EPILEPSY CURRENTS

Current Literature

In Basic Science

Runaway Dendrites: Blame the Older Siblings

Abnormalities of Granule Cell Dendritic Structure Are a Prominent Feature of the Intrahippocampal Kainic Acid Model of Epilepsy Despite Reduced Postinjury Neurogenesis.

Murphy BL, Hofacer RD, Faulkner CN, Loepke AW, Danzer SC. *Epilepsia* 2012;53:193–199.

PURPOSE: Aberrant plastic changes among adult-generated hippocampal dentate granule cells are hypothesized to contribute to the development of temporal lobe epilepsy. Changes include formation of basal dendrites projecting into the dentate hilus. Innervation of these processes by granule cell mossy fiber axons leads to the creation of recurrent excitatory circuits within the dentate. The destabilizing effect of these recurrent circuits may contribute to hyperexcitability and seizures. Although basal dendrites have been identified in status epilepticus models of epilepsy associated with increased neurogenesis, we do not know whether similar changes are present in the intrahippocampal kainic acid model of epilepsy, which is associated with reduced neurogenesis. METHODS: In the present study, we used Thy1-YFP–expressing transgenic mice to determine whether hippocampal dentate granule cells develop hilar-projecting basal dendrites in the intrahippocampal kainic acid model. Brain sections were examined 2 weeks after treatment. Tissue was also examined using ZnT-3 immunostaining for granule cell mossy fiber terminals to assess recurrent connectivity. Adult neurogenesis was assessed using the proliferative marker Ki-67 and the immature granule cell marker calretinin. KEY FINDINGS: Significant numbers of cells with basal dendrites were found in this model, but their structure was distinct from basal dendrites seen in other epilepsy models, often ending in complex tufts of short branches and spines. Even more unusual, a subset of cells with basal dendrites had an inverted appearance; they completely lacked apical dendrites. Spines on basal dendrites were found to be apposed to ZnT-3 immunoreactive puncta, suggestive of recurrent mossy fiber input. Finally, YFP-expressing abnormal granule cells did not colocalize Ki-67 or calretinin, indicating that these cells were more than a few weeks old, but were found almost exclusively in proximity to the neurogenic subgranular zone, where the youngest granule cells are located. SIGNIFICANCE: Recent studies have demonstrated in other models of epilepsy that dentate pathology develops following the aberrant integration of immature, adult-generated granule cells. Given these findings, one might predict that the intrahippocampal kainic acid model of epilepsy, which is associated with a dramatic reduction in adult neurogenesis, would not exhibit these changes. Herein we demonstrate that hilar basal dendrites are a common feature of this model, with the abnormal cells likely resulting from the disruption of juvenile granule cell born in the weeks before the insult. These studies demonstrate that postinjury neurogenesis is not required for the accumulation of large numbers of abnormal granule cells.

Commentary

Temporal lobe epilepsy (TLE) is one of the most common intractable epilepsy syndromes encountered in the clinic. Hippocampal pathology in TLE patients includes loss of hilar cells and pyramidal neurons in CA1 and CA3, astro- and microgliosis, and architectural abnormalities of the dentate granule cells (DGCs). The latter comprises three main structural alterations. First is an abnormal localization of the DGC soma, resulting in dispersion of the granule cell layer and hilar ectopic DGCs. Second, the axonal projections of DGCs (termed "mossy fibers") remodel abnormally as well, resulting in mossy fiber sprouting into the dentate inner molecular layer, where DGCs normally receive synaptic inputs (1). Third, many DGCs exhibit abnormal dendritic trees with dendrites projecting into the hilus; these are known as hilar basal dendrites (HBDs) (2, 3).

Multiple animal models of TLE exist. These models are typically created by local or systemic administration of chemicals, toxins, or electrical stimulation to induce status epilepticus (4). Although they differ in the degree to which they recapitulate the aforementioned neuropathologic abnormalities, the presence of mossy-fiber sprouting seems to be a constant finding (5). The presence of HBDs, however, has only been examined and identified in a subset of TLE models to date, including those involving intraperitoneal application of either kainic acid (KA) or pilocarpine (2, 3). Of interest, in both of these animal models, the induction of epilepsy markedly increases DGC neurogenesis (6). Furthermore, only DGCs maturing during, or especially those generated after, the epileptogenic insult exhibit persistent HBDs (7–9).

