The Spine Loss Paradox: Clues to Mechanisms and Meaning

A Cellular Mechanism for Dendritic Spine Loss in the Pilocarpine Model of Status Epilepticus. Kurz JE, Moore BJ, Henderson SC, Campbell JN, Churn SB. *Epilepsia* 2008 May 8. [Epub ahead of print] PURPOSE: Previous studies have documented a synaptic translocation of calcineurin (CaN) and increased CaN activity following status epilepticus (SE); however, the cellular effect of these changes in CaN in the pathology of SE remains to be elucidated. This study examined a CaN-dependent modification of the dendritic cytoskeleton. CaN has been shown to induce dephosphorylation of cofilin, an actin depolymerization factor. The ensuing actin depolymerization can lead to a number of physiological changes that are of interest in SE. METHODS: SE was induced by pilocarpine injection, and seizure activity was monitored by video-EEG. Subcellular fractions were isolated by differential centrifugation. CaN activity was assayed using a paranitrophenol phosphate (pNPP) assay protocol. Cofilin phosphorylation was assessed using phosphocofilin-specific antibodies. Cofilin–actin binding was determined by coimmunoprecipitation, and actin polymerization was measured using a triton-solubilization protocol. Spines were visualized using a single-section rapid Golgi impregnation procedure. RESULTS: The immunoreactivity of phosphocofilin decreased significantly in hippocampal and cortical synaptosomal samples after SE. SE-induced cofilin dephosphorylation could be partially blocked by the preinjection of CaN inhibitors. Cofilin activation could be further demonstrated by increased actin–cofilin binding and a significant depolymerization of neuronal actin, both of which were also blocked by CaN inhibitors. Finally, we demonstrated a CaN-dependent loss of dendritic spines histologically. DISCUSSION: The data demonstrate a CaN-dependent, cellular mechanism through which prolonged seizure activity results in loss of dendritic spines via cofilin activation. Further research into this area may provide useful insights into the pathology of SE and epileptogenic mechanisms.

COMMENTARY

For many years—indeed, more than a 100 years—it has been known that neurons in the brains of humans and animals with epilepsy have a decreased density of spines on dendrites (1). Dendritic spines are the major sites of excitatory synaptic transmission onto hippocampal and neocortical pyramidal cells. The loss of spines might be interpreted as a reduction in the number of excitatory synapses impinging on these cells and therefore, a decrease in neuronal excitability. Yet, just the opposite is observed in epilepsy, a chronically hyperexcitable state—thus, the paradox. How can one reconcile an apparent decrease in excitatory synaptic transmission with epilepsy? Most often, network, cellular, and molecular remodeling are studied in an attempt to explain this apparent paradox.

In this paper, Kurz and colleagues use biochemical and anatomical techniques to investigate the mechanisms of acute dendritic spine loss following pilocarpine-induced status epilepticus (SE) (2). Video-EEG recordings stage the time course and severity of SE in each rat, ensuring that rats entered into the study had comparable seizures. The authors report that the calcium-regulated phosphatase, calcineurin, is increased in synaptic membrane fractions shortly after (15–20 minutes) the onset of SE, and this effect results in the dephosphorylation of proteins that are found in spines. One of these proteins, cofilin, is a small peptide that is thought to regulate actin in dendritic spines. Dephosphorylation of cofilin leads to enhanced binding to filamentous actin and to its depolymerization, which conceivably could result in the collapse of dendritic spines since filamentous actin is the major cytoskeletal protein in spines. Importantly, the effects of SE on cofilin phosphorylation and its binding to actin are blocked by two different calcineurin inhibitors, FK-506 and cyclosporine A. Moreover, decreases in spine density on hippocampal and neocortical neurons produced by 1 hour of SE were prevented by FK-506.

These results implicate that the activation of calcineurin during prolonged seizures in dendritic spine loss and suggest it is a key molecular participant in this phenomenon. The findings are complemented by another recent report by Zeng and colleagues that also showed that dendritic spine loss during SE was suppressed by pretreatment with the calcineurin inhibitor, FK-506 (3). This study employed multiphoton imaging in intact mice that express green florescent protein (GFP) in neocortical pyramidal cells, as previously described in an *Epilepsy Currents* commentary (4). One major difference between the results reported by Kurz et al. and Zeng at al. is that in the latter study a marked, but transient beading of dendrites also was observed. Indeed, the dendrites took on the appearance of a string of beads, at which time few spines were apparent. Kurz et al. do not report the presence of dendritic beading in their Golgi stained sections, leaving the reader to consider reasons for the disparate outcomes. A major difference between the two studies is that Kurz et al. used a rat pilocarpine model and Zeng et al. a mouse kainate model. Another distinguishing feature may be the severity of SE, since Zeng et al. only observed beading once an animal underwent 1 hour of stage 5 SE. Nonetheless, both studies report major decreases in dendritic spine density and implicate calcineurin in this process. While the Zeng et al. study is primarily an imaging study, Kurz and colleagues rely on

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numerous biochemical assays to convincingly show calcineurin enrichment in synaptic membrane fractions following SE and to elucidate the downstream effects of this phosphatase on spine proteins.

The results of these studies revisit the long-standing question in epilepsy research: what is the meaning of spine loss, and for this particular study, of the acute effects of SE on dendrites? Instances of dendritic beading concurrent with spine loss might suggest excitotoxic dendritic injury; that is, spine loss may merely be a consequence of the severe seizures. However, for situations in which beading is not seen but spine loss is prominent, a neuroprotective or adaptive role for spine loss is possible and may be equated to a loss of excitatory synapses on dendrites. Earlier studies in dissociated neuronal cultures showed that when NMDA was briefly applied to neurons, spine loss occurred without beading (also in a calcineurin-dependent manner) (5). This finding suggests that neurons may attempt to compensate for excessive excitatory synaptic input by eliminating their spines.

There has been much speculation as to whether there are long-term consequences of spine loss following SE. One possibility is that spine loss is permanent and contributes to cognitive deficits seen in chronic epilepsy. A decrease in the normal complement of glutamatergic synapses on hippocampus or neocortex would be expected to impact learning and memory, given their roles in long-lasting forms of synaptic plasticity. However, an earlier study using the pilocarpine model suggested that spine number recovers after SE, only to decrease again once spontaneous seizures emerge (6). While these results have yet to be confirmed, they call into question the importance of acute spine loss in cognitive deficits seen in chronic epilepsy. In contrast, Kurz et al. suggest that acute spine loss could be an early step in epileptogenesis. Spines could regrow, yet the complement of spines might be quite different than that before SE, with remodeling of the molecular make-up and biophysical properties of spines contributing to the neuronal hyperexcitability of epilepsy.

While clearly there is much more to be learned, the importance of the Kurz et al. and similar studies is that they provide important clues to potential molecular mechanisms responsible for spine loss and offer experimental tools to unravel the role of spine loss in epileptogenesis and/or in cognitive deficits associated with some forms of epilepsy. Many experiments can be envisioned in this regard. For instance, would pretreatment with a calcineurin inhibitor not only prevent spine loss during SE but also the development of spontaneous seizures? Results from an earlier study suggest this may be the case (7). If so, this information would provide a unique point of entry for new molecular studies—hopefully leading to the day when the spine loss paradox is no longer paradoxical.

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