Title: Oops!...Glutamate Did it Again

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Increasing astrocyte glutamate uptake is protective in a model of epilepsy

Peterson, Allison R., et al. *Targeted overexpression of glutamate transporter-1 reduces seizures and attenuates pathological changes in a mouse model of epilepsy*. Neurobiology of Disease, 2021. P157.

Astrocytic glutamate transporters are crucial for glutamate homeostasis in the brain, and dysregulation of these transporters can contribute to the development of epilepsy. Glutamate transporter-1 (GLT-1) is responsible for the majority of glutamate uptake in the dorsal forebrain and has been shown to be reduced at epileptic foci in patients and preclinical models of temporal lobe epilepsy (TLE). Current antiepileptic drugs (AEDs) work primarily by targeting neurons directly through suppression of excitatory neurotransmission or enhancement of inhibitory neurotransmission, which can lead to both behavioral and psychiatric side effects. This study investigates the therapeutic capacity of astrocyte-specific AAV-mediated GLT-1 expression in the intrahippocampal kainic acid (IHKA) model of TLE. In this study, we used Western blot analysis, immunohistochemistry, and long-term–video EEG monitoring to demonstrate that cell-type–specific upregulation of GLT-1 in astrocytes is neuroprotective at early time points during epileptogenesis, reduces seizure frequency and total time spent in seizures, and eliminates large behavioral seizures in the IHKA model of epilepsy. Our findings suggest that targeting glutamate uptake is a promising therapeutic strategy for the treatment of epilepsy.

Commentary

Glutamate is the primary excitatory neurotransmitter in the central nervous system, and is critical to brain function. Under normal conditions, once glutamate is released from presynaptic neuronal terminals, astrocytes remove it from the extracellular space via the glutamate transporters GLT-1 and GLAST. GLT-1 is extremely abundant (approximately 1% of total brain protein), rapidly binds extracellular glutamate, and transports it into astrocytes. This important protein is responsible for ~90% of glutamate uptake in the adult dorsal forebrain, is crucial for the maintenance of low extracellular glutamate concentrations, and ensures that synaptic transmission occurs with spatiotemporal precision.[1] Not surprisingly, when glutamate signaling is excessive, neuronal activity can be enhanced, contributing to the initiation, and spread of seizure activity, as well as epileptogenesis.[2] Microdialysis studies reveal that the extracellular concentration of glutamate is indeed elevated both before and during seizure onset, suggesting that enhanced glutamate release and/or impaired uptake may occur in epilepsy.[3,4] It is well known that following a variety of brain insults including injury, infections, and acute seizures, astrocytes become "reactive," changing their morphology, protein expression, and function. In line with elevated glutamate signaling in the epileptic brain, GLT-1 levels can be decreased in "reactive" astrocytes which would lead to elevated glutamate levels.[5] Increasing evidence suggests that changes in astrocytes can contribute in many ways to the development of epilepsy.[6-8] Astrocytes are involved in ionic homeostasis, regulation of extracellular space volume, and clearance of neurotransmitters, many of which are disrupted in the epileptic brain. Could enhancing astrocyte glutamate uptake, by increasing GLT-1 expression, lead to novel treatment options for patients with refractory epilepsies?

In this exciting study, Peterson et al developed and employed a "gene therapy" approach using an adeno-associated viral (AAV) vector to promote the expression of GLT-1 specifically in astrocytes. This viral vector (AAV-Gfap-GLT-1) allows the expression of GLT-1 specifically in astrocytes by driving GLT-1 expression using the astrocyte-specific promoter Gfap. This is important because although the vast majority of GLT-1 is expressed by astrocytes, it is also expressed by neurons, where it can have unique functions.[9] Therefore, precise cell-type–specific expression ensures virally expressed GLT-1 acts to enhance astrocyte glutamate uptake and limit excitatory neuronal transmission. To determine if overexpression of GLT-1 in astrocytes could have a disease-modifying effect on epilepsy, the authors used the intrahippocampal kainic acid (IHKA) rodent model of temporal lobe epilepsy. Prior to the induction of seizures, animals were infected with AAV-Gfap-GLT-1; epilepsy was then induced using IHKA, and seizure phenotypes were quantified.

The authors first used a combination of Western blot and immunohistochemistry to confirm that the expression of GLT-1 was increased specifically in astrocytes. Viral overexpression resulted in increased GLT-1 expression both 7 and 30 days after

kainic acid–induced status epilepticus in astrocytes, but not in neurons. These changes occurred only in the ipsilateral hippocampus and were not seen in the contralateral hippocampus, confirming that the increases were regionally specific and due to viral transduction. Next, the authors showed that pretreatment with AAV-Gfap-GLT-1 affected multiple seizure-induced anatomical changes in the hippocampus. AAV-Gfap-GLT-1 reduced dentate granule cell dispersion and a decreased density of neurons in area CA3 (NeuN labeling) was observed when examined 7 days after IHKA injection. Surprisingly, these effects were not seen when examined 30 days after IHKA. The most exciting results show that pretreatment with AAV-Gfap-GLT-1 reduced seizure frequency and severity in the IHKA model of epilepsy at the 7-day post-status timepoint. The electrographic seizure burden was significantly decreased in animals pre-treated with AAV-Gfap-GLT-1, with seizure frequency and total seizure time reduced. Additionally, cell-type–specific upregulation of GLT-1 in astrocytes greatly reduced large behavioral seizures ($\geq$ 20 s, Racine score $\geq$3) 7 days post–IHKA-induced SE. Seizures were not examined at 30 days.

