Could Astrocytes Be Used to Beat Epilepsy? Experiments in dnSNARE Mice Drum Up New Hope

Commentary
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The greatest challenge faced by those studying the role of astrocytes in epilepsy has been a lack of astrocyte-specific tools to dissect the contribution of these cells to neuronal networks. Fortunately, in the last few years, the increased accessibility of cell type-specific inducible transgenic mice and optogenetic sensors, such as the GCaMP family of proteins, offers hope that exciting breakthroughs lay just beyond the horizon (7). In this vein, a recent study by Clasadonte and colleagues used astrocyte-specific transgenic mice to investigate the role that astrocyte-mediated transmitter release may play in the progression of TLE.

Previously this group developed a line of inducible dominant negative SNARE (dnSNARE) transgenic mice, wherein the vesicular machinery required for the release of transmitters is selectively disrupted in astrocytes (8). In the current study, 2-month-old dnSNARE and WT male littermate mice were administered low-dose injections of pilocarpine and allowed to undergo status epilepticus (SE) for 90 minutes before terminating the SE with diazepam. After a latent period following SE, these mice went on to develop TLE, characterized by spontaneous recurrent seizures. Appropriately, this progressive development of seizures was tracked using long-term video EEG recording. The dnSNARE mice had a longer latency to the development of spontaneous recurrent seizures than did wild-type age-matched controls. Furthermore, during the chronic epilepsy period, the dnSNARE mice had less severe seizures, a slower progression of seizure severity over time, and a reduction in the number of interictal spikes as compared to wild-type controls, suggesting that the loss of vesicular release of signaling molecules in the dnSNARE mice could modify epileptogenesis.

Astrocyte Control of Synaptic NMDA Receptors Contribute to the Progressive Development of Temporal Lobe Epilepsy.

Astrocytes modulate neuronal activity, synaptic transmission, and behavior by releasing chemical transmitters in a process termed gliotransmission. Whether this process impacts epilepsy in vivo is not known. We show that genetic impairment of transmitter release from astrocytes by the expression of a glial dominant negative SNARE domain in mice reduced epileptiform activity in situ, delayed seizure onset after pilocarpine-induced status epilepticus, and attenuated subsequent progressive increase in seizure frequency in vivo. The reduced seizure frequency was accompanied by attenuation of hippocampal damage and behavioral deficits. As the delay in seizure onset and the reduced seizure frequency were mimicked by intracerebroventricular delivery of the NMDA receptor (NMDAR) antagonist D-(-)-2-amino-5-phosphonopentanoate in WT littermates and because dominant-negative SNARE expression leads to a hypofunction of synaptic NMDARs, we conclude that astrocytes modulate epileptogenesis, recurrent spontaneous seizures, and pathophysiological consequences of epilepsy through a pathway involving NMDARs.

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The investigators also looked at behavioral and histological markers associated with TLE 8 months after SE. They assessed locomotor activity using an open field behavior test. Intriguingly, wild-type mice treated with pilocarpine showed less locomotor activity than their dnSNARE counterparts. Clasadonte et al. also quantified the relative number of neurons using an antibody for NeuN. Profound neuronal cell death was associated with SE in the hilar region of the dentate gyrus in WT mice but not in dnSNARE mice suggesting that the dnSNARE mutation is neuroprotective. The authors also investigated reactive gliosis using immunohistochemistry for glial fibrillary acidic protein (GFAP, a protoplasmic astrocytic marker) and found less GFAP expression in the dnSNARE mice after SE compared to controls. These studies pointed to a reduced pathology of brain regions that are normally quite sensitive to SE.

They then turned to an in vitro acute brain slice model of epileptiform activity to investigate changes in synaptic physiology. In brain slices prepared from dnSNARE mice there was a reduced latency to onset of epileptiform activity, and a decrease in the number of ictal-like events compared to WT mice. Furthermore, patch-clamp recordings from CA1 pyramidal neurons 5 months after pilocarpine treatment indicated that NMDA currents were significantly reduced in the dnSNARE mice. Finally, Clasadonte and coauthors found that treatment with D-AP5, an NMDA receptor antagonist, during SE dramatically reduced the latency of epileptiform activity in the hilar region of the dentate gyrus in WT mice but not in dnSNARE mice suggesting that the dnSNARE mutation is neuroprotective. The authors also investigated reactive gliosis using immunohistochemistry for glial fibrillary acidic protein (GFAP, a protoplasmic astrocytic marker) and found less GFAP expression in the dnSNARE mice after SE compared to controls. These studies pointed to a reduced pathology of brain regions that are normally quite sensitive to SE.

