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Back to Basics: A Role for Astrocyte Alkalization in Epileptogenesis

Exacerbation of Epilepsy by Astrocyte Alkalization and Gap Junction Uncoupling

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Seizures invite seizures. At the initial stage of epilepsy, seizures intensify with each episode; however, the mechanisms underlying this exacerbation remain to be solved. Astrocytes have a strong control over neuronal excitability and the mode of information processing. This control is accomplished by adjusting the levels of various ions in the extracellular space. The network of astrocytes connected via gap junctions allows a wider or more confined distribution of these ions depending on the open probability of the gap junctions. K^+ clearance relies on the K^+ uptake by astrocytes and the subsequent diffusion of K^+ through the astrocyte network. When astrocytes become uncoupled, K^+ clearance becomes hindered. Accumulation of extracellular K^+ leads to hyperexcitability of neurons. Here, using acute hippocampal slices from mice, we uncovered that brief periods of epileptiform activity result in gap junction uncoupling. In slices that experienced short-term epileptiform activity, extracellular K^+ transients in response to glutamate became prolonged. Na^+ imaging with a fluorescent indicator indicated that intercellular diffusion of small cations in the astrocytic syncytium via gap junctions became rapidly restricted after epileptiform activity. Using a transgenic mouse with astrocyte-specific expression of a pH sensor (Lck-E^2GFP), we confirmed that astrocytes react to epileptiform activity with intracellular alkalization. Application of Na^+/HCO_3^- cotransporter blocker led to the suppression of intracellular alkalization of astrocytes and to the prevention of astrocyte uncoupling and hyperactivity intensification both in vitro and in vivo. Therefore, the inhibition of astrocyte alkalization could become a promising therapeutic strategy for countering epilepsy development.

Commentary

The human brain is made up of neurons and glial cells. Glia are a diverse group of cells that provide structural and nutritional support for neurons. It is becoming clear that glia can also play critical roles in regulating neuronal activity. Astrocytes are glial cells that perform many functions in the central nervous system, including secretion of neurotransmitters and maintenance of the blood–brain barrier. They are also key regulators of brain homeostasis. Astrocytes express channels and transporters that help maintain proper extracellular levels of glutamate, potassium, and water during synaptic activity. Disruptions in astrocyte function have been associated with hyperexcitability and epileptogenesis.[1] For example, high extracellular potassium (K^+) levels can cause epileptiform activity in acute brain slices. Astrocytes clear K^+ generated during synaptic activity from the extracellular space. One mechanism that astrocytes use to clear K^+ is called spatial buffering. This process relies on inward-rectifying potassium (K_{ir}) channels and connections between astrocytes called gap junctions to transfer K^+ from regions of elevated K^+ concentration to regions of lower K^+ concentration in a network of functionally coupled astrocytes. Astrocyte uncoupling through gap junctions can therefore hinder K^+ clearance.

Decreased astrocytic coupling has been documented in sclerotic hippocampal tissue from individuals with mesial temporal lobe epilepsy (mTLE).[2] Mice injected with kainic acid exhibited decreased astrocytic coupling and impaired K+ clearance that preceded onset of spontaneous seizures.[2] Uncoupling of astrocytes was also seen in a febrile seizure model,[3] suggesting that astrocytic uncoupling may be a general mechanism underlying epileptogenesis. Preventing uncoupling or restoring connectivity in astrocytes that have uncoupled due to seizure activity may inhibit epilepsy progression. A new study by Onodera and colleagues[4] has revealed that intracellular alkalization of astrocytes contributes to astrocyte uncoupling after seizures. Blocking this alkalization prevents uncoupling and may represent a novel anti-epileptogenic therapeutic strategy.

In this study, a brief bout of epileptiform activity was induced in hippocampal brain slices from mice. This treatment was sufficient to cause astrocytic uncoupling and impairment of K^+ clearance. Using a genetically encoded pH sensor

expressed specifically in astrocytes, the researchers found that epileptiform activity resulted in significant alkalization of astrocytes. In an independent experiment, they demonstrated that chemical induction of alkalization also resulted in gap junction uncoupling. These findings suggested that alkalization of astrocytes after epileptiform activity could provide a mechanism for seizure-associated astrocyte uncoupling. They hypothesized that inward activity of the $Na^+/HCO3^-$ cotransporter (NBC) could account for the alkalization through influx of the alkaline ion $HCO3^-$. Blockade of NBC reduced alkalization of astrocytes, indicating that the alkalization was caused in part by NBC activity. The NBC blocker also prevented astrocytic uncoupling and suppressed neuronal excitability in the slices exposed to epileptiform activity. The authors extended these findings to an in vivo mouse model of rapid hippocampal kindling. Administration of the NBC blocker into the mouse hippocampus prevented the development of hyperactivity from short-term electrical stimulation, suggesting that blockade of NBC activity could be an effective antiepileptogenic therapy. However, NBC blockade did not prevent hyperexcitability following prolonged stimulation, indicating that NBC activity-mediated astrocyte alkalization is only partially responsible for kindling and represents one of multiple mechanisms underlying epileptogenesis.

