



Tangled Roots: Digging Deeper Into Astrocyte or Interneuron Dysfunction in Temporal Lobe Epilepsy

Selective Induction of Astrocytic Gliosis Generates Deficits in Neuronal Inhibition.

Ortinski PI, Dong J, Mungenast A, Yue C, Takano H, Watson DJ, Haydon P, Coulter DA. *Nat Neurosci* 2010;13:584–591.

Reactive astrocytosis develops in many neurologic diseases, including epilepsy. Astrocytotic contributions to pathophysiology are poorly understood. Studies examining this are confounded by comorbidities accompanying reactive astrocytosis. We found that high-titer transduction of astrocytes with enhanced green fluorescent protein (eGFP) via adeno-associated virus induced reactive astrocytosis without altering the intrinsic properties or anatomy of neighboring neurons. We examined the consequences of selective astrocytosis induction on synaptic transmission in mouse CA1 pyramidal neurons. Neurons near eGFP-labeled reactive astrocytes had reduced inhibitory, but not excitatory, synaptic currents. This inhibitory postsynaptic current (IPSC) erosion resulted from a failure of the astrocytic glutamate-glutamine cycle. Reactive astrocytes downregulated expression of glutamine synthetase. Blockade of this enzyme normally induces rapid synaptic GABA depletion. In astrocytotic regions, residual inhibition lost sensitivity to glutamine synthetase blockade, whereas exogenous glutamine administration enhanced IPSCs. Astrocytosis-mediated deficits in inhibition triggered glutamine-reversible hyperexcitability in hippocampal circuits. Thus, reactive astrocytosis could generate local synaptic perturbations, leading to broader functional deficits associated with neurologic disease.

Transplant of GABAergic Precursors Restores Hippocampal Inhibitory Function in a Mouse Model of Seizure Susceptibility.

Zipancic I, Calcagnotto ME, Piquer-Gil M, Mello LE, Álvarez-Dolado M. *Cell Transplant* 2010;19:549–564.

Defects in GABAergic function can cause epilepsy. In the last years, cell-based therapies have attempted to correct these defects with disparate success on animal models of epilepsy. Recently, we demonstrated that medial ganglionic eminence (MGE)-derived cells grafted into the neonatal normal brain migrate and differentiate into functional mature GABAergic interneurons. These cells are able to modulate the local level of GABA-mediated synaptic inhibition, which suggests their suitability for cell-based therapies. However, it is unclear whether they can integrate in the host circuitry and rescue the loss of inhibition in pathological conditions. Thus, as proof of principle, we grafted MGE-derived cells into a mouse model of seizure susceptibility caused by specific elimination of GABAergic interneuron subpopulations in the mouse hippocampus after injection of the neurotoxic saporin conjugated to substance P (SSP-Sap). This ablation was associated with significant decrease in inhibitory postsynaptic currents (IPSC) on CA1 pyramidal cells and increased seizure susceptibility induced by pentylenetetrazol (PTZ). Grafting of GFP⁺ MGE-derived cells in SSP-Sap-treated mice repopulates the hippocampal ablated zone with cells expressing molecular markers of mature interneurons. Interestingly, IPSC kinetics on CA1 pyramidal cells of ablated hippocampus significantly increased after transplantation, reaching levels similar to the normal mice. More importantly, this was associated with reduction in seizure severity and decrease in post-seizure mortality induced by PTZ. Our data show that MGE-derived cells fulfill most of the requirements for an appropriate cell-based therapy, and indicate their suitability for neurological conditions where a modulation of synaptic inhibition is needed, such as epilepsy.



