Exploring cysteine transport by the human glutamate transporter, EAAT3

R. Bashour, R.J. Vandenberg and R.M. Ryan, Discipline of Pharmacology, D06 Blackburn building, University of Sydney, NSW 2006, Australia.

The concentration of glutamate in the brain is regulated by the excitatory amino acid transporters (EAATs). Elevated levels of glutamate lead to excitotoxicity which may be associated with obsessive compulsive disorder (OCD) and Alzheimer's disease. Therefore, regulation of glutamate by the EAATs is vital for maintaining normal brain function (Danbolt, 2001). The EAATs, which transport the acidic amino acids glutamate and aspartate are part of the SLC1A family which also includes the alanine serine cysteine transporter (ASCT). There are five subtypes of EAATs (EAAT1-5) and EAAT3 is unique in that it can transport glutamate, aspartate and the neutral amino acid cysteine (Zerangue & Kavanaugh, 1996). Cysteine is the main component as well as the rate limiting step in glutathione production, which plays a vital role in preventing oxidative stress and eventually cell toxicity (Aoyama *et al.*, 2005; Chen & Swanson, 2003).

The aim of this study was to elucidate the molecular basis for cysteine transport by EAAT3. Two residues close to the substrate binding site that are conserved in EAAT3 and ASCT1 but not in EAAT1,2,4,5 are Ile330 and Val411. These residues were mutated to the equivalent residues in EAAT1 (Thr362 and Ile444) (I330T, V411I). Wild type EAAT3 and the mutant transporters I330T and V411I were expressed in *Xenopus laevis* oocytes and transport properties were examined using the two electrode voltage clamp technique. I330T displays reduced maximal transport and increased affinity for cysteine ($K_{0.5}$ of 69 ± 9 μM, n=7) compared to wildtype EAAT3 ($K_{0.5}$ of 122 ± 12 μM, n=7). Similarly I330T had reduced maximal transport and increased glutamate affinity ($K_{0.5}$ of 15 ± 1 μM, n=7) compared to wildtype EAAT3 ($K_{0.5}$ of 39 ± 3 μM, n=14); while V411I displays similar maximal transport and affinity for cyteine and glutamate ($K_{0.5}$ of 79 ± 9 μM, n=6; $K_{0.5}$ of 24 ± 2 μM, n=6) respectively compared to wildtype EAAT3. Although I330T displayed a subtle increase in substrate affinity, we conclude that neither I330 nor V411 are responsible for the ability of EAAT3 to transport cysteine. We are currently investigating other residues in the substrate binding domain that are unique to EAAT3 and ASCT1.

Aoyama K, Suh SW, Hamby AM, Liu J, Chan WY, Chen Y, Swanson RA. (2005) Neuronal glutathione deficiency and age-dependent neurodegeneration in the EAAC1 deficient mouse. *Nature Neuroscience* **9:** 119-126.

Chen Y, Swanson RA. (2003) The glutamate transporters EAAT2 and EAAT3 mediate cysteine uptake in cortical neuron cultures. *Journal of Neurochemistry* **84:** 1332-1339.

Danbolt NC. (2001) Glutamate uptake. *Progress in Neurobiology* **65:** 1-105.

Zerangue N, Kavanaugh MP. (1996) Interaction of L-cysteine with a human excitatory amino acid transporter. *The Journal of Physiology* **493:** 419-23.