

## NEUROTRANSMITTER SUPPLY AND DEMAND IN EPILEPSY

### **Block of Glutamate-Glutamine Cycle Between Astrocytes and Neurons Inhibits Epileptiform Activity in Hippocampus**

Bacci A, Sancini G, Verderio C, Armano C, Pravettoni E, Fesce R, Franceschetti S, Matteoli M

J Neurophysiol 2002;88:2302–2310

Recurrent epileptiform activity occurs spontaneously in cultured CNS neurons and in brain slices in which  $\gamma$ -aminobutyric acid (GABA) inhibition has been blocked. We demonstrate here that pharmacologic treatments, resulting in either the block of glutamine production by astrocytes or the inhibition of glutamine uptake by neurons, suppress or markedly decrease the frequency of spontaneous epileptiform discharges both in primary hippocampal cultures and in disinhibited hippocampal slices. These data point to an important role for the neuron-astrocyte metabolic interaction in sustaining episodes of intense rhythmic activity in the CNS, and thereby reveal a new potential target for antiepileptic treatments.

### **A Neuronal Glutamate Transporter Contributes to Neurotransmitter GABA Synthesis and Epilepsy**

Sepkuty JP, Cohen AS, Eccles C, Rafiq A, Behar K, Ganel R, Coulter DA, Rothstein JD

J Neuroscience 2002;22:6372–6379.

The predominant neuronal glutamate transporter, EAAC1 (excitatory amino acid carrier-1), is localized to the dendrites and somata of many neurons. Rare pre-synaptic localization is restricted to  $\gamma$ -aminobutyric acid (GABA) terminals. Because glutamate is a precursor for GABA synthesis, we hypothesized that EAAC1 may play a role in regulating GABA synthesis and thus could cause epilepsy in rats when inactivated. Reduced expression of EAAC1 by antisense treatment led to behav-

ioral abnormalities, including staring-freezing episodes and electrographic (EEG) seizures. Extracellular hippocampal and thalamocortical slice recordings showed excessive excitability in antisense-treated rats. Patch-clamp recordings of miniature inhibitory postsynaptic potentials (mIPSCs) conducted in CA1 pyramidal neurons in slices from EAAC1 antisense-treated animals demonstrated a significant decrease in mIPSC amplitude, indicating decreased tonic inhibition. A 50% loss of hippocampal GABA levels was associated with knockdown of EAAC1, and newly synthesized GABA from extracellular glutamate was significantly impaired by reduction of EAAC1 expression. EAAC1 may participate in normal GABA neurosynthesis and limbic hyperexcitability, whereas epilepsy can result from a disruption of the interaction between EAAC1 and GABA metabolism

## COMMENTARY

**A**re particular biochemical pathways necessary for the sustained release of neurotransmitters responsible for epileptiform neuronal activity during seizures? If so, might it be possible to manipulate such systems to modify seizure susceptibility? Two intriguing recent reports suggest that this might be true by providing information regarding specific molecular systems that are required for the maintenance of the neurotransmitter content at a level sufficient for normal and pathologic synaptic transmission.

In general, excitatory glutamatergic neurotransmission is responsible for the initiation and spread of seizure activity, even if it is not necessarily the primary underlying pathogenic mechanism. Similarly,  $\gamma$ -aminobutyric acid (GABA)-mediated synaptic inhibition is known to be critical in regulating epileptic activity, as even a minor disinhibition can trigger hyperexcitability (1). Thus a dysfunction in either GABA or glutamate availability will have important consequences regarding seizure genesis.

The duration of excitation during glutamatergic neurotransmission relies on specific transporters, which terminate the action of glutamate and control its extracellular level by

clearing the synaptic cleft, thus preventing excitotoxicity and hyperexcitability. Glutamate transporters are present in the plasmalemma of neurons and astrocytes ensheathing the synapses, and can be divided into five subtypes: GLAST (or EAAT-1) and GLT-1 (or EAAT-2) are astroglial transporters, whereas EAAC1 (or EAAT-3), EAAT-4, and EAAT-5 are neuronal proteins (2). In the hippocampus, astroglial glutamate transporters are responsible of ~80% of glutamate clearance during synaptic transmission resulting in synaptic inactivation (2,3).

