapse (Nagel et al., 1990) is also essential for sustaining astrocyte polarization (Frigeri et al., 2001, 2002; Howard et al., 1998; Vajda et al., 2002). Mice with targeted disruption of the dystrophin gene as well as dystrophic mice (mdx) exhibit a number of anomalies in brain, including loss of perivascular aquaporin-4 (AQP4) (Frigeri et al., 2001; Vajda et al., 2002). This loss is reproduced in animals with deletion of the gene encoding α-syntrophin, a PDZ-domain containing scaffold protein that forms an integral part of DAP (Neely et al., 2001).

The picture that has emerged from recent studies is that perivascular endfeet of macroglia are equipped with a DAP that is attached to the perivascular basal lamina by way of α- and β-dystroglycan (Blake et al., 1999; Claudepierre et al., 1999; Howard et al., 1998; Szabo et al., 2004; Ueda et al., 1998; Zaccaria et al., 2001). This complex is responsible for the polarized expression of AQP4 in brain astrocytes (Amiry-Moghaddam and Ottersen, 2003; Amiry-Moghaddam et al., 2004; Nicchia et al., 2004) and possibly also for a number of other molecules known to be preferentially expressed at the endfeet. Recent biochemical studies of brain homogenates have indicated an association between Kir4.1 and α-syntrophin (Connors et al., 2004; Leonoudakis et al., 2004), although α-syntrophin deletion does not lead to a significant decrease of perivascular Kir4.1 immunogold signal in the hippocampus (Amiry-Moghaddam et al., 2003b).

Retinal glial cells differ from those of the brain in their geometry and in their repertoire of plasma membrane proteins. Müller cells contain a different complement of glutamate transporters and K^+ channels than brain astrocytes (Newman, 1986). Functionally, the Müller cells are involved in transretinal water transport and K^+ siphoning (Newman et al., 1984), functions that are likely to be critically dependent on AQP4 and Kir4.1, respectively. Furthermore, studies of knockout animals have shown that AQP4 and Kir4.1 are also involved in retinal signal transduction and in the pathophysiology of retinal edema and

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Abbreviations: AQP4, aquaporin-4; DAP, dystrophin-associated protein complex.

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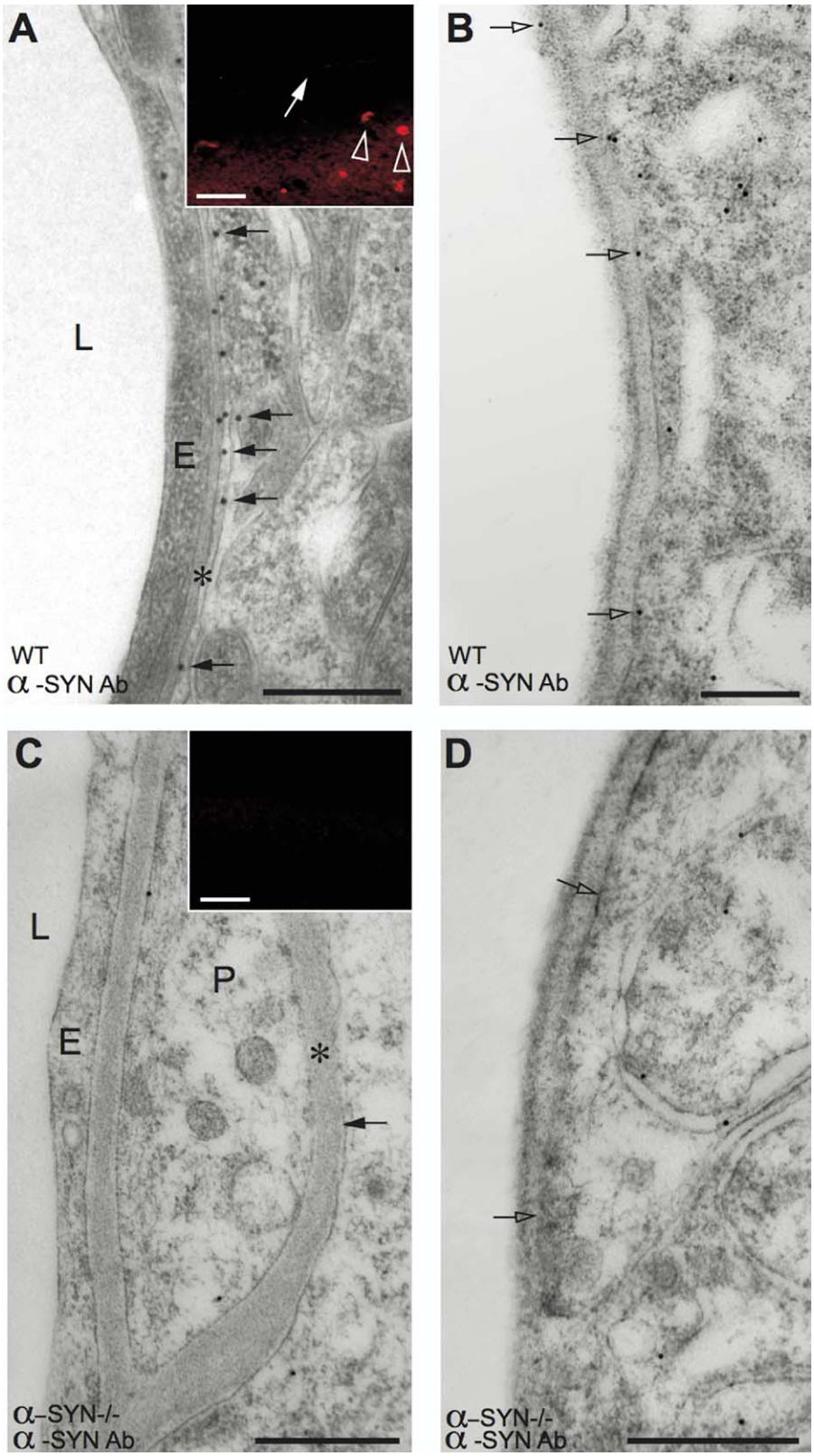

Fig. 1. α-Syntrophin immunolabeling in WT (A, B) and α-syntrophin knockout (C, D) mouse retina. (A, B) Polarized α-syntrophin immunogold labeling at the perivascular (black arrows) and subvitreal (open arrows) macroglial membrane domains. Inset in A: α-syntrophin immunofluorescence at the perivascular (arrowheads) and subvitreal (white arrow) membranes. (C, D) Perivascular (black arrow) and subvitreal (open arrows) α-syntrophin labeling is lost following deletion of α-syntrophin. The complete loss of α-syntrophin immunofluorescence (inset in C) confirms the immunogold data. E, endothelium; L, capillary lumen; P, pericyte; asterisk, basal lamina. Scale bars=500 nm, inset scale bar=50 μm.