CURRENT LITERATURE

A NOVEL ANIMAL MODEL OF EPILEPSY CAUSED BY INHIBITING NEURONAL ACTIVITY DURING DEVELOPMENT

Blockade of Neuronal Activity during Hippocampal Development Produces a Chronic Focal Epilepsy in the Rat

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During brain development, neuronal activity can transform neurons characterized by widely ranging axonal projections to ones with more restricted patterns of synaptic connectivity. Previous studies have shown that an exuberant outgrowth of local recurrent excitatory axons occurs in hippocampal area CA3 during postnatal weeks 2 and 3. Axons are remodeled with maturation, and nearly half of the branches are eliminated. Postnatal weeks 2 and 3 also coincide with a "critical" period of development, when CA3 networks have a marked propensity to generate electrographic seizures. In an attempt to prevent axonal remodeling, local circuit activity was blocked unilaterally in dorsal hippocampus by continuous infusion of tetrodotoxin (TTX). Field potential recordings from behaving animals were dramatically altered when TTX infusion was initiated at the beginning of the critical period, week 2, but not later in life. Spontaneous, synchronized spikes and electrographic seizures with behavioral accompaniments were observed after 4 weeks of TTX infusion and persisted into adulthood. When recordings were made during TTX infusion, synchronized spiking was recorded in ventral hippocampus as early as 2 weeks after infusate introduction. At this same time, extracellular field recordings from in vitro slices demonstrated spontaneous network-driven "mini-bursts" arising from ventral hippocampal slices. These were abolished by glutamate receptor antagonists. Whole-cell recordings from CA3 neurons revealed bursts of excitatory synaptic potentials coincident with the network bursts recorded extracellularly. Thus, local assemblies of mutually excitatory CA3 pyramidal cells are hyperexcitable in these rats. Whether alterations in developmental axonal remodeling mediate these effects awaits further studies.

COMMENTARY

One of the problems in epilepsy research today is the availability of good animal models that are clearly related to the clinical condition. Ideally, the model should shed light on the clinical problem, suggesting new therapeutic strategies. Galvan et al. provide a new animal model of epilepsy that, at the very least, elucidates potential causes of epileptogenesis that are relevant to human epilepsy.

Previous studies in this laboratory had shown that area CA3 pyramidal neurons have extremely large axon arbors in the immature animal, and that these are pruned gradually during maturation, similar to other neuronal types in the CNS. They also showed that there was abnormal excitability in area CA3 of hippocampus at the time during development when CA3 axons had not yet been pruned. This led to the hypothesis that excess axonal connections might be the reason for the hyperexcitable state, and if the pruning process could be prevented, this hyperexcitability might persist, leading to epilepsy.

To test this hypothesis, the investigators made use of the large literature on the effects of blocking neuronal activity to impair normal circuit development. They used the sodium channel antagonist tetrodotoxin (TTX) to block neuronal activity, and infused the drug into one hippocampus at an early age. Subsequently animals were evaluated both electrographically and area CA3 neurons were recorded in hippocampal slices. The results were clear: electrographic and behavioral seizures followed TTX infusion, and perhaps just as important, they continued into adult life. In hippocampal slices, spontaneous synchronized discharges occurred in area CA3.

This was a thorough study that carefully tested the animal model. For example, 2-deoxyglucose studies showed that indeed there was a region of hypometabolism in the hippocampus where TTX infusion had occurred, but in the contralateral hippocampus, ipsilateral ventral hippocampus, and other brain

areas, metabolism appeared normal. This suggests that TTX acted within the hippocampus, and did not spread outside the hippocampus. However, all three subfields appeared to have reduced metabolism after TTX infusion, so effects may not have been due entirely to effects within area CA3.

Unfortunately, proving axon arbors have changed in neurons with as complex and far-reaching an axon as CA3 pyramidal cells is a daunting task. Therefore, whether blockade of pruning occurred, and whether it might also have occurred in area CA1 or the dentate gyrus, is unclear at this time. The authors raise the point that TTX blockade may have had other actions, such as effects on dendritic spine formation, as has been described in other studies. Also mentioned is the work of Niesen and Ge (1) who showed that cultures exposed to TTX develop abnormal T-type calcium currents. So there are still many possible effects of TTX that could have contributed to the results, besides blockade of pruning.

Several aspects of this study were intriguing. First, the results seemed most robust if TTX was infused at a particular time during development, supporting the hypothesis of the authors that there is a "critical period" during which insults or injury may be particularly effective in altering excitability with long term consequences. Also of interest was the progression of the abnormalities, which started with one apparent focus ipsilateral to the infusion site, followed by the development of contralateral hippocampal foci, and ultimately foci located outside the hippocampus. This emphasizes the evidently widespread effects that can develop from an action that is initially relatively small, both spatially and temporally. Finally, abnormal discharges were primarily from the ventral hippocampus, although TTX was infused dorsally, and seemed to be restricted to dorsal hippocampus based on 2DG studies. This underscores previous suggestions of a dorsal-ventral axis of excitability in the hippocampus, with the ventral hippocampus most prone to seizures. It also suggests, as the authors point out, that in fact the TTX infusion may have acted indirectly to alter the ventral hippocampus by inhibiting dorsal hippocampal neurons that are synaptically-connected to ventral hippocampus.

This study has several implications, many of which are discussed by the authors. For example, the results suggest that a stimulus that reduces neuronal activity during development may have long lasting effects on excitability, even if the reduction in activity is transient. Indeed, this provides a potential mechanistic basis for epilepsies that are due to abnormalities during development. Of course, one of the next questions is whether something like activity blockade ever occurs in young children, but it may at the very least be reduced, for example after inflammation releases cytokines that depress synaptic transmission.

If one is skeptical whether the results of this study could apply to many different types of epilepsy, i.e., apply to areas outside the hippocampus, the authors remind us of previous studies in inferior colliculus that showed very similar results. Temporary hearing loss at 2–3 weeks led to a permanent susceptibility to audiogenic seizures and excess innervation of the inferior colliculus (2,3). In addition, visual deprivation in young children can result in cortical interictal spikes (4). Therefore, this article provides a novel animal model of epilepsy that allows research into phenomenon that may have widespread clinical relevance.

References

1. Niesen CE, Ge S. Chronic epilepsy in developing hippocampal neurons: electrophysiologic and morphologic features. Dev Neurosci 1999; 21:328–338.
2. Pierson M, Swann JW. The sensitive period and optimum dosage for induction of audiogenic seizure susceptibility by kanamycin in the Wistar rat. Hear Res 1988;32:1–10.
3. Pierson M, Snyder-Keller A. Development of frequency selectivity in inferior colliculus of normal and neonatally noise-exposed rats. Brain Res 1994;636:55–67.
4. Kellaway P. Introduction to plasticity and sensitive periods. In: Kellaway P, Noebels JL, eds. Problems and concepts in developmental neurophysiology. London: The John Hopkins University Press; 1989: 3–28.

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