Commentary

Hippocampal tissue resected from patients with intractable temporal lobe epilepsy (TLE) often shows mesial temporal sclerosis, with reactive astrogliosis, diminished numbers of interneurons, and characteristic mossy fiber sprouting of dentate granule neurons into the inner molecular layer of the dentate gyrus (1–4). These anatomic changes are also seen in several experimental rodent models of TLE that result in spontaneous recurrent seizures. TLE patients as well as rodents with severe TLE, may also exhibit metabolic alterations and neuroinflammatory changes. It has been controversial whether interneuron loss or mossy fiber sprouting is sufficient to cause epileptogenesis, but there is general agreement that decreased synaptic inhibition and increased synaptic excitation in the dentate gyrus are key features of TLE. Due to the concomitant emergence of neuropathological, metabolic, immunologic, and electrophysiologic changes, sorting out which changes cause epileptogenesis has been a challenge in the field of epilepsy research.

Now, two studies have used molecular approaches to induce reactive astrogliosis or cull interneurons to advance our understanding of pathophysiology of temporal lobe epilepsy. Ortinski and colleagues induced reactive gliosis with a novel adenoviral approach. In contrast, Zipancic and colleagues culled GABAergic interneuron populations in the hippocampus with a selective neurotoxin. Despite the different cell types that were targeted, both approaches generate a decrease in inhibitory synaptic currents and hyperexcitability in the hippocampus. Together, these studies are aiding efforts to disentangle the contributions of different cell types and synaptic dysfunction in temporal lobe epilepsy.

Astrocytes serve a key role in supplying glutamine to neurons and rapidly clearing extracellular glutamate. This process appears to be disrupted in patients with TLE, as a six-fold rise in the extracellular concentrations of glutamate in the hippocampus occurs during temporal lobe seizures, and the elevated levels persist for nearly half an hour following seizures (5). The enzyme glutamine synthetase is selectively expressed by astrocytes, allowing them to convert glutamate into glutamine. Glutamine transporters then allow the transfer of glutamine from glia to neurons, where it is converted to glutamate. In inhibitory interneurons, this process is taken a step further—glutamate is converted into the inhibitory neurotransmitter GABA. When reactive astrocytes hypertrophy in TLE, they reduce expression of glutamine synthetase, creating a shortage in the supply of glutamine for GABA synthesis within interneurons. Higher levels of extracellular glutamate have been proposed to trigger seizures and excitotoxic neuronal injury.

While reduced glutamine synthetase seems to be an important factor contributing to loss of synaptic inhibition and increased excitation in the hippocampus, a breakdown of the glutamine-glutamate cycle had not been demonstrated to reduce synaptic inhibition in the hippocampus and increase excitability, in the absence of any overt neuronal losses or mossy fiber sprouting. By transducing hippocampal astrocytes with an adeno-associated virus that triggers marked astrocyte hypertrophy, Ortinski and colleagues were able to explore the link between reactive gliosis, reduced glutamine synthetase, and inhibitory synaptic function.

Specific transduction of astrocytes in the hippocampal CA1 region was achieved by stereotactic delivery of an adeno-associated virus (AAV2/9) containing a vector in which the Glial Fibrillary Acidic Protein (GFAP) promoter controls expression of enhanced green fluorescent protein (eGFP). High titers of this virus cause astrocytes to hypertrophy at the site of injections and enhance levels of eGFP expression in these cells, allowing visualization of the reactive astrocytes in brain slices. To examine synaptic transmission in regions of reactive gliosis, the authors examined evoked inhibitory postsynaptic currents (eIPSCs) and spontaneous IPSCs in CA1 pyramidal neurons proximal to reactive astrocytes infected with AAV2/9. Whole cell electrophysiological recordings showed smaller eIPSCs in CA1 pyramidal neurons with lower amplitudes and reduced frequencies of spontaneous miniature inhibitory postsynaptic currents (mIPSCs). These findings indicate that inhibitory neurotransmission in CA1 neurons is impaired when the neurons are near reactive astrocytes. In contrast to these results, the excitatory postsynaptic potentials (EPSPs) recorded from CA1 neurons were comparable in control and virally transduced slices, showing that excitatory signaling was not altered by reactive gliosis. They further show that reactive gliosis produces more widespread excitability across broad expanses of the CA1 pyramidal layer, an effect reversed by supplying extra glutamine to the solutions bathing the slices. These results demonstrate that the hippocampal network becomes hyperexcitable during reactive gliosis associated with reduced levels of glutamine synthetase and less synaptic GABA release.

