

THE ROLE OF GLUTAMATE TRANSPORTERS IN DEVELOPMENTAL EPILEPSY: A CONCEPT IN FLUX

Glutamate Transporters Prevent the Generation of Seizures in the Developing Rat Neocortex

Demarque M, Villeneuve N, Manent JB, Becq H, Represa A, Ben-Ari Y, Aniksztejn L

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Glutamate transporters are operative at an early developmental stage well before synapse formation, but their functional significance has not been determined. We now report that blockade of glutamate transporters in the immature neocortex generates recurrent *N*-methyl-D-aspartate (NMDA) receptor-mediated currents associated with synchronous oscillations of $[Ca^{2+}]_i$ in the entire neuronal population. Intracerebroventricular injections of the blocker to pups generate seizures that are prevented by coinjections of NMDA-receptor blockers. Therefore the early expression of glutamate transporters plays a central role to prevent the activation by local glutamate concentrations of NMDA receptors and the generation of seizures that may alter the construction of cortical networks. A dysfunction of glutamate transporters may be a central event in early infancy epilepsy syndromes.

COMMENTARY

To understand the pathophysiology of seizures in the developing brain, we must cast a wide net in search of potential mechanisms. Some of the mechanisms are shared by the mature brain, and others are unique to the developing brain (1). Examples of potential mechanisms include neurotransmitters (synthesis, receptors), voltage- and transmitter-gated ionic channels, and intrinsic membrane properties—each of which has a profile that could produce excessive excitability during development. Yet the goal of identifying an age-specific mechanism that can be manipulated to therapeutic benefit has remained elusive.

Neurotransmitter transporters have only recently been recognized as another possible mechanism. In the adult brain, glutamate transporters function to reduce the synaptic concentration of glutamate after its release, holding in check its potentially excitotoxic action (2,3). At least five distinct glutamate transporters have been identified. Some transporters are present on neurons; others reside on glial membranes. Transporters on neurons prevent the excessive accumulation of glutamate and provide a source of γ -aminobutyric acid (GABA) precursor for interneurons; those on glia help to recycle glutamate via the glutamine shuttle.

The importance of glutamate transporters in epilepsy is suggested by the occurrence of spontaneous seizures in animals with genetic deletions of the transporters (reviewed in a previous issue of *Epilepsy Currents*, (Huguenard J, 2003) (4), which would theoretically prolong the exposure of the synapse to this excitatory transmitter. Deficiency of a glutamate transporter protein has not been linked to seizures in a clinical setting, although in studies of human patients with temporal lobe epilepsy, specific alterations in glutamate transporters (EAAT2 and EAAT3 mRNA) and in protein levels have been reported (5). After lithium-pilocarpine-induced status epilepticus in the developing brain (P10), an upregulation of the glutamate transporter EAAC1 occurs in adulthood, suggesting a possible compensatory action to dampen seizure occurrence (6).

In the developing brain, the roles of neurotransmitter transporters are less well defined. Blockade of the GABA transporter GAT-1 in immature hippocampus enhances GABA-mediated depolarizing potentials, suggesting a role for this transporter in the regulation of excitability during development (7). Glutamate transporters are present and functional at a time when glutamate receptors are not yet expressed. What role could these glutamate transporters play in such immature neural networks? And could such transporters be playing a role in preventing excessive neuronal discharges in the immature brain?

Demarque et al. explored the role of glutamate transporters in very young rat neocortex (P0-5), at a time when transporters are present but synaptic glutamate receptors are underdeveloped. They found that a blocker of glutamate transport, DL-threo- β -benzoyloxyaspartate (TBOA), caused synchronized

slow *N*-methyl-D-aspartate (NMDA)-receptor-mediated currents in a network of immature neocortical neurons in vitro (slices). These slow oscillations are blocked by tetrodotoxin (TTX) but not by the intracellular sodium channel blocker QX314, suggesting that this is a network phenomenon, not one intrinsic to individual neurons. The slow oscillations are dependent on NMDA-receptor activation and are tightly linked to calcium fluctuations in the cellular network. The oscillations were blocked by both TBOA and NMDA-receptor antagonists, but not by the GABA-receptor antagonist bicuculline. When TBOA was injected intraventricularly into freely moving rats, seizures developed; these seizures were prevented by the NMDA-receptor antagonist D(-)-2-amino-5-phosphopentanoic acid (D-APV). The authors concluded that glutamate transporters play an important function in regulating excitability in developing neocortex and, without such control, hyperexcitability ensues in the form of seizures.

The hypothesis is appealing: When glutamate transport is blocked, excess glutamate accumulates in the synaptic space, leading to increased NMDA-receptor activation, further glutamate release, and seizures. One missing piece of information is whether this scenario occurs clinically. The authors are cautious in proposing that some infantile epilepsies might be related, in part, to glutamate transporter dysfunction. This supposition requires experimental evidence that is more definitive, but comprises an intriguing putative mechanism for seizure control. One

important future step is to show that enhancement of glutamate transporter function can protect an animal from seizures.

by Carl E. Stafstrom, M.D., Ph.D.

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