Losing Touch With Your Astrocytes Can Cause Epilepsy

Reactive Astrogliosis Causes the Development of Spontaneous Seizures.

Robel S, Buckingham SC, Boni JL, Campbell SL, Danbolt NC, Riedemann T, Sutor B, Sontheimer H. *J Neurosci* 2015;35(8):3330–3345.

Epilepsy is one of the most common chronic neurologic diseases, yet approximately one-third of affected patients do not respond to anticonvulsive drugs that target neurons or neuronal circuits. Reactive astrocytes are commonly found in putative epileptic foci and have been hypothesized to be disease contributors because they lose essential homeostatic capabilities. However, since brain pathology induces astrocytes to become reactive, it is difficult to distinguish whether astrogliosis is a cause or a consequence of epileptogenesis. We now present a mouse model of genetically induced, widespread chronic astrogliosis after conditional deletion of β1-integrin (Itgβ1). In these mice, astrogliosis occurs in the absence of other pathologies and without BBB breach or significant inflammation. Electroencephalography with simultaneous video recording revealed that these mice develop spontaneous seizures during the first six postnatal weeks of life and brain slices show neuronal hyperexcitability. This was not observed in mice with neuronal-targeted β1-integrin deletion, supporting the hypothesis that astrogliosis is sufficient to induce epileptic seizures. Whole-cell patch-clamp recordings from astrocytes further suggest that the heightened excitability was associated with impaired astrocytic glutamate uptake. Moreover, the relative expression of the cation-chloride cotransporters (CCC) NKCC1 (Slc12a2) and KCC2 (Slc12a5), which are responsible for establishing the neuronal Cl^- gradient that governs GABAergic inhibition were altered and the NKCC1 inhibitor bumetanide eliminated seizures in a subgroup of mice. These data suggest that a shift in the relative expression of neuronal NKCC1 and KCC2, similar to that observed in immature neurons during development, may contribute to astrogliosis-associated seizures.

Commentary

It is now widely accepted that epilepsy (and many other neurologic disorders) can occur as a result of combined neuronal and astrocytic dysfunction (1, 2). Astrocytes, long thought of as supportive cells in the brain, clean up after messy neurons who dump glutamate and potassium into the extracellular space with wild abandon. But, astrocytes are more than just the cleanup crew for these cavalier neurons. They actively release neuroactive substances, have complex cell–cell interactions with multiple cell types, and are poised to quickly react to multiple forms of brain insult. A significant challenge in understanding the role of astrocytes in epilepsy has been the complex relationship between seizure-provoking insult (e.g., fever, injury, genetic lesion), neuronal dysfunction, and reactive astrocytosis. Reactive astrocytosis is an umbrella term that refers to a bevy of changes in astrocyte properties following brain insult (3). These include upregulation of the astrocyte intermediate filament protein glial fibrillary acidic protein (GFAP), cell hypertrophy, alterations in the protein expression profile, loss of homeostatic function, and changes in the ability of astrocytes to enzymatically recycle neurotransmitters (4).

Epilepsy Currents, Vol. 15, No. 6 (November/December) 2015 pp. 349–350

As reactive astrocytosis is caused by seizures, and many of the molecular changes that occur during reactive astrocytosis may be proconvulsive, it has been difficult to delineate whether reactive astrocytosis is a cause or an effect of epilepsy. While the question may be viewed as one of semantics, it is not. Multiple studies have shown that astrocyte-specific manipulations, on their own, can induce seizures and significant neuronal pathology (5, 6). Furthermore, the vast majority of modern antiepileptic drugs (AEDs) target synaptic function and voltage-gated ion channels. As a third or more of people suffering from epilepsy are not adequately managed by current AEDs, finding new molecular targets is critical. Perhaps astrocytes may provide previously unappreciated molecular targets for restoring normal brain function in epilepsy.

To directly test the question of whether reactive astrocytosis can cause epilepsy, Robel and colleagues designed an elegant series of experiments to produce reactive astrocytosis without injury or insult. Using a mouse genetic approach, they selectively deleted a protein known as β1-integrin in GFAP-expressing cells. By targeting this cell population, the authors removed β1-integrin from all astrocytes. It should be noted that GFAP is also expressed by neural stem cells, so their manipulation likely affected all forebrain neural lineages. Deleting β1-integrin resulted in profound and progressive reactive astrocytosis, increased neuronal excitability, interictal spiking, and spontaneous seizures. While full-blown seizures

were not frequent in β1-integrin–lacking animals, spontaneous seizures were recorded in over 50% of animals (likely an underestimation owing to limited EEG recording time), and interictal spiking occurred at a high frequency. Of importance, this genetic manipulation did not cause a breach of the blood–brain barrier or induce significant inflammation. Furthermore, these effects were striking, as deletion of β1-integrin only in neurons (by targeting Nex-expressing cells) did not replicate any of the effects.

