Epileptogenesis: A New Twist on the Balance of Excitation and Inhibition

Rapid Plasticity at Inhibitory and Excitatory Synapses in the Hippocampus Induced by Ictal Epileptiform Discharges. Lopantsev V, Both M, Draguhn A. *Eur J Neurosci* 2009;29(6):1153–1164. Epileptic seizures can induce pathological processes of plasticity in the brain that tend to promote the generation of further seizures. However, the immediate impact of epileptic seizures on cellular excitability remains poorly understood. In order to unravel such early mechanisms of epilepsy-induced plasticity, we studied synaptic transmission before and shortly after three ictal discharges induced by transient elevation of extracellular K^+ in mouse hippocampal slices. Discharges were initiated in the CA3 region and propagated via the Schaffer collaterals into CA1 where they were associated with sustained membrane depolarization and bursts of action potentials in CA1 pyramidal cells. Subsequently, discharges were followed by long-term potentiation (LTP) of Schaffer collateral-evoked field excitatory postsynaptic potentials (EPSPs) in the CA1. The ability to generate epileptiform activity in response to repetitive stimulation was enhanced during LTP. Changes in both inhibitory and excitatory synaptic transmission contributed to LTP in CA1 pyramidal cells. Discharges reduced γ-aminobutyric acid-A receptor-mediated hyperpolarizing inhibitory postsynaptic potentials by shifting their reversal potentials in a positive direction. At the same time, the amplitudes of Schaffer collateral-evoked RS-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor-mediated EPSPs and action potential-independent miniature EPSPs were enhanced. However, *N*-methyl-d-aspartate receptor-mediated EPSPs remained unchanged. Paired-pulse stimulation revealed a reduced probability of glutamate release. Together, these changes in synaptic transmission produce a sustained increase in hippocampal excitability. We conclude that a few seizure-like ictal episodes are sufficient to cause fast and lasting changes in the excitation/inhibition balance in hippocampal networks, and therefore may contribute to early phases of progressive epileptogenesis.

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COMMENTARY

The report by Lopantsev and coworkers describes increases in glutamatergic excitation and decreases in GABAergic inhibition after repetitive seizures. At first glance, this study might be seen as just one more example of the decades-old adage that epilepsy is due to a disruption of the balance of excitation and inhibition. Although the experiments described here support this long-standing hypothesis, they also lead to two other concepts that are worthy of further discussion and experimentation. The two interrelated concepts involve a shift in the balance of excitation and inhibition that could play a role in: 1) the positive-feedback or self-sustaining component of status epilepticus and 2) seizure clusters.

The key result from Lopantsev et al., who performed in vitro brain-slice experiments on the CA1 area of the hippocampus, is that a few seizure-like discharges—induced by rapid bath application of artificial cerebrospinal fluid containing an increased concentration of extracellular potassium ($[K^+]_o$) of 9 mM—caused a long-lasting increase in the amplitude of glutamatergic EPSPs and a decrease in the amplitude of $GABA_A$-receptor–mediated IPSPs. In this protocol, epileptiform bursts of electrical activity in the CA3 area activated the seizure-like events in CA1, via the Schaffer collateral pathway. Importantly, the effects on EPSPs and IPSPs persisted long after the high-$[K^+]_o$ bathing-solution was removed and the slice had been in normal solution for tens of minutes. As reiterated by the authors, increased excitation and/or decreased inhibition caused by intense electrical activity have been well documented over many years. However, two of the more interesting aspects of their work are that only a *few* seizure-like events were needed to cause the shift in the balance of excitation and inhibition and that the effects were relatively long lasting after a return to normal solution (i.e., they persisted for over an hour). Thus, it is reasonable to propose that more intense and long-lasting periods of repetitive seizures would cause even more dramatic and persistent effects on EPSPs and IPSPs.

