Mechanism of cation binding to the glutamate transporter EAAC1 probed with mutation of the conserved amino acid residue Thr101.

Abstract:

The glutamate transporter excitatory amino acid carrier 1 (EAAC1) catalyzes the co-transport of three Na(+) ions, one H(+) ion, and one glutamate molecule into the cell, in exchange for one K(+) ion. Na(+) binding to the glutamate-free form of the transporter generates a high affinity binding site for glutamate and is thus required for transport. Moreover, sodium binding to the transporters induces a basal anion conductance, which is further activated by glutamate. Here, we used the [Na(+)] dependence of this conductance as a read-out of Na(+) binding to the substrate-free transporter to study the impact of a highly conserved amino acid residue, Thr(101), in transmembrane domain 3. The apparent affinity of substrate-free EAAC1 for Na(+) was dramatically decreased by the T101A but not by the T101S mutation. Interestingly, in further contrast to EAAC1(WT), in the T101A mutant this [Na(+)] dependence was biphasic. This behavior can be explained by assuming that the binding of two Na(+) ions prior to glutamate binding is required to generate a high affinity substrate binding site. In contrast to the dramatic effect of the T101A mutation on Na(+) binding, other properties of the transporter, such as its ability to transport glutamate, were impaired but not eliminated. Our results are consistent with the existence of a cation binding site deeply buried in the membrane and involving interactions with the side chain oxygens of Thr(101) and Asp(367). A theoretical valence screening approach confirms that the predicted site of cation interaction has the potential to be a novel, so far undetected sodium binding site.