These findings represent 2 significant advances for the field. First, it shows that overexpression of GLT-1 can delay cell loss in epilepsy. Any therapy that "preserves the brain" and helps maintain neuronal integrity is likely beneficial in preserving long-term brain health and function. Second, it demonstrates that viral overexpression of GLT-1 can reduce early seizure burden caused by status epilepticus. Again, an intervention that can reduce the number of seizures would be an important new tool in treating epilepsy.

To really test whether this potential therapy has long-term benefit, it would be extremely interesting to see if the anti-seizure effects of AAV-Gfap-GLT-1 are present 30 days after IHKA beyond the 7-day window examined here. Because granule cell dispersion and CA3 cell density are not rescued by AAV-Gfap-GLT-1 at 30 days post-IHKA, collecting EEG at this time point would help us understand the link between the anatomical and electrographic changes. In addition, the potential impact of this approach would be increased if AAV-Gfap-GLT-1 was administered after chronic epilepsy was already established. AAV pretreatment, before the induction of epilepsy, is not a real-world clinical scenario and could impact the initial seizure intensity caused by IHKA itself. If the authors can show that AAV-Gfap-GLT-1 prevents or reduces ongoing seizures in a model of chronic epilepsy, that would represent a true disease-modifying therapy. Another intriguing question revolves around GLT-1 function during pathological glutamate challenges. GLT-1 is hugely abundant in healthy tissue; therefore, it is somewhat surprising that overexpressing GLT-1 is so protective against seizures and epilepsy. This suggests that transient loss of GLT-1 with seizures is extremely detrimental and having a "back-up reserve" of GLT-1 is beneficial. This also suggests that the amount of glutamate released in this model is tremendous because it likely overwhelms normally expressed GLT-1. It also helps show why having such high levels of GLT-1 under normal conditions is important to maintaining brain health. Intriguingly, other studies have shown that GLT-1 is increased during the chronic phase of epilepsy,[10] although these studies used slightly different animal models. This suggests that how GLT-1 expression responds to seizures might not be universal and is in line with increased activity leading to increased GLT-1 expression.[11] Also, important to consider is whether GLT-1 expression is so low at the more chronic time point (30 days post-IHKA) that overexpression strategies may no longer be sufficient to bring levels back into a protective range.

Overall, this work provides evidence that taking advantage of endogenous glutamate uptake mechanisms can be useful in preventing or reducing seizures. This study also shows how far viral-based gene therapies have come toward becoming realistically useful in a clinical setting. GLT-1 overexpression has previously been examined and was shown to not be protective.[12] New viral technologies allow increased viral titers and cell-type–specific expression of candidate therapeutic molecules, which may make viral-based gene therapy a realistic clinical option in the not-too-distant future. However, there are still questions that need to be addressed before viruses can be used with patients suffering from epilepsy regarding safety, efficacy, and duration of expression. All-in-all, this study highlights the clinical promise of enhancing or restoring astrocyte glutamate uptake in the brain and is an exciting proof-of-concept study for anyone interested in engaging astrocytes in the fight against epilepsy.

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References

1. Danbolt NC. Glutamate uptake. *Prog Neurobiol*. 2001;65(1):1-105.
2. Zhou Y, Danbolt NC. Glutamate as a neurotransmitter in the healthy brain. *J Neural Transm*. 2014;121(8):799-817.
3. Ong C, Damisah EC, Gruenbaum SE, Dhaher R, Deng Y, Sandhu MRS, et al. Increased branched-chain amino acids at baseline and hours before a spontaneous seizure in the human epileptic brain. *Epilepsia*. 2021;62(6):e88-e97.
4. Dhaher R, Gruenbaum SE, Sandhu MRS, Ottestad-Hansen S, Tu N, Wang Y, et al. Network-Related Changes in Neurotransmitters and Seizure Propagation During Rodent Epileptogenesis. *Neurology*. 2021;96(18):e2261-e2271.
5. Hubbard JA, Szu JI, Yonan JM, Binder DK. Regulation of astrocyte glutamate transporter-1 (GLT1) and aquaporin-4 (AQP4) expression in a model of epilepsy. *Exp Neurol*. 2016;283(Pt A):85-96.
6. Proper EA, Hoogland G, Kappen SM, Jansen GH, Rensen MGA, Schrama LH, et al. Distribution of glutamate transporters in the hippocampus of patients with pharmaco-resistant temporal lobe epilepsy. *Brain*. 2002;125(1):32-43.
7. Sarac S, Afzal S, Broholm H, Madsen FF, Ploug T, Laursen H. Excitatory amino acid transporters EAAT-1 and EAAT-2 in temporal lobe and hippocampus in intractable temporal lobe epilepsy. *Apmis*. 2009;117(4):291-301.

8. Peterson AR, Binder DK. Regulation of synaptosomal GLT-1 and GLAST during epileptogenesis. *Neuroscience*. 2019;411:185-201.
9. Rimmele TS, Rosenberg PA. GLT-1: The elusive presynaptic glutamate transporter. *Neurochem Int*. 2016;98:19-28.
10. Takahashi DK, Vargas JR, Wilcox KS. Increased coupling and altered glutamate transport currents in astrocytes following kainic-acid-induced status epilepticus. *Neurobiol Dis*. 2010;40(3): 573-585.
11. Morel L, Higashimori H, Tolman M, Yang Y. VGluT1+ neuronal glutamatergic signaling regulates postnatal developmental maturation of cortical protoplasmic astroglia. *J Neurosci*. 2014;34(33): 10950-10962.
12. Young D, Fong DM, Lawlor PA, Wu A, Mouravlev A, McRae M, et al. Adenosine kinase, glutamine synthetase and EAAT2 as gene therapy targets for temporal lobe epilepsy. *Gene Therapy*. 2014; 21(12):1029-1040.