In the last decade, glutamate transporters have become a focus of epilepsy research. Although human studies have not proven a direct role of defective glutamate uptake in epilepsy, a reduced expression of glutamate transporters was found to produce or to be associated with seizure activity in several animal models. For example, mice lacking the astroglial-type GLT-1 develop spontaneous seizures (4), and a decreased expression of glutamate transporters GLAST, GLT-1, and EAAC1 was found in a rat model of absence epilepsy (GAERS) (5). Rothstein et al. (6) previously showed that antisense treatments that knock down expression of GLAST, GLT-1, or EAAC1 have differential effects. Whereas impairment of the expression of the glial subtypes (GLAST and GLT-1) caused massive excitotoxicity and neurodegeneration, a deficit in the neuronal subtype (EAAC1) was responsible for only mild neurotoxicity and epilepsy.

In cortical structures, although the function of astroglial glutamate transporter subtypes is clear, the role of their neuronal counterpart, EAAC1, has remained elusive, mostly because of its localization. Indeed, EAAC1 is expressed in neurons in the dendritic compartment perisynaptically (i.e., outside the synaptic specialization), both *in vitro* (7) and *in vivo* (8,9). These data rule out a major role for EAAC1 in clearing glutamate during fast synaptic transmission and make its function unclear. EAAC1 was found in the dendritic perisynaptic location of a nonnegligible number of GABAergic interneurons both in the hippocampus and in the neocortex (8,9). This suggests that GABAergic cells might take up glutamate via this transporter and refuel inhibitory neurotransmission after conversion to GABA via glutamate decarboxylase.

Starting with this hypothesis, Sepkuty et al. (10) tested whether perturbed GABA homeostasis might be responsible for the epileptic phenotype shown by rats with EAAC1 knock-down. The authors used a variety of techniques ranging from EEG to whole-cell patch-clamp recording and tissue microdialysis to address this question. They found that spontaneous epileptic seizure activity and associated staring spells occurred after several days of treatment. Further, thalamocortical and hippocampal-entorhinal cortical slices from knockdown animals both showed increases in spontaneous epileptiform activity compared with controls (sense treated). Patch-clamp re-

cordings from CA1 neurons revealed that synaptic inhibition is defective in EAAC1-treated animals, as shown by a slightly reduced amplitude of miniature inhibitory postsynaptic currents (mIPSCs). This was accompanied by a more prominent reduction of total tissue GABA concentration (detected with high-performance liquid chromatography [HPLC]), especially in the hippocampus. It is of note that GABA synthesis rates were decreased by either antisense treatment or glutamate-uptake blockade. This supports the conclusion that EAAC1 activity provide GABAergic interneurons with glutamate as a GABA substrate and that, if not present, the net reduction in GABA availability disrupts the balance between excitation-inhibition balance and increases seizure susceptibility.

The idea that GABAergic neurotransmission is fueled by glutamate is novel and provocative, although, as in all good stories, there are now more questions than answers. Is the mild, although significant mIPSC amplitude reduction sufficient to produce the intense and robust seizure activity recorded in slices and *in vivo*? Perhaps this assay, obtained under conditions of low release probability, underestimates the net circuit defect that would occur during sustained activity, as is discussed later. Another question arises from the article (i.e., what is the role of EAAC1 in glutamatergic neurons?). EAAC1 is expressed mostly by excitatory neurons (8,9), and the proepileptic effect generated by antisense EAAC1 administration might also derive from a lack of expression of this transporter in excitatory neurons.

A recent report from Bacci et al. (11) perhaps more directly addresses the issue of sustained neurotransmitter precursors delivery during epileptiform activity. Here the authors target astrocyte metabolism and, more specifically, glutamine homeostasis, to protect neurons from excessive glutamate availability during epileptiform activity. Although efficient glutamate uptake performed by astrocytes in the context of simple synaptic excitation is a desired feature, as it ends excitation, it can backfire with pathologic consequences during hyperexcitability and seizures, and thus be instrumental in sustaining such excitability. Indeed, once internalized by astrocytes, glutamate is converted to glutamine by glutamine synthetase. Glutamine is then delivered to neuronal presynaptic terminals where it is transformed back into glutamate and eventually packed into synaptic vesicles. This scheme, known as the glutamate-glutamine shuttle, represents one important example of the crosstalk between glia and neurons during synaptic transmission (12).

With neuronal/glial cocultures, Bacci et al. (11) showed that the ability to sustain high-frequency repetitive discharge is inhibited by fluoroacetate. This glial-specific metabolic poison disrupts the tricarboxylic acid (TCA) cycle in astrocytes and increases their reliance on glutamine as a carbon source for energy metabolism. The result is that glial cells presumably hoard glutamine, making it unavailable for release, and neu-

rons are thus starved of this important source of neurotransmitter precursor. In support of this idea, replenishment of the cultures with glutamine completely restores neuronal excitability such that the epileptiform activity, monitored by either intracellular recordings or  $\text{Ca}^{2+}$  microfluorimetry, is maintained for extended periods.