The results from this study are in agreement with a study by a different group showing that epileptiform discharges weaken astrocyte coupling and decrease spatial redistribution of K^+ ions.[5] Genetic deletion of astrocyte gap junction results in spontaneous epileptiform activity and worsens seizures in mouse models of temporal lobe epilepsy (TLE).[6] In contrast, other studies have found pro-epileptic effects of enhanced gap junction connectivity during epileptiform activity.[7] For example, one study found that elevated levels of extracellular K^+ produced during epileptiform bursting can enhance gap junction connectivity and potentially promote seizures by synchronizing neural activity via nonsynaptic mechanisms such as glial calcium waves propagating through the astrocyte network. Another study found that glucose uptake was increased during epileptiform activity, and distribution of glucose via gap junctions was necessary to sustain the epileptiform activity. Currently, the preponderance of data from human samples and experimental models strongly suggest that gap junction uncoupling is pathogenic in mTLE with hippocampal sclerosis. More studies will be needed in models of other types of epilepsy to assess the role of gap junction functionality more broadly.

These findings are intriguing because they suggest that targeting astrocyte function, rather than directly targeting neuronal function, may be a promising new therapeutic strategy. Identification of new treatments is especially important for the 30% of epilepsy patients who do not respond to current treatments. To demonstrate whether NBC blockade could be a potential epilepsy treatment, the NBC blocker would need to be tested in additional epilepsy models, including models of acquired spontaneous epilepsy. These studies would determine whether prevention of astrocyte alkalization could indeed prevent epileptogenesis after traumatic brain injury or status epilepticus. It would also be interesting to test the generality of NBC blockade in suppressing hyperexcitability by testing it in models of genetic epilepsy where hyperexcitability is driven by a known molecular mechanism.

Uncoupling of astrocytes is only one aspect of astrocyte dysfunction that may contribute to epileptogenesis. Although it is now widely accepted that astrocytes play a role in mediating excitability in the brain, it remains unclear whether changes in astrocyte function after seizures are pathological or compensatory and which changes represent targets for therapeutic intervention. Given that astrocytes are key regulators of homeostasis in the brain, the response of astrocytes to neuronal hyperexcitability is sure to be complex. The finding that epileptiform discharges provoke astrocyte alkalization and uncoupling of astrocytes that impedes K^+ clearance is only one piece of this puzzle. In addition to mediating extracellular ion and water balance, astrocytes are actively involved in processes including neuroinflammation, energy supply and metabolism, and maintenance of the blood–brain barrier.[1] Astrocytes also release many molecules, including glutamate, GABA, glycine, ATP, and BDNF, that can function as "gliotransmitters" to regulate neuronal excitability and synaptic transmission. It will be important to study multiple aspects of astrocyte activity in normal and hyperexcitable networks, as well as after seizures, to understand how dysregulation of astrocyte function contributes to the development of epilepsy. Then, specific types of astrocytic dysfunction can be interrogated as new targeted therapies for epilepsy and prevention of epileptogenesis.

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References

1. Verhoog QP, Holtman L, Aronica E, van Vliet EA. Astrocytes as guardians of neuronal excitability: mechanisms underlying epileptogenesis. *Front Neurol*. 2020;11:591690.
2. Bedner P, Dupper A, Hüttmann K, et al. Astrocyte uncoupling as a cause of human temporal lobe epilepsy. *Brain*. 2015;138(pt 5):1208-1222.
3. Khan D, Dupper A, Deshpande T, Graan PNED, Steinhäuser C, Bedner P. Experimental febrile seizures impair interastrocytic gap junction coupling in juvenile mice. *J Neurosci Res*. 2016;94(9):804-813.
4. Onodera M, Meyer J, Furukawa K, et al. Exacerbation of epilepsy by astrocyte alkalization and gap junction uncoupling. *J Neurosci*. 2021;41(10):2106-2118.
5. Wang Q, Wang W, Aten S, Kiyoshi CM, Du Y, Zhou M. Epileptiform neuronal discharges impair astrocyte syncitial isopotentiality in acute hippocampal slices. *Brain Sci*. 2020;10(4):208.
6. Deshpande T, Li T, Henning L, et al. Constitutive deletion of astrocytic connexins aggravates kainate-induced epilepsy. *Glia*. 2020;68(10):2136-2147.
7. Seifert G, Steinhäuser C. Neuron-astrocyte signaling and epilepsy. *Exp Neurol*. 2013;244:4-10.