

SEEING THE FOREST AND THE TREES: DENDRITIC INJURY AFTER STATUS EPILEPTICUS

Kainate Seizures Cause Acute Dendritic Injury and Actin Depolymerization In Vivo. Zeng LH, Xu L, Rensing NR, Sinatra PM, Rothman SM, Wong M. *J Neurosci* 2007;27(43):11604–11613. Seizures may cause brain injury via a variety of mechanisms, potentially contributing to cognitive deficits in epilepsy patients. Although seizures induce neuronal death in some situations, they may also have “nonlethal” pathophysiological effects on neuronal structure and function, such as modifying dendritic morphology. Previous studies involving conventional fixed tissue analysis have demonstrated a chronic loss of dendritic spines after seizures in animal models and human tissue. More recently, in vivo time-lapse imaging methods have been used to monitor acute changes in spines directly during seizures, but documented spine loss only under severe conditions. Here, we examined effects of secondary generalized seizures induced by kainate, on dendritic structure of neocortical neurons using multiphoton imaging in live mice in vivo and investigated molecular mechanisms mediating these structural changes. Higher-stage kainate-induced seizures caused dramatic dendritic beading and loss of spines within minutes, in the absence of neuronal death or changes in systemic oxygenation. Although the dendritic beading improved rapidly after the seizures, the spine loss recovered only partially over a 24 h period. Kainate seizures also resulted in activation of the actin-depolymerizing factor, cofilin, and a corresponding decrease in filamentous actin, indicating that depolymerization of actin may mediate the morphological dendritic changes. Finally, an inhibitor of the calcium-dependent phosphatase, calcineurin, antagonized the effects of seizures on cofilin activation and spine morphology. These dramatic in vivo findings demonstrate that seizures produce acute dendritic injury in neocortical neurons via calcineurin-dependent regulation of the actin cytoskeleton, suggesting novel therapeutic targets for preventing seizure-induced brain injury.

COMMENTARY

Neuronal loss and reorganization of synaptic connectivity are two well-described consequences of chronic epilepsy, both in humans and experimental animal models. This type of neuronal injury can be seen at a macroscopic level, for example, in mesial temporal sclerosis. However, are there more subtle alterations in the structure and function of surviving neurons? The principal neuronal subtype of neocortex and hippocampus, the pyramidal neuron, is an obvious target of investigation, with its striking apical dendrites that arborize over many hundreds of microns, like the branches of an oak tree. The dendrites of pyramidal neurons are the main sites of excitatory synaptic input and comprise greater than 90% of the membrane

surface area of the cell. Electrophysiological studies of pyramidal dendrites in the past 10 years or so have disclosed the remarkable signaling occurring in these structures under normal conditions: dendrites support retrograde action potential propagation from the soma, which serves as a signal for synaptic plasticity, and localized activity-induced dendritic calcium transients activate the biochemical machinery of learning and memory (1).

Recent work has shown that epilepsy alters dendritic physiology. In the pilocarpine animal model of temporal lobe epilepsy, patch clamp recordings in the dendrites of hippocampal pyramidal neurons demonstrate a loss of A-type potassium channels and a concurrent enhancement of dendritic action potential propagation, both producing a potentially proconvulsant increase in neuronal excitability (2). Similarly, the onset of epilepsy is associated with a loss of dendritic hyperpolarization-activated cation channels, a situation that likewise predisposes to hyperexcitability (3). These studies, among others, suggest

that in epilepsy, dendrites are a locus of change for the intrinsic excitability properties of pyramidal neurons.

The present study asks whether morphological change in the dendrites also occurs in epilepsy. In some ways, this is a question with a decades-old answer. Observations dating to the 19th century described two main pathologies in dendrites from human epileptic tissue: varicose “swellings” along the dendritic shaft and loss of spines—the sites of excitatory synaptic contacts (4). Similar findings have been replicated in animal models of epilepsy. However, a limitation to these earlier studies is that they were performed in fixed tissue under conditions of chronic epilepsy and represented purely histological observations without investigation of underlying mechanisms. The work of Zeng et al. seeks to address the same issues by observing changes in dendritic structure in living animals mere hours after an episode of status epilepticus (SE). Their findings confirm that dendritic injury occurs on an acute timescale, and they begin to dissect the underlying mechanisms that depend on phosphorylation signaling.

A remarkable feature of the present study is the use of in vivo multiphoton imaging to visualize neocortical pyramidal neurons in a restrained, anesthetized but otherwise intact animal. In brief, a window of skull is removed in a transgenic mouse expressing a fluorescent protein in a subset of pyramidal neurons (mostly layer V cells). A microscope objective is positioned above the cortex, and stimulation with light at infrared wavelengths allows visualization of the most distal 100 μm or so of the dendritic tree. In essence, it is a 21st century glow-in-the-dark version of the Golgi stain, but in a living animal. The investigators were able to visualize dendritic shafts and spines in submicron resolution, first in untreated animals, and then after 30 min of kainate-induced SE.

Using this demanding technique, the authors found that swelling or beading of dendrites occurred within the first hour after SE, accompanied by the apparent obliteration of about 50% of the spines. Some of the micrographs show a dramatic conversion of a spine-studded dendritic shaft to a form resembling a series of blobby pearls on a string, in which the spines could no longer be seen. The pathology was identical to that seen in the classical Golgi studies over 100 years ago. Interestingly, the changes seen in this study occurred only with the most severe (Racine stage 5) examples of SE; stage 4 SE failed to provoke any dendritic changes. The dendritic beading and spine loss partially resolved within a few hours post-SE and persisted to some extent as late as 24 h.

The authors made several key observations about the underlying mechanisms. Reasoning that dendrite and spine structure is dependent on the filamentous, polymerized form of actin (a structural protein), they found that the depolymerized form increased after SE. Cofilin is a protein that depolymerizes actin

after it is activated by dephosphorylation. Dephosphorylated cofilin levels were indeed elevated after seizures, and when calcineurin (a key phosphatase) was inhibited, cofilin activation decreased as did acute dendritic pathology. These results suggest that SE sets into a motion a biochemical cascade that alters the phosphorylation status of proteins maintaining dendrite structure. The end result, at least acutely, is a collection of sickly-appearing dendrites.

The findings show that SE causes acute structural changes to dendrites. While the images are graphic testimony to the deleterious effects of prolonged seizures, it is worthwhile considering what is not yet known about the causes and consequences of this dendritic pathology. It appears self-evident that such morphological change is bad for neuronal function in that the presence of fewer synaptic spines probably implies diminished neuronal information processing. However, it actually is not known whether reduced spine number is a cause of the diminished cognitive function seen in human epilepsy (5). Nor for that matter, is it clear that the acute changes in dendritic structure are the same as those seen at chronic time points. Also, the distinction between pathology following SE and that following recurrent seizures must be kept in mind; the changes seen here occurred only after the most severe grade of SE and thus, may be distinct from neuronal pathology observed in animals with epilepsy without antecedent SE.

One finding that coincides with other recent work is the involvement of calcineurin in post-SE pathology. Calcineurin is a recurring theme in other studies examining epileptogenic changes in intrinsic neuronal excitability, suggesting that the existence of a common initial biochemical pathway that leads to diverse cellular alterations (6,7). If so, then there may be reason for optimism that an antiepileptogenic intervention can be found, as calcineurin inhibitors have long been in clinical use as immunosuppressant drugs; in which case, “save the trees” will take on a whole new meaning after a neurological insult.

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References

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