They further showed that interference with separate steps in the glutamate-glutamine shuttle—inhibition of glutamine synthetase (by methionine sulfoximine, MSO), or blockade of glutamine uptake into neurons (by  $\alpha$ -[methylamino]isobutyric acid, MEAIB)—each replicated the effect of fluoroacetate, with the former being reversed by glutamine supplement to an extent similar to that occurring with fluoroacetate effect. Finally, to generalize these findings, the effects of MSO and MEAIB were tested on interictal-like spike activity induced in acute hippocampal slices by disinhibition. Each compound produced a reduction in interictal spike frequency, and the effects of MSO were reversed by glutamine supplementation.

These novel findings regarding (a) an interaction between excitatory amino acid transporters and inhibitory neurotransmission, and (b) the requirement of high level of glutamate-glutamine shuttle functioning for sustained seizure-like activity, open new perspectives and suggest that therapeutic alterations in the *supply* of neurotransmitters may decrease seizure activities that have high a *demand* on such resources. Although it would not be practical to use a pharmacologic agent that would modulate all glutamate-uptake subtypes, it would be certainly interesting to test whether a putative enhancer of EAAC1 would diminish or possibly stop seizure activity in experimental models. Similarly, it will be interesting to test whether mild disruption of the glutamate-glutamine shuttle might effect a specific inhibition of epileptic hyperexcitability.

by John Huguenard, Ph.D.

## References

1. Chagnac-Amitai Y, Connors BW. Horizontal spread of synchronized activity in neocortex and its control by GABA-mediated inhibition. *J Neurophysiol* 1989;61:747–758.
2. Danbolt NC. Glutamate uptake. *Prog Neurobiol* 2001;65:1–105.
3. Bergles DE, Jahr CE. Synaptic activation of glutamate transporters in hippocampal astrocytes. *Neuron* 1997;19:1297–1308.
4. Tanaka K, Watase K, Manabe T, Yamada K, Watanabe M, Takahashi K, Iwama H, Nishikawa T, Ichihara N, Kikuchi T, Okuyama S, Kawashima N, Hori S, Takimoto M, Wada K. Epilepsy and exacerbation of brain injury in mice lacking the glutamate transporter GLT-1. *Science* 1997;276:1699–1702.
5. Dutuit M, Touret M, Szymocha R, Nehlig A, Belin MF, Didier-Bazes M. Decreased expression of glutamate transporters in genetic absence epilepsy rats before seizure occurrence. *J Neurochem* 2002; 80:1029–1038.
6. Rothstein JD, Dykes-Hoberg M, Pardo CA, Bristol LA, Jin L, Kuncl RW, Kanai Y, Hediger MA, Wang Y, Schielke JP, Welty DE. Knock-out of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. *Neuron* 1996;16: 675–686.
7. Coco S, Verderio C, Trotti D, Rothstein JD, Volterra A, Matteoli M. Non-synaptic localization of the glutamate transporter EAAC1 in cultured hippocampal neurons. *Eur J Neurosci* 1997;9:1902–1910.
8. Conti F, DeBiasi S, Minelli A, Rothstein JD, Melone M. EAAC1, a high-affinity glutamate transporter, is localized to astrocytes and GABAergic neurons besides pyramidal cells in the rat cerebral cortex. *Cereb Cortex* 1998;8:108–116.
9. He Y, Janssen WG, Rothstein JD, Morrison JH. Differential synaptic localization of the glutamate transporter EAAC1 and glutamate receptor subunit GluR2 in the rat hippocampus. *J Comp Neurol* 2000;418:255–269.
10. Sepkuty JP, Cohen AS, Eccles C, Rafiq A, Behar K, Ganel R, Coulter DA, Rothstein JD. A neuronal glutamate transporter contributes to neurotransmitter GABA synthesis and epilepsy. *J Neurosci* 2002;22: 6372–6379.
11. Bacci A, Sancini G, Verderio C, Armano S, Pravettoni E, Fesce R, Franceschetti S, Matteoli M. Block of glutamate-glutamine cycle between astrocytes and neurons inhibits epileptiform activity in hippocampus. *J Neurophysiol* 2002;88:2302–2310.
12. Pfrieger FW, Barres BA. New views on synapse-glia interactions. *Curr Opin Neurobiol* 1996;6:615–621.