Identification of glutamate transporter glast splice variants in hypoxic neonatal pig brain

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Glutamate homeostasis is critical to normal brain function and deficiencies in regulation of extracellular glutamate are thought to be a major determinant of damage in hypoxic brains. Extracellular levels of glutamate are regulated mainly by plasma membrane glutamate transporters. In this study, RNA from hypoxic neonatal pig brains were analysed for isoforms of the glutamate transporter GLAST using reverse transcription PCR and cDNA cloning/sequencing methods. As reported previously in the human (Vallejo-Illarramendi *et al.*, 2005), a splice variant lacking exon 9 (GLASTex9skip) was also detected in hypoxic pig brain. Sequence alignment revealed that GLASTex9skip shared ~90% sequence similarity (at the nucleotide and amino acid level) with the human orthologue EAAT1ex9skip. We also isolated a novel splice variant which lacks both exons 5 and 6 (GLASTex5+6skip) resulting in the loss of 112 amino acids. RT-PCR analysis indicated that both these splice variants are expressed in the hypoxic brain, at levels ranging between 10% and 20% of the level of full length pig GLAST. Immunohistochemical analysis revealed that GLASTex9skip was expressed in the plasma membrane of neurons following mild hypoxic insult. Uptake studies using hypoxic brain slices demonstrated accumulation of a glutamate analogue (D-Glu) into neurons. This uptake of D-glu is unlikely to be due to EAAC1 (EAAT3), the only other candidate neuronal transporter of glutamate in the cortex since D-Glu is not a substrate for EAAC1. Furthermore, we observed no accumulation of D-Glu into neurons in brain slices from control animals that did not express GLASTex9skip. Our data support a model in which GLASTex9skip is a glutamate transporter in hypoxic neurons. We propose that the utilization of GLASTex9skip rather than a multi-step transport process (glutamate-glutamine cycle) with multiple energy demands may be an adaptation of the brain to reduce energy burden while maintaining glutamate homeostasis, at least under conditions of low energy availability. Further *in vitro* transport studies are underway using cloned GLASTex9skip and GLASTex5+6skip to characterise the glutamate transporting capacity of these GLAST isoforms.

Vallejo-Illarramendi A, Domercq M, Matute C. (2005) *Journal of Neurochemistry* **95**: 341-348.