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This investigation offered a valuable new perspective on understanding the role of astrocyte-derived chemical transmission on the process of epileptogenesis. A particular strength of the study was the use of astrocyte-specific inducible genetic techniques, as selectively distinguishing astrocyte versus neuronal contributions to networks has been a continuous limitation in other studies attempting to understand astrocyte function in epileptogenesis.

Furthermore, the use of long-term chronic EEG and behavioral monitoring was another considerable strength of the study and provided important relevant data on the various phases of TLE disease progression. One concerning aspect of the experimental design was the lack of electrophysiologic monitoring during pilocarpine-induced SE for all mice in the study. Although behavioral seizures were assessed, this evidence alone is inadequate to conclude that the severity of SE was the same in both the dnSNARE and their wild-type counterparts. Thus, it cannot be ruled out that the severity of SE was reduced in some of the dnSNARE mice possibly explaining the reductions in seizures, as well as histologic and behavioral differences between the two groups. In addition, analysis of seizures was performed only on 4 to 6 individual mice. Given the variability in number of seizures among the different cohorts, forthcoming studies should include larger sample sizes when assessing behavioral and electrographic seizure measures.

Future directions will hopefully address the selective mechanisms that control glutamate release from astrocytes in wild-type mice as this still remains a contentious topic within the field of astrocyte physiology. In addition, caution should be taken not to conflate phenocopying with the elucidation of a direct mechanism. Furthermore, specific elaboration of the NMDAR-dependent pathway in which astrocyte-selective inhibition of vesicle release is effective in diminishing progression of TLE is warranted. While the approach taken in this study suggests that glutamate released from astrocytes may be contributing to the progression of TLE, the authors did not rule out contributions from other gliotransmitters, such as D-serine, a required co-agonist of the NMDAR receptor.

Most excitingly, the study by Clasadonte and colleagues provides evidence suggesting that astrocytes could serve as potential disease-modifying targets in TLE, the Holy Grail for epilepsy researchers. Thus, our goal should be to conduct experiments that provide nuanced information about the mechanistic role of astrocytes during epileptogenesis. With so many new and exciting emerging technologies, obtaining robust data that clarifies and strengthen our models is well within reach.

by Meredith B. Gibbons and Karen S. Wilcox, PhD

References

American Epilepsy Society
Epilepsy Currents Journal
Disclosure of Potential Conflicts of Interest

Instructions
The purpose of this form is to provide readers of your manuscript with information about your other interests that could influence how they receive and understand your work. Each author should submit a separate form and is responsible for the accuracy and completeness of the submitted information. The form is in four parts.

1. Identifying information.
   Enter your full name. If you are NOT the main contributing author, please check the box “no” and enter the name of the main contributing author in the space that appears. Provide the requested manuscript information.

2. The work under consideration for publication.
   This section asks for information about the work that you have submitted for publication. The time frame for this reporting is that of the work itself, from the initial conception and planning to the present. The requested information is about resources that you received, either directly or indirectly (via your institution), to enable you to complete the work. Checking “No” means that you did the work without receiving any financial support from any third party – that is, the work was supported by funds from the same institution that pays your salary and that institution did not receive third-party funds with which to pay you. If you or your institution received funds from a third party to support the work, such as a government granting agency, charitable foundation or commercial sponsor, check “Yes”. Then complete the appropriate boxes to indicate the type of support and whether the payment went to you, or to your institution, or both.

3. Relevant financial activities outside the submitted work.
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1. Today's Date: September 21, 2014

2. First Name  Karen     Last Name Wilcox  Degree PhD

3. Are you the Main Assigned Author? ☑ Yes ☐ No

If no, enter your name as co-author:

4. Manuscript/Article Title: Could Astrocytes Be Used to Beat Epilepsy? Experiments in dnSNARE Mice Drum Up New Hope

5. Journal Issue you are submitting for: 14.5

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Did you or your institution at any time receive payment or services from a third party for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc.)?

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**Section #4 Other relationships**

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