Insights gained from these mechanistic studies go beyond the age-old concept that reduced inhibitory neurotransmission in the hippocampus contributes to hyperexcitability of hippocampal circuits. These studies show for the first time that astrogliosis occurring in the hippocampus in the absence of overt interneuron death and mossy fiber sprouting, can induce widespread deficits in inhibitory neurotransmission. The authors speculate upon the possibility that this effect is due to reduced quantal release of GABA at inhibitory synapses. Because synaptic GABAergic neurotransmission appears to rely more upon astrocyte-supplied glutamine than glutamatergic transmission, astrocytic changes would likely impact synaptic release of GABA onto both principal neurons and interneurons, creating less inhibition throughout the region of reactive gliosis.

These findings raise additional questions. Does induction of regional hippocampal astrogliosis in mice with this experimental approach enhance seizure susceptibility, and lead to spontaneous recurrent seizures? Would a gene therapy targeted to increase the production of glutamine synthetase in the hippocampus suppress seizures in TLE? Determining the molecular mechanisms for how reactive gliosis is triggered by the specific adeno-associated virus used in these studies may also indicate molecular targets for drug therapies to prevent hippocampal sclerosis.

In a separate study, by Zipancic and colleagues, inhibitory neurotransmission was reduced by a method that selectively targets interneurons with a substance P analogue conjugated to a neurotoxin called saporin, a ribosome-inactivating protein (6). Because diverse types of hippocampal interneurons express substance P receptors, this method eliminates



most hippocampal interneurons that express neuropeptide Y, somatostatin, or substance P but does not destroy parvalbumin- or calretinin-expressing interneurons, excitatory pyramidal neurons, or astrocytes. Zipancic and colleagues injected the toxin at multiple sites along the septo-temporal axis of the hippocampus to ablate interneurons throughout the dentate gyrus and CA regions. One week following ablation by the toxin, they prepared hippocampal slices from the mice and measured whole cell currents in CA1 pyramidal neurons. They found significant reductions in spontaneous IPSCs and mIPSCs in CA1 pyramidal neurons, similar to the findings reported by Ortinski and colleagues after viral induction of astrogliosis. While the mice did not develop spontaneous recurrent seizures in the 2 months following interneuron ablation, they had lower seizure thresholds after injections of the proconvulsive agent, pentylenetetrazol. The authors further investigated whether supplementing synaptic levels of GABA in this model by means of GABA cell transplantation could restore normal levels of synaptic inhibition and raise the threshold for inducing seizures. Following transplantation of neural progenitors obtained from the medial ganglionic eminence, the site where genesis of most telencephalic GABAergic neurons occurs, sIPSCs and mIPSCs were restored to levels found in control mice. When transplanted into regions of the hippocampus depleted of interneurons, the grafted GABAergic progenitors restored normal levels of inhibitory synaptic currents within 2 months. The findings suggest that transplanted fetal GABAergic neurons differentiate after they are transplanted and augment the number of GABAergic synapses in CA1, bringing excitatory and inhibitory synaptic transmission back into balance.

Taken together, these new treatments to grow or diminish different hippocampal cell populations emphasize the point that different pathological mechanisms may cultivate similar deficits at inhibitory synapses and produce hyperexcitability. As these novel approaches are applied to study the causes and treatments for temporal lobe epilepsy, we can anticipate bountiful harvests in the field.

by Janice R. Naegele, PhD

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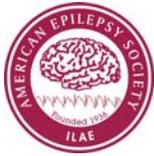
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Dr Coulter, one of the co-authors on one of the papers that I reviewed in my commentary, serves on an NIH study section that recently reviewed one of my grant applications (it was not funded). I wrote the commentary subsequent to the grant panel recommendations to not fund my grant application.