Identification of components of a glutamate homeostasis complex in astrocytes

P. Poronnik,[2] A. Lee[1] and D.V. Pow,[1] [1]UQCCR and Perinatal Research Centre, The University of Queensland, QLD 4072, Australia and [2]School of Medical Sciences, RMIT University, VIC 3083, Australia.

Efficient glutamate transport into astrocytes is essential for termination of glutamatergic neurotransmission. Uptake by proteins such as GLAST (EAAT1) requires a sodium gradient, formed by the actions of a sodium-potassium ATPase (NKA), and the rapid catabolism of the accumulated glutamate by glutamine synythetase (GS). We demonstrate, by co-immunoprecipitation experiments (n=12) that GLAST associates with NKA α 1 in the rat brain. GST-pulldown assays demonstrate that the cytoplasmic loop region (residues 350 775) of NKA α 1 pulls down GLAST from brain lysate. Similarly, the extreme C-terminal tail (residues 513 -543) of GLAST pulls down NKA α 1 from brain lysate. Confocal microscopy studies revealed that GLAST co-localised with NKA α 1. We have examined D-aspartate uptake in transfected Cos-7 cells to study the effects of co-expression of GLAST and a construct encoding the cytoplasmic loop region (residues 350 775) of NKA α 1. The construct was predicted to block normal interactions between GLAST and NKA α 1. Co-expression led to a significant decrease (~20%) in D-aspartate uptake when compared to the control. The interaction between the cytoplasmic loop region of NKA α 1 and the C-terminal tail of GLAST is likely to involve other accessory proteins. Similarly we demonstrate by immunoprecipitation studies that the enzyme GS is part of this multi-molecular complex. We have previously demonstrated that NHERF1 anchors GLAST to the cytoskeleton, and confirm here that NHERF1 is part of the complex containing GS, GLAST and NKA α 1. These findings indicate that multiple protein interactions may be required for efficient glutamate transport and that these interactions may represent novel pharmacological targets in conditions such as stroke.