

NEW EVIDENCE FOR AN IMPORTANT ROLE OF ENDOGENOUS GABA IN SEIZURE GENERATION IN THE IMMATURE HIPPOCAMPUS

Excitatory Actions of Endogenously Released GABA Contribute to Initiation of Ictal Epileptiform Activity in the Developing Hippocampus

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In the developing rat hippocampus, ictal epileptiform activity can be elicited easily *in vitro* during the first 3 postnatal weeks. Changes in neuronal ion transport during this time cause the effects of γ -aminobutyric acid subtype A (GABA_A) receptor (GABA_A-R) activation to shift gradually from strongly depolarizing to hyperpolarizing. It is not known whether the depolarizing effects of GABA and the propensity for ictal activity are causally linked. A key question is whether the GABA-mediated depolarization is excitatory, which we defined operationally as being sufficient to trigger action potentials. We assessed the effect of endogenous GABA on ictal activity and neuronal firing rate in hippocampal slices from postnatal day 1 (P1) to P30. In extracellular recordings, a strong correlation was noted between the postnatal age at which GABA_A-R antagonists decreased action-potential frequency (P23) and the age at which ictal activity could be induced by elevated potassium levels (P23). In addition, a strong correlation was found between the fraction of slices in which ictal activity was induced by elevated potassium concentrations and the fractional decrease in action-potential firing when GABA_A-Rs were blocked in the presence of ionotropic glutamate receptor antagonists. Finally, ictal activity induced by elevated potassium levels was blocked by the GABA_A-R antagonists bicuculline and SR-95531 (gabazine) and increased in frequency and duration by GABA_A-R agonists isoguvacine and muscimol. Thus the propensity of the developing hippocampus for ictal activity is highly correlated with the effect of GABA on action-potential probability and reversed by GABA_A antagonists, indicating that GABA-mediated excitation is causally linked to ictal activity in this developmental window.

COMMENTARY

The study by Dzhala and Staley addresses the issue of why neonates and young children, as well as immature animals, are more susceptible to seizures. The hypothesis that the depolarizing actions of γ -aminobutyric acid (GABA) contribute to or underlie the increased seizure susceptibility of the immature brain has been studied for more than a decade (1) but remains controversial (2). Two issues have limited the acceptance of the hypothesis. First, it has been determined that GABA responses can be depolarizing, yet still be inhibitory, when they shunt more strongly depolarizing glutamate-mediated currents (3). Second, recent studies demonstrated that antiepileptic drugs (AEDs) that enhance GABA_A receptor-mediated currents still have anticonvulsant effects in the immature central nervous system (CNS). Staley and Dzhala avoid the problem of shunting by measuring the net effect of the postsynaptic potentials as action-potential frequency. The second issue appears more difficult to resolve: if drugs that increase the opening of GABA_A-receptor channels reduce seizure activity in the neonatal hippocampus, how could GABA be excitatory in this preparation? The authors propose that small amounts of endogenously released GABA may “ignite” seizures, and that GABAergic anticonvulsants reduce this ignition capacity. They propose an activity-dependent mechanism, based on the understanding that immature neurons normally accumulate chloride and that GABA_A-receptor activation results in chloride efflux and neuronal depolarization. Therefore, if an AED prolongs the openings of GABA_A-receptor channels, it will eventually exhaust the store of chloride in the neuron (2). Under these conditions, the equilibrium potential for GABA_A-receptor activation will approach resting membrane potential and produce an efficient inhibition, via shunting of excitatory currents. Thus AEDs that act on GABA_A receptors will provide seizure protection—even in animals and for preparations in which brief GABA_A-receptor activation is excitatory. Desensitization of GABA_A receptors could also factor into the GABAergic AED effect, although this possibility is not addressed in the article.

In addition, these experiments combine the use of extracellular recordings and GABA_A-receptor antagonists as a means to evaluate whether *endogenous* GABA is excitatory and contributes to high-potassium-induced seizures in the

CA3 area of the hippocampus. The observation that GABA_A-receptor antagonists can depress epileptiform activity, whereas GABA_A-receptor agonists enhance it, provides additional evidence that endogenously released GABA is proconvulsant in the immature hippocampus. When comparing these results with those of prior investigations (2), the reader should bear in mind that some significant experimental differences are found across studies. The current study used high extracellular potassium rather than low extracellular magnesium to trigger seizures. High extracellular potassium can enhance the excitatory actions of GABA by reducing the ability of neurons to extrude chloride via the potassium–chloride cotransporter (5).

The effects of GABA_A-receptor activation also may be relevant to ictal epileptiform activity in adult tissue. Acute cellular injury and increased extracellular potassium are among several events that occur during seizures in adults and can alter the transmembrane chloride gradient in a manner that may lead to a depolarizing action of GABA. In addition, numerous other cellular and network mechanisms could theoretically contribute to the increased seizure susceptibility of the immature brain, including increased recurrent excitatory circuits, altered *N*-methyl-D-aspartate (NMDA) receptors, or differences in nonsynaptic mechanisms (e.g., increased gap junctions between neurons and/or compromised regulation of the concentration of extracellular potassium). The normal GABA_A receptor-mediated mechanisms present in the adult brain are likely, at least partially, to mask all of these hypothetical mechanisms. Any alterations in the mechanism of action of GABA could thus have important secondary effects on seizure susceptibility.

In conclusion, the experiments of Dzhala and Staley lend support to the hypothesis that the depolarizing actions of GABA contribute to the enhanced seizure susceptibility of the immature brain. The authors delineate the ability of GABA_A-receptor antagonists to depress seizure activity under some experimental conditions in vitro and document the potential contribution of endogenous, synaptically released GABA to seizure generation. No one is suggesting that GABA antagonists should be used to treat neonatal seizures! However, learning more about the nuances of GABAergic signaling in the neonatal hippocampus already is allowing researchers to resolve apparent scientific controversies, and future research may ultimately lead to the development of more effective anticonvulsant strategies.

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References

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