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 synaptical 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-[^{14}C]-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_M 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 Ca^{2+} ions on glutamate transport. Unlike Na^+, Ca^{2+} ions are not transported by GLT-1 but affect the transport of L-glutamate. These observations expand our view of the effect of intracellular Ca^{2+} 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 α-position – as in L-α-aminoadipate - turned out to be essential for the binding properties of ligands. Uncharged amino acids or β-amino acids, as L-β-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-aspartat as GLAST-1.