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Assessin' the Vexin' Connexin Between Severity of Epilepsy and Hippocampal Gliosis

Constitutive Deletion of Astrocytic Connexins Aggravates Kainate-Induced Epilepsy

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The astroglial gap junctional network formed by connexin (Cx) channels plays a central role in regulating neuronal activity and network synchronization. However, its involvement in the development and progression of epilepsy is not yet understood. Loss of interastrocytic gap junction (GJ) coupling has been observed in the sclerotic hippocampus of patients with mesial temporal lobe epilepsy (MTLE) and in mouse models of MTLE, leading to the suggestion that it plays a causative role in the pathogenesis. To further elucidate this clinically relevant question, we investigated consequences of astrocyte disconnection on the time course and severity of kainate-induced MTLE with hippocampal sclerosis (HS) by comparing mice deficient for astrocytic Cx proteins with wild-type (WT) mice. Continuous telemetric EEG recordings and video monitoring performed over a period of 4 weeks after epilepsy induction revealed substantially higher seizure and interictal spike activity during the chronic phase in Cx deficient versus WT mice, while the severity of status epilepticus was not different. Immunohistochemical analysis showed that, despite the elevated chronic seizure activity, astrocyte disconnection did not aggravate the severity of HS. Indeed, the extent of CA1 pyramidal cell loss was similar between the experimental groupsx, while astrogliosis, granule cell dispersion, angiogenesis, and microglia activation were even reduced in Cx deficient as compared to WT mice. Interestingly, seizure-induced neurogenesis in the adult dentate gyrus was also independent of astrocytic Cxs. Together, our data indicate that constitutive loss of GJ coupling between astrocytes promotes neuronal hyperexcitability and attenuates seizure-induced histopathological outcomes.

Keywords
gap junction, temporal lobe epilepsy, gliosis, astrocyte, hippocampus

Commentary

The past 2 decades have brought increased attention to the specific roles that glial cells, including astrocytes, play in the pathophysiology of epilepsy.[1] Reactive astrogliosis is a histopathological hallmark in most epilepsies and is present in the majority of animal models of acquired epilepsy, including mesial temporal lobe epilepsy (MTLE). However, it remains unclear whether astrogliosis is a cause or effect of seizure activity. On one hand, targeted transgenic induction of astrogliosis can drive spontaneous recurrent seizures,[2] and virally induced astrogliosis can result in neuronal hyperexcitability,[3] indicating a causative role for gliosis in the development of epilepsy. However, seizure activity is not always accompanied by gliosis,[4] suggesting that gliosis is not necessarily required for epilepsy development. Furthermore, it remains unclear if a bidirectional interaction exists between seizures and the severity of gliosis.

Astrocytes are notable for their extensive intercellular coupling through gap junctions, constructed primarily by connexin (Cx) proteins Cx43 and Cx30.[5] Whether this astrocyte coupling has pro- or antiepileptic effects is currently debated. Astrocytes play a critical role in ionic and biochemical buffering, in which gap junctions likely reduce excitability by allowing intercellular redistribution of potassium and glutamate across coupled astrocytes. Unsurprisingly, the uncoupling of astrocytes via constitutive deletion of astrocytic Cx43 and Cx30 (termed double knockout, or DKO mice) results in reduced potassium clearance and a reduced threshold for epileptiform events.[6] However, a separate study demonstrated that DKO animals lack the ability to transfer the necessary energy metabolites required to maintain epileptiform activity due to gap junction loss,[7] suggesting that astrocyte coupling can also have proconvulsant consequences. Altogether, our understanding of the complex interrelationships between astrogliosis, gap junction coupling, and seizure activity remains insufficient.

Previous work from the Bedner et al and Steinhäuser et al group demonstrated that the loss of gap junction coupling in hippocampal astrocytes precedes the onset of neurodegeneration and chronic seizure activity in an intracortical kainate model of MTLE,[8] suggesting that astrocyte uncoupling directly contributes to the development of MTLE. In this study, the same group investigated whether the constitutive loss of astrocytic gap junction channels in DKO animals impacts the pathogenesis and severity of MTLE.[9] Wild-type (WT) and DKO mice underwent the same procedure of kainate microinjection into the cortex directly above the right dorsal hippocampus, together with skull surface EEG electrode implantation. This intracortical injection induces MTLE while preserving the hippocampal integrity for histochemical analysis.[8] Twenty-four-hour telemetric EEG recordings were made during the initial status epilepticus (SE) and continued for four weeks alongside continuous video monitoring.

