Riding the Calcium Wave to a Better Understanding of Ictal Events

Direct Imaging of Hippocampal Epileptiform Calcium Motifs Following Kainic Acid Administration in Freely Behaving Mice.

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Prolonged exposure to abnormally high calcium concentrations is thought to be a core mechanism underlying hippocampal damage in epileptic patients; however, no prior study has characterized calcium activity during seizures in the live, intact hippocampus. We have directly investigated this possibility by combining whole-brain electroencephalographic (EEG) measurements with microendoscopic calcium imaging of pyramidal cells in the CA1 hippocampal region of freely behaving mice treated with the pro-convulsant kainic acid (KA). We observed that KA administration led to systematic patterns of epileptiform calcium activity: a series of large-scale, intensifying flashes of increased calcium fluorescence concurrent with a cluster of low-amplitude EEG waveforms. This was accompanied by a steady increase in cellular calcium levels (>5 fold increase relative to the baseline), followed by an intense spreading calcium wave characterized by a 218% increase in global mean intensity of calcium fluorescence (n = 8, range [114-349%], p < 10(-4); t-test). The wave had no consistent EEG phenotype and occurred before the onset of motor convulsions. Similar changes in calcium activity were also observed in animals treated with 2 different proconvulsant agents, N-methyl-D-aspartate (NMDA) and pentylenetetrazol (PTZ), suggesting the measured changes in calcium dynamics are a signature of seizure activity rather than a KA-specific pathology. Additionally, despite reducing the behavioral severity of KA-induced seizures, the anticonvulsant drug valproate (VA, 300 mg/kg) did not modify the observed abnormalities in calcium dynamics. These results confirm the presence of pathological calcium activity preceding convulsive motor seizures and support calcium as a candidate signaling molecule in a pathway connecting seizures to subsequent cellular damage. Integrating in vivo calcium imaging with traditional assessment of seizures could potentially increase translatability of pharmacological intervention, leading to novel drug screening paradigms and therapeutics designed to target and abolish abnormal patterns of both electrical and calcium excitation.

Commentary

The consequences of seizures and epilepsy are well known. Outcomes such as mossy fiber sprouting (1), neuronal death (2), and other types of brain injury (3) have been studied for decades and are now well characterized. But a central question in the field of epilepsy remains unanswered: what are the cellular dynamics that cause seizures in the first place? Most observations of the events preceding ictal activity up to this point have been confined to either EEG findings (4) or, at most, small groups of unit recordings (5). EEG gives a broad picture of the average activity of large groups of cells, but incomplete information about how individual cell types act, whereas single unit and tetrode recordings give precise information of the activity of only a small group of cells. As a result, research needs to push beyond EEG and unit recordings to the direct observations of larger networks of individual cells to make sense of the cellular dynamics that give rise to what we observe as seizures. Berdyyeva and colleagues took a critical first step in this direction by directly observing calcium dynamics in the build-up to a seizure event.

Using cutting-edge imaging techniques, Berdyyeva and colleagues were able to visualize calcium dynamics within the hippocampal CA1 region before, during, and after a kainic acid (KA)-induced seizure. The calcium-imaging methodology employed by the team made use of a miniature head-mounted microscope weighing less than 3 ounces. A calcium-responsive fluorescent protein (GCaMP6) was virally introduced to the hippocampus of mice, and the microscope apparatus was implanted above the CA1 region to allow chronic in vivo imaging of hippocampal calcium dynamics in unanesthetized mice. To capture traditional electrographic seizures, the authors also implanted EEG wires into the optic tectum and prefrontal cortex. Together, these techniques allowed alignment of the novel calcium imaging data with the established EEG seizure onset and visualization of the events leading up to and following the

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seizure. The team then administered KA to induce seizures and simply watched what happened.

Consistent with previous observations using the KA model (6), the team observed an immediate decrease in EEG activity, which correlated with a decrease in spontaneous Ca^{2+} activity following KA administration. Approximately 20 to 30 minutes after administration, they observed a steady build-up of spontaneous activity as well as baseline fluorescence. Interestingly, this build-up was consistently followed within 10 seconds by a slow "wave" of calcium-induced fluorescence that spread across the imaging field and dissipated over the course of a few minutes. The peak fluorescence of this wave event was associated with Stage 1 and 2 seizures on the Racine scale (low-amplitude EEG spikes and behavioral twitching). In six of eight mice tested, this wave reoccurred immediately before the onset of a convulsive seizure, which happened on average at approximately 30 minutes post injection. To determine whether this observation is specific to KA-induced seizures, the authors used pentylenetetrazol (PTZ) and NMDA to induce seizures in four other mice. They found a similar pattern of activity followed by a wave in both of the NMDA-treated mice, but only in one of the two PTZ-treated mice. Thus, the authors conclude that this is a general feature of seizures, rather than a specific effect of KA.

Finally, to assess how anticonvulsants affect the observed Ca^{2+} dynamics, they treated the animals with valproate (VA) 15 minutes before administration of KA. Although VA seemed to reduce the likelihood of the mice experiencing a convulsive seizure, they all still exhibited the build-up and wave dynamics. Thus, VA treatment was effective in reducing the convulsive seizure phenotype, but did not seem to be effective in preventing the observed aberrant neural dynamics.

This study is noteworthy because it gives an unprecedented direct visual observation of the neural calcium dynamics preceding epileptiform activity in vivo. Although the EEG correlates of seizures have been known and thoroughly documented for decades, the actual neural dynamics preceding seizure onset were largely unknown. This study provides novel insight into seizure generation, and dissection of the underlying mechanisms producing the calcium wave has the potential to lead to new pharmacotherapies that target the mechanisms of seizure onset instead of treating the convulsive event itself.

Interestingly, this study also demonstrates that at least one common antiepileptic drug stops the convulsive seizure event without overtly altering the underlying neural activity. It remains unclear whether the calcium wave event plays a causative role in the generation of seizures, but it is clear that the wave is unperturbed by anticonvulsive treatment. Pathological calcium dynamics, such as the observed build-up and wave, may play a prominent role in epileptogenesis and the comorbidities associated with epilepsy, and treatments targeted at mitigating or eliminating the effects of excess calcium influx may serve to both prevent seizures and lead to better long-term treatment outcomes.

Determining the cause of this large and prolonged global calcium transient, and perhaps the functional consequences, has the potential to fundamentally change what we know about both the generation of seizures, epileptogenesis, and the associated comorbidities, such as cognitive impairment. However, to realize the potential, it must be determined whether similar calcium dynamics are present in spontaneous seizures and epileptic animals, including genetic models, and whether these calcium dynamics persist or change during the course of epileptogenesis. The next step will then be to determine whether the calcium dynamics are pathogenic, benign, or perhaps even protective. Understanding what prevents this event in some animals may provide insight into individual differences in seizure generation as well as long-term outcomes. If this event is protective in nature, it may be possible to induce such events in epileptic individuals to control seizure events. Alternatively, if it is pathogenic, prevention of the event itself would be a novel target for seizure treatment.

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