NHERF1 - a novel scaffold protein for the astroglial glutamate transporter GLAST

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The Na^+/H^+ exchanger regulatory factor (NHERF) proteins, NHERF1 and NHERF2 are renal epithelial PSD95/Dlg/ZO-1 (PDZ) domain containing proteins (Weinman *et al.*, 1995; Yun *et al.*, 1997). NHERF proteins contain two tandem PDZ domains (PDZ1 and PDZ2), which associate with specific C-terminal motifs of target peptides. NHERF proteins also contain a C-terminal region able to interact with the Ezrin-Radixin-Moesin (ERM) proteins which provides a link to the actin cytoskeleton.

While NHERF proteins have been extensively characterised in kidney and other tissues, very little is known regarding NHERF expression in the central nervous system (CNS) and its possible-binding partners in the CNS. Tissues used for immunohistochemical and biochemical analyses were isolated from euthanised adult Wistar rats, following procedures approved by the University of Queensland Animal Ethics Committee. In this study, we performed immunohistochemical characterisation of the cellular distribution of NHERF1 and NHERF2 in the adult rat brain. Immunohistochemistry was performed on adult rat brain sections using polyclonal antibodies specific for NHERF1 and NHERF2. Expression of NHERF1 was shown to be widespread in brain, most prominently in hippocampus, thalamus, choroid plexus and cerebellum. In these different regions of the brain, NHERF1 was primarily restricted to astrocytes. NHERF2 expression was primarily restricted to endothelial cells of blood vessels and capillaries. The distribution in adult rat brain was similar to that of GLAST, an astroglial glutamate transporter that also contains a potential PDZ binding consensus sequence (ETKM) in its COOH-terminus (Lehre, *et al.*, 1995). Double immunofluorescence labelling studies were performed using antibodies specific for GLAST and NHERF1 and imaged by confocal microscopy, co-localisation of GLAST and NHERF1 was detected in astroglial cells. Using solubilised adult rat brain lysate, co-immunoprecipitation experiments demonstrated that GLAST, NHERF1 and ezrin co-associate *in vivo*. To determine which domains of NHERF1 and GLAST interact we performed pull-down assays using solubilised adult rat brain lysate and GST-fusion proteins of the GLAST COOH-terminus, full-length NHERF1 and various domains of NHERF1 (PDZ1, PDZ2 and ERM domain). These experiments revealed that the GLAST-NHERF1 interaction requires the COOH-terminal ETKM sequence of GLAST and utilises the PDZ1 domain of NHERF1.

Therefore we have demonstrated that NHERF1 links GLAST to the actin cytoskeleton through ezrin, leading to the formation of a multi-protein complex. Linkage of GLAST to NHERF1 may serve as an important mechanism for localising the GLAST transporter to specialised membrane sites in astrocytes or for regulating transport activity.

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