The authors first investigated if SE severity was altered in animals lacking astrocytic Cx proteins. Intriguingly, DKO animals experienced roughly half the number of seizures compared with WT animals, but the durations of individual seizures were nearly twice as long. This finding suggests that the loss of astrocyte gap junctions may initially be anticonvulsant, but once seizures manifest, lack of astrocyte coupling may be proconvulsant, facilitating sustained epileptiform activity and prolonging the duration of each seizure. Overall, the counteracting effects on seizure number and duration resulted in a similar amount of time in seizure activity during SE in both WT and DKO mice. By contrast, in the case of chronic spontaneous seizures, DKO mice experienced approximately twice the number of spontaneous seizures compared with WT mice, and seizure duration did not differ between groups. Additionally, DKO animals experienced increased interictal activity compared to WT, as demonstrated by more large-amplitude EEG spikes and higher δ-frequency band power. Altogether, the loss of astrocyte coupling in DKO animals significantly augments chronic seizure burden, and this phenomenon is not due to increased SE severity.

Next, the authors investigated whether astrocyte coupling impacts the severity of hippocampal degeneration and/or gliosis following development of MTLE. Histochemical analysis revealed that in the ipsilateral hippocampus, there was a nearly 90% reduction of CA1 neurons in both genotypes, demonstrating that the lack of coupled astrocytes did not alter the extent of seizure-induced neuronal death. Interestingly, however, the CA1 region in DKO animals showed an attenuation of several hallmark characteristics of MTLE compared with WT, including astrogliosis, microglial activation, and angiogenesis. Furthermore, seizure-induced granule cell dispersion (GCD) was significantly diminished in DKO mice. Taken together, it appears that astrocytic coupling contributes to the severity of both gliosis and GCD in this model of MTLE.

It is often assumed that seizure burden and gliosis are closely interrelated in MTLE and that worsening one aspect likely exacerbates the other. However, this study reveals a surprising dichotomy in which astrocyte uncoupling results in increased seizure burden during chronic epilepsy but an attenuation of astrogliosis and other histopathological hallmarks of MTLE. These data suggest that astrocyte gap junctions play critical, yet diametrically opposed, roles in mediating the respective severity of epilepsy and gliosis. One mechanism to explain this phenomenon is that gap junctions may also allow the intercellular transmission of toxic signaling molecules that propagate cell injury,[10] thus exacerbating gliosis. It is important to consider, however, that in DKO animals, astrocytes are uncoupled via the genetic removal of astrocytic Cx proteins, whereas in human resected tissue, increased expression of Cx proteins is often seen.[11] Additional evidence also suggests that astrocyte uncoupling in human and experimental MTLE is likely a result of subcellular reorganization and posttranslational modifications of Cx43.[12] Therefore, one could argue that the constitutive deletion of astrocytic Cx proteins does not functionally recapitulate the uncoupling observed in MTLE, and the astrogliosis typically seen in MTLE may be at least partially attributed to the maladaptive expression and functionality of Cx proteins. Nevertheless, the model used in this study may prove useful for future investigations probing the dissociation between seizure burden and gliosis development.

One caveat is that only male mice were utilized in this study. It would be interesting to determine whether females display similar results, especially since hippocampal astrocyte morphology can show sex differences and fluctuate across the estrous cycle in rodents.[13] Additionally, the number of recorded seizures seen in this study is relatively low for an intracranial kainate model (for example, see the study by Zeidler et al[14]), likely attributed to the use of cortical surface EEG electrodes, which may miss focal hippocampal electrographic seizures that do not spread to the cortex. Although the use of hippocampal depth electrodes could interfere with analysis of hippocampal sclerosis, it would be interesting to determine whether astrocyte uncoupling has differential effects on focal versus generalized seizure activity. Overall, this study highlights the critical nature of the syncytium-like network of astrocytes in the regulation of seizure activity while demonstrating that the extent and severity of seizures and gliosis in MTLE do not necessarily correlate with one another.

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