Abstract

Glutamate transporter GLT-1 is a transmembrane protein, which is mainly localized in astrocytes of the CNS. GLT-1 is a high affinity Na⁺ dependent transporter, which removes the neurotransmitter glutamate from the synaptic cleft, an essential process required for defined signal transduction and to avoid excitatory damage.

Glutamate transporter GLT-1 and fluorescent GLT-1 as well as GLAST-1 and EAAC1, which contained the transporter-polypeptide fused to the fluorescent protein EGFP at the N-terminal end of the glutamate transporter were constructed for heterologous expression in Xenopus laevis oocytes and HEK293 (human embryonic kidney) cells. The experiments described here prove, that GLT-1 and likewise the fusionprotein are targeted during the cellular transport to and functionally incorporated into the the plasmamembrane. Therefore the fluorescent-labeled GLT-1 and likewise GLAST-1 and EAAC1, two other members of the glutamate transporter family, heterologously expressed in different cell system, like Xenopus oocytes and HEK293 cells, proved to be valuable tools for cell biological, biochemical and electrophysiological studies in the characterization of these excitatory glutamate transporters.

Neurotransmitter uptake studies in GLT-1 transfected Xenopus oocytes and HEK293 cells used radioactive labeled L-[¹⁴C]-glutamate associated with the method of choice, the electrophysiological whole cell voltage- and patch-clamp experiment, were applied to the characterization of GLT-1. The ion specificity for Na⁺ ions, K⁺ values of glutamate and Na⁺ ions, the kinetics of glutamate uptake and several inhibitors of the GLT-1 transporter were established. The present work also describes the influence of Ca²⁺ ions on glutamate transport. Unlike Na⁺, Ca²⁺ ions are not transported by GLT-1 but affect the transport of L-glutamate. These observations expand our view of the effect of intracellular Ca²⁺ on the GluT family.
The structural requirements of ligands with structures analogous to the genuine neurotransmitter glutamate have been classified more exactly. In addition to the terminal charged carboxyl-group or the SH-group in ligands, the amino group in $\alpha$-position – as in L-$\alpha$-aminoadipate - turned out to be essential for the binding properties of ligands. Uncharged amino acids or $\beta$-amino acids, as L-$\beta$-aminoadipate, do not show any influence on the transport of L-glutamate. It is shown that GLT-1 has a similar ability to transport L-aspartate as GLAST-1.