An important component of the study was that the effects were induced with 9-mM $[K^+]_o$, a level of extracellular potassium that is within a realistic pathophysiological range for epileptogenic tissue. A long history of experimental studies using both brain slices and intact animals has shown that seizure-like and even interictal-like activity leads to an increase in $[K^+]_o$ (1). Furthermore, increased $[K^+]_o$ causes seizure-like events (as seen in the Lopantsev et al. study), which many investigators believe forms a positive-feedback loop that is an important component of seizure generation. In addition, 9-mM $[K^+]_o$ can be rapidly washed out of the brain slice, which distinguishes the experiments of Lopantsev and coworkers from previous studies that have used $GABA_A$-receptor antagonists (e.g., bicuculline) or K^+-channel blockers (e.g., 4-aminopyridine) to induce seizure-like activity—both of which are more difficult to wash out of the extracellular space. The experiments by Lopantsev et al. would have been compromised if the ionic treatment that generated the seizure-like activity had long-lasting effects of their own.

Lopantsev and coworkers propose that the mechanistic changes they identified may be important in the early stages of epileptogenesis. They cite two, now classic epidemiological studies that describe the probability of developing chronic epilepsy after a patient has had one or more unprovoked seizures (2,3). The rationale implied by the authors is that if one or only a few seizures in otherwise normal patients is sometimes followed by the development of chronic epilepsy, then the few seizures induced in this in vitro study may be equivalent to an epileptogenesis-inducing event. A potential flaw in the logic in this interpretation is that the epidemiological observations describe a predictive ability, in a probabilistic or statistical sense, of the clinical observation of a single, unprovoked seizure (versus a greater number of unprovoked seizures) to assess whether a patient will develop epilepsy as a function of time. The two epidemiological studies do not define (or claim to define) the minimal seizure substrate for inducing epileptogenesis, and in fact, it is highly unlikely that a few seizures in an otherwise normal human patient or rat would lead to epileptogenesis. In the epidemiological studies, patients experiencing a few seizures may have had a previous brain insult; therefore, any subsequent development of epilepsy may stem not so much from the few unprovoked seizures, but rather reflect the long-term effects of the previous, unrecognized brain insult. Furthermore, even in rat models of acquired epileptogenesis after status epilepticus, the apparent latent period to the first electrographic seizure based on continuous recording is typically several days and can be weeks in some animals (4). Thus, if the effects described by Lopantsev and coworkers persist for <1 h and the latent period is as short as 4 days (roughly estimated to be about 7 days to the first spontaneous, recurrent electrographic seizure in the repeated low-dose kainate model), the mechanisms would be two orders of magnitude too short to be directly relevant to epileptogenesis. However, the investigators were careful to state that the mechanisms "may contribute to [the] early phases of progressive epileptogenesis," and since the sequence of mechanisms that underlie epileptogenesis is not understood, their assertion may be true.

The mechanisms reported by Lopantsev and colleagues might play an important but indirect role in progressive epileptogenesis either by contributing to 1) the self-sustaining component of status epilepticus (5) or 2) seizure clusters, often seen in patients with epilepsy, particularly patients with intractable epilepsy (6). Hypothetically, the contribution to status epilepticus would involve playing an early role, while the contribution to clustering would be a late role. During electrographically recorded status epilepticus after lithium–pilocarpine treatment, as an example, seizures evolve to a point where they appear to

merge together (5); thus, the mechanisms described here could represent a positive-feedback process responsible for this phenomenon. Similarly, one or a few seizures theoretically could promote further seizures, raising the probability of prolonged seizure clusters by increasing glutamatergic excitation and decreasing GABAergic inhibition. These changes would be in addition to an elevated $[K^+]_o$ and a decrease in the concentration of extracellular calcium ions ($[Ca^{2+}]_o$), both of which have long been known to occur during and after intense electrical activity (1). If the continuous occurrence of electrographic spikes during status epilepticus arises from the mechanisms presented by Lopantsev et al., they may indeed contribute to epileptogenesis. Similarly, if seizure clusters represent a mechanism by which seizures beget seizures (4), it would suggest that the Lopantsev et al. mechanisms could indirectly be involved in the physiological processes that cause progressive epileptogenesis. Future studies based on the data from these *in vitro* experiments will need to determine how to test these hypotheses in intact animals with spontaneous seizures.

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