

SAMPLE ABSTRACT

Neuronal Glutamine Transporters in Hippocampal Neurons are Subject to Substrate-Induced Adaptive Regulation

Norah Defamie*, H el ene Varoqui, and Jeffrey D. Erickson
Neuroscience Center, Louisiana State University Health Sciences Center,
New Orleans, Louisiana 70112

This laboratory has recently cloned and functionally identified cDNAs encoding SAT1 and SAT2, the first members of the system A family of Na⁺-coupled glutamine transporters. Since glutamine is a critical precursor for neurotransmitter glutamate *via* the glutamate/glutamine cycle, we propose that SAT transporters function to regulate the synthesis of the major excitatory transmitter in the brain. We examined the cellular and subcellular distribution of SAT1 and SAT2 in primary cultures of hippocampal neurons by double fluorescence deconvolution microscopy against various markers. Both SAT1 and SAT2 immunoreactivity is found in neuronal cell bodies and processes that contain VGLUT1, the dominant vesicular glutamate transporter isoform expressed in the hippocampus. We next examined whether the functional activity of system A in hippocampal neurons is regulated following treatments that inhibit amino acid flux through the glutamate/glutamine cycle. A unique characteristic of system A is its ability to recognize N-alkylated amino acids as substrates and alpha-(methylamino)isobutyric acid (MeAIB) is routinely used as a model substrate. Transport of ¹⁴C-MeAIB (50 M) in differentiated hippocampal cultures was examined at various times following total amino acid deprivation. An approximate two-fold increase in ¹⁴C-MeAIB uptake is observed with total amino acid deprivation for 1, 3 or 4.5 hr. After 6 hr incubation without amino acids, system A activity increases more than 4-fold. Interestingly, both the rapid and delayed increases in system A activity are completely blocked when the neurons are supplied with the system A substrate glutamine. To determine whether the increase in system A activity following acute and chronic total amino acid deprivation is due to changes in mRNA and protein synthesis, we examined the effects of cycloheximide (75 g/ml) or actinomycin D (7.5 g/ml). Our data show that chronic exposure (>6hr) of hippocampal neurons to an amino acid-free medium induces the system A activity most likely by enhancing *de novo* synthesis of the transporter proteins and consequently increasing the density of the transporter proteins in the plasma membrane. These drugs however have no effect on the acute increase in system A activity that is observed after only 1 hr of deprivation. Furthermore, they do not completely block the increase in system A activity that is observed following 6 hr of incubation. Taken together, these data suggest that the acute response of system A in neurons in culture to amino acid deprivation likely arises by increasing the cycling of transporter from intracellular pools to the plasma membrane. The availability of molecular tools will now allow us to identify whether this acute and chronic substrate-induced adaptive regulation is specific to SAT1 or to SAT2 in hippocampal neurons in current studies.

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