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Glutamate transporters: Adding new tunes to the neuron-glia orchestra.

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Nowadays it is widely accepted that astrocytes are actively engaged in a bidirectional communication with neurons. However, are neuron and astrocyte the equal partners at the synapse? Is the astroglia's only function to provide structural and metabolic support to its "big brother" - neuron that is solely in charge of synaptic transmission? Recent advances in the field cast serious doubts on this traditional view.

A good illustration of this notion is astroglia ensheathing the glutamatergic synapse. Astrocytes, like neurons, express functional metabotropic glutamate receptors, as well as ionotropic glutamate receptors represented by both kainate- and AMPA-preferring subtypes [1, 2, 3]. Among the most exciting findings reported this year is a demonstration of functional astroglial NMDA receptors [4] which had been widely acknowledged to be exclusively expressed in neurons.

Another neuronal "stronghold"-calcium-dependent vesicular release of neuro-transmitters - is also quaking these days. Several elegant studies demonstrate that in response to different stimuli (eg glutamate, bradykinin, prostaglandins, calcium ionophores, alpha-Latrotoxin, etc) astrocytes are capable of releasing glutamate and other messenger substances in a calcium-dependent manner. [5-9]

These observations are supported by the detection of the components of the exocytotic apparatus in astrocytes - secretory organelles and proteins (eg syntaxin I, synaptobrevin II, synaptophysin, SNAP-25, synaptotagmin I, etc.) that are essential for the initiation of exocytosis and that are typically associated with the pre-synaptic compartment [7, 10,11].

Furthermore, synaptically released glutamate is taken up and biotransformed by astrocytes much more efficiently than by neurons [12,13]. Five types of high-affinity glutamate transporters, designated GLT1, EAAC1, GLAST, EAAT4 and EAAT5, have been cloned since 1992 (for reviews, see [14, 15]). However, no cloned transporter has been so far detected wthin the synaptic cleft - seemingly a most convenient location for the uptake of the released neurotransmitter, although at least two transporter subtypes, EAAC1 and EAAT4, are expressed in neurons, predominantly on cell bodies and dendrites. In fact, the majority of glutamate uptake in the brain is executed by a tandem of two astroglial transporters - GLT1 and GLAST which are highly expressed at the sites ensheathing the synaptic cleft [16-18]. Their operational capacity is dramatically increased due to a rapid transformation of glutamate to glutamine by glutamine synthetase, an enzyme which is expressed in astrocytes, but not in neurons [12, 19]. Thus, recent studies support an emerging view that astroglial cells are equipped with practically all major molecular components required for glutamate signalling at the synapse and, as compared to neurons, express somewhat more advanced machinery for handling this major excitatory neurotransmitter.

All components of the system dealing with glutamate seem to act in concert on both cell types, involve additional factors, and, thus, ensure complex bidirectional tuning of synaptic function and other vital processes. For example, astroglial glutamate transporters are upregulated by macromolecular soluble factors that are predominantly expressed in neurons (e.g. BDNF; PACAP, VIP) [20-25] and recent studies have revealed involvement of PI3- and p42/44 MAP kinase pathways, as well as essential roles of the CREB and NF-kB transcription factors in these processes [22,23,26]. Neuronal factors regulate also expression of metabotropic glutamate receptors (mGluR) 3 and -5 in astroglia. In turn, the activation of astroglial mGluR3 by neuronally released glutamate can induce expression of factors that protect neurons against glutamate toxicity or other insults [27]. The induction and activation of the astroglial group I and II mGluRs by neuronal factors have important consequences for glutamate uptake as well. Our recent studies demonstrate that mGluR3 mediates upregulation of GLAST , while activation of mGluR5 results in the decreased levels of this transporter [23].

It seems, that glutamate regulates glutamate transporter expression via different mechanisms. For example, the GLAST levels can be controlled not only by mGluRs, but also via the astroglial kainate receptor activation [28]. Moreover, glutamate, as well as other transport substrates, can increase cell surface expression of GLAST without involvment of glutamate receptors [29]. In other words, when the active pool of transporters is kept busy with carrying glutamate or other transport substrates, more and more latent transporter molecules show up at the cell surface. The analogous

process - activity-dependent trafficking of transporter protein from the cytoplasmic pool to the cell surface - has been demonstrated for another carrier protein - EAAT4 [23]. Although the mechanism of this phenomenon is highly enigmatic, unique properties of EAAT4 may be in charge.

EAAT4 functions not only as a glutamate carrier, but also exhibits features of a glutamate-gated chloride- and arachidonate-activated proton channel [30]. In 1998, based on the dual function of EAAT4, analysis of structural motifs of glutamate transporters, and some unexplained observations, we argued that functions of glutamate transporters might not be restricted solely to glutamate uptake but could also comprise some receptor-like activities (e.g. interaction with G- proteins) [15]. The most recent discoveries in the field support our prediction. For example, the activation of EAAT4 by substrates that feature very low affinity to glutamate receptors apparently triggers a chain of yet unidentified intracellular events that result in phosphorylation of one of the key factors involved in translational processes - Phas-1 (Gegelashvili et. al. Unpublished observation). Furthermore, two cytosolic EAAT4-binding proteins, designated GTRAP41 and GTRAP48, have recently been cloned and characterized. Several domains required for the activation of the Rho family of G proteins, including a PDZ motif, have been identified in GTRAP48 and an actual interaction with G 13 subunit.has been demonstrated.[31]. It is noteworthy, that the cytoplasmic C-terminus of the glutamate transporter GLAST also bears some similarity to the PDZ motif [32] . Another glutamate transporter, GLT1, binds to a novel cytosolic LIM protein, Ajuba, that interacts with Grb2, augments MAP kinase signalling and mediates embryonal cell differentiation [33]. Finally, transport substrates for glutamate carriers, but not glutamate receptor agonists or antagonists, appear to increase p44/p42 MAP phosphorylation in astrocytes [34]. Thus, glutamate transporters apparently display the properties of signal transducing molecules, featuring both ionotropic and metabotropic activities. Future studies will reveal whether glutamate transporters, besides their carrier function, could be considered as novel glutamate receptors, or they are just adapter molecules for other receptors. In any case, these findings add more complexity to the glutamatergic machinery and highlight new roles of glutamate carrier proteins in neuron-glia cross talk

References

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