The astrocyte-specific effects of deletion of β1-integrin were next investigated. Both potassium buffering, by K_{ir} channels, and glutamate uptake, by excitatory amino acid transporters, were disrupted. Loss of these essential astrocyte processes was not due to decreased expression of the relevant proteins but rather to functional changes likely caused by mislocalization or improper molecular trafficking. As both astrocyte morphology and cytoskeleton are disrupted in the reactive state, it is highly likely that potassium channels and glutamate transporters are not present at their normal site of action in β1-integrin conditional knockouts. β1-Integrin mediates membrane–membrane interactions (see below) and therefore may directly, or indirectly, disrupt the trafficking of potassium buffering and glutamate transport proteins. The neuronal effects of β1-integrin deletion were also investigated. Of interest, disruptions were found in NKCC1 and KCC2, important chloride transporters, which set the chloride reversal potential, thus dictating the effects of GABA receptor activation (7, 8). These transporters are developmentally regulated. In the immature brain, NKCC1 predominates, while mature neurons express more KCC2. This leads to higher intracellular chloride levels in the neonatal brain, associated with depolarization upon GABA receptor activation, and lower intracellular chloride levels in the adult brain, giving GABA receptors their powerful inhibitory effects. When β1-integrin was conditionally deleted in these experiments, the KCC2:NKCC1 ratio was disrupted in animals older than 6 weeks. These changes are consistent with the immature state and could lead to increased intracellular chloride levels, decreased inhibitory GABA receptor currents, and seizures. To demonstrate the relevance of these changes to the seizure phenotype seen in β1-integrin–lacking animals, the authors treated the mice with bumetanide. This attenuated the seizure phenotype in approximately half the animals.

Together these studies clearly show that deletion of β1-integrin in GFAP-expressing cells induces reactive astrocytosis without other confounds normally associated with brain injury, stroke, and the like, and that this leads to spontaneous seizures. This is an important result as it highlights the role of astrocytes in the epileptogenic process and is a wake-up call to all those focusing exclusively on neuronal dysfunction in epilepsy. The obvious next question is, How does loss of β1-integrin do this? Integrins are transmembrane receptor proteins that mediate cell–cell and cell–extracellular matrix interactions. They link to intracellular signaling cascades that control pathways from cell cycle regulation to cell motility. It is highly likely that deletion of β1-integrin directly leads to changes in important cell–cell interactions and signaling pathways. When cell–cell contacts are lost or altered, cell morphology and protein localization are likely disrupted. These could directly lead to the functional changes described by Robel et al. There are a number of important studies examining the role of β1-integrin in astrocytes (9) and many other cell types (10). More recently, the specific downstream targets of β1-integrin signaling in astrocytes have also been studied, implicating integrin-linked kinase (11). Understanding astrocyte-specific signaling pathways, and the profound effect of changes in astrocyte morphology on their function, is of critical importance. The link between conditional loss of β1-integrin and changes in neuronal chloride handling is another important question raised by this study. Neurons and astrocytes are known to interact, but the mechanistic link between β1-integrin and KCC2:NKCC1 has yet to be determined. Perhaps studies like this one can utilize a reductionist approach to understanding reactive astrogliosis by manipulating individual components of the complex and dynamic cellular phenotype. Whether this will lead to novel astrocyte-directed therapies remains to be seen, but current animal studies suggest there may be a wealth of opportunity waiting to be tapped.

by Chris G. Dulla, PhD

References

1. Wetherington J, Serrano G, Dingledine R. Astrocytes in the epileptic brain. *Neuron* 2008;58:168–178.
2. Seifert G, Schilling K, Steinhauser C. Astrocyte dysfunction in neurological disorders: A molecular perspective. *Nat Rev Neurosci* 2006;7:194–206.
3. Sofroniew MV. Molecular dissection of reactive astrogliosis and glial scar formation. *Trends Neurosci* 2009;32:638–647.
4. Ortinski PI, Dong J, Mungenast A, Yue C, Takano H, Watson DJ, Haydon PG, Coulter DA. Selective induction of astrocytic gliosis generates deficits in neuronal inhibition. *Nat Neurosci* 2010;13:584–591.
5. Wong M, Ess KC, Uhlmann EJ, Jansen LA, Li W, Crino PB, Mennerick S, Yamada KA, Gutmann DH. Impaired glial glutamate transport in a mouse tuberous sclerosis epilepsy model. *Ann Neurol* 2003;54:251–256.
6. 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.
7. Delpire E, Staley KJ. Novel determinants of the neuronal Cl(-) concentration. *J Physiol* 2014;592:4099–4114.
8. Kaila K, Price TJ, Payne JA, Puskarjov M, Voipio J. Cation-chloride cotransporters in neuronal development, plasticity and disease. *Nat Rev Neurosci* 2014;15:637–654.
9. Robel S, Mori T, Zoubaa S, Schlegel J, Sirko S, Faissner A, Goebbels S, Dimou L, Götz M. Conditional deletion of beta1-integrin in astroglia causes partial reactive gliosis. *Glia* 2009;57:1630–1647.
10. Hall DE, Reichardt LF, Crowley E, Holley B, Moezzi H, Sonnenberg A, Damsky CH. The alpha 1/beta 1 and alpha 6/beta 1 integrin heterodimers mediate cell attachment to distinct sites on laminin. *J Cell Biol* 1990;110:2175–2184.
11. Pan L, North HA, Sahni V, Jeong SJ, Mcguire TL, Berns EJ, Stupp SI, Kessler JA. β1-Integrin and integrin linked kinase regulate astrocytic differentiation of neural stem cells. *PLoS One* 2014; 9:e104335. doi:10.1371/journal.pone.0104335.