The study by Murphy et al. aims to investigate whether HBDs might be present in the only known adult rodent model

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of TLE associated with reduced DGC neurogenesis: the mouse intrahippocampal KA model (10). In this model, nanomolar amounts of the ionotropic glutamate receptor agonist KA are unilaterally injected stereotactically into the dorsal hippocampus of adult mice, allowing the use of the contralateral hippocampus as a relative control. Soon after the injection, epileptiform discharges occur in the ipsilateral hippocampus, followed by discharges in the cortex, and then by convulsive status epilepticus that lasts for hours. Spontaneous seizures that originate in the ipsilateral hippocampus begin after a few weeks (11).

In the present study, the authors took advantage of the H line of Thy1–yellow fluorescent protein (YFP)–expressing mice. This line shows YFP expression in the soma and dendrites of subsets of hippocampal cells, including mature (more than 4 weeks old) DGCs, allowing precise characterization of dendritic structure with the use of confocal microscopy. Brains were examined by immunohistochemistry 2 weeks after KA injections. Initially, the authors validated their use of the model by confirming the appearance in the treated hippocampus of pathologic changes previously reported in this model. These abnormalities include granule cell dispersion, hypertrophy of DGC somata, and mossy-fiber sprouting; in addition, the changes were absent in the contralateral hippocampus, in naive animals, and in those that received intrahippocampal vehicle injection.

The authors next examined the dendritic structure of DGCs in the injected hippocampus, focusing on persistent HBDs. While none of the DGCs in the hippocampus contralateral to the KA injection exhibited HBDs, 20% of those in the ipsilateral side did. HBDs in other animal models of TLE tend to be thin and long, with limited branching; also, they are present in addition to the normal apical dendrites that DGCs commonly display. In mice experiencing intrahippocampal KA-induced seizures, HBDs conformed to this classical appearance, but 30% of DGCs with HBDs (6% of the total DGC population) exhibited what the authors called "tufted" HBDs. These are very short and thick dendrites that terminate in a rich arborization pattern. Furthermore, DGCs with tufted HBDs lacked apical dendrites.

HBDs have been proposed to participate in epileptogenesis by receiving excitatory synaptic inputs from DGC mossy fibers projecting to the hilus, thereby creating a source of recurrent excitation. The authors evaluated the possibility that tufted HBDs are also innervated by mossy fibers using immunohistochemistry for the zinc transporter ZnT-3, which is enriched in mossy-fiber terminals. They found that dendritic spines of both typical and tufted HBDs are in close apposition to ZnT-3 immunoreactive puncta. Although this finding is very suggestive of mossy fibers innervating HBDs in this model, definite proof of synapse formation will require more detailed anatomic (i.e., electron microscopy) or electrophysiological experiments.

The location of the DGCs with HBDs within the granule cell layer was examined , and the cells were found to be closer to the hilar border than DGCs without HBDs. Cells expressing calretinin, a marker of immature DGCs in mice, also exhibited disorganized dendritic processes in the ipsilateral hippocampus, while showing normal apical dendrites contralaterally. Both results, along with the finding that the majority of DGCs have normal dendritic trees, suggest that only DGCs at a younger developmental stage are susceptible to development of persistent HBDs after the epileptogenic insult, as has been found after systemic KA- or pilocarpine-induced status epilepticus (7–9). Thus, although neurogenesis is decreased in this TLE model, DGCs that are still differentiating during KA injection, the "older siblings" of neural stem cell progeny that are probably suppressed by KA-induced damage to the germinal zone, are likely responsible for HBD formation rather than the preexisting population of mature DGCs generated in the perinatal period. Stronger evidence to support this idea should be relatively easy to obtain using conditional transgenic reporter mice or retroviral reporters to birth-date DGCs.

A main finding of this work, in addition to identifying HBDs in the intrahippocampal KA model, is that the HBDs may exhibit more varied morphologic characteristics such as the tufted appearance seen in DGCs with no apical dendrites. Along with previous studies, this report shows that HBDs and other pathologic characteristics of TLE are present in models associated with and in those without increased neurogenesis. The results also suggest that during the neural differentiation process, susceptibility periods exist during which epileptogenic insults may cause cells to acquire an abnormal structure. Delineating such susceptibility windows and the mechanisms underlying them could help in the development of preventive, as opposed to symptomatic, treatments for TLE.

The presence of HBDs and their innervation by mossy fibers are a possible pathway for increased auto/paracrine excitation of DGCs leading to overall hippocampal hyperexcitability. However, whether HBDs contribute to or are merely an epiphenomenon of the epileptogenic process in TLE remains to be determined. Future studies will need to focus on this question and, ideally, also compare the relative importance of recurrent mossy-fiber excitation of HBDs, DGC apical dendrites, and hilar ectopic DGCs in abnormal network function.

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