Learning Through Silence: Amping up Cognition After Neonatal Hypoxic Seizures Through AMPA Receptor Inhibition

Hypoxia-Induced Neonatal Seizures Diminish Silent Synapses and Long-Term Potentiation in Hippocampal CA1 Neurons.


Neonatal seizures can lead to epilepsy and long-term cognitive deficits into adulthood. Using a rodent model of the most common form of human neonatal seizures, hypoxia-induced seizures (HS), we aimed to determine whether these seizures modify long-term potentiation (LTP) and silent NMDAR-only synapses in hippocampal CA1. At 48–72 h after HS, electrophysiology and immunofluorescent confocal microscopy revealed a significant decrease in the incidence of silent synapses, and an increase in AMPARs at the synapses. Coincident with this decrease in silent synapses, there was an attenuation of LTP elicited by either tetanic stimulation of Schaffer collaterals or a pairing protocol, and persistent attenuation of LTP in slices removed in later adulthood after P10 HS. Furthermore, postseizure treatment in vivo with the AMPAR antagonist 2,3-dihydroxy-6-nitro-7-sulfonyl-benzo[f]quinoxaline (NBQX) protected against the HS-induced depletion of silent synapses and preserved LTP. Thus, this study demonstrates a novel mechanism by which early life seizures could impair synaptic plasticity, suggesting a potential target for therapeutic strategies to prevent long-term cognitive deficits.

Commentary

Hypoxia-induced seizures are among the commonest causes of neonatal seizures and raise significantly the risk for subsequent epilepsy and poor neurodevelopmental outcomes (1). Experimental models of early life encephalopathies with seizures are indispensable in the effort to develop effective disease-modifying treatments. The research group authoring this report has pioneered the characterization of a rat model of neonatal hypoxia-induced seizures. Postnatal day 10 (PN10) rats exposed to hypoxia develop acute seizures, subsequent cognitive deficits, increased seizure susceptibility, and spontaneous seizures later in life (2).

Prior studies have highlighted the role of AMPA receptors in the pathogenesis of cognitive dysfunction and pro-epileptogenic diathesis of rats subjected to neonatal hypoxic seizures (3, 4). Starting within the first hour following PN10 hypoxic seizures, AMPA receptor spontaneous excitatory postsynaptic currents (sEPSCs) increase in the hippocampus, partially as a result of the enhanced phosphorylation of AMPA receptor subunits, GluA1 and GluR2. This observation correlates nicely with the parallel, but transient, early enhancement of long-term potentiation (LTP) in this model. Furthermore, early systemic administration of AMPA receptor inhibitors, such as NBQX or topiramate, during the two first posthypoxia days prevents the appearance of cognitive impairment and seizure susceptibility following hypoxia. Even though these results provided a strong case for the pathogenic role of AMPA receptors in the early stages of this model, little was known about the molecular and pathophysiological changes during the period of cognitive impairment.

Zhou et al. report that 2 to 3 days following hypoxic seizures in PN10 pups, the frequency of sEPSCs is still increased in the CA1 stratum radiatum, through further enrichment of the NMDA receptor (NR)-positive synapses with AMPA receptors, such as GluA1. Through an elegant series of experiments, they demonstrate an increased ratio of AMPA receptor/NR-type sEPSCs, and increased synaptic colocalization of GluA1 and NR1, the obligate NR subunit. It is known that the presence of functional AMPA receptors in NR-containing synapses “unsilences” the synapses in the face of glutamate release, through activation of AMPA receptors, depolarization, and release of the Mg²⁺ blockade of NRs (5). Indeed, the authors proceed to show that 2 to 3 days following PN10 hypoxic seizures, the failure rates of eliciting glutamatergic sEPSCs after synaptic stimulation are selectively lower for AMPA receptor-mediated sEPSCs but not for NR-mediated sEPSCs. As the authors comment, such results indicate a reduced number of “silent” glutamate synapses following post-hypoxic seizures. Given the importance of silent synapses for the generation of LTP, the authors demonstrate that LTP is significantly impaired in the hippocampus at the time when silent glutamatergic synapses are reduced. Exposure to hypoxia, in the absence of seizures, fails to elicit similar effects, indicating that synaptic unsilencing is triggered by...
hypoxic seizures or the factors that predispose to them. More interestingly, prior treatment with the AMPA receptor inhibitor NBQX, given 12 to 36 hours following hypoxic seizures, unsilences glutamatergic synapses and improves LTP; with the benefit lasting until adulthood.

The clinical importance of such results is obvious, as anyone caring for neonates with hypoxic seizures readily appreciates the importance of improving long-term outcomes. The immediate clinical relevance is strengthened by the availability of approved antiseizure medications with AMPA receptor inhibitory potential, like topiramate. The study by Zhou et al. is well conducted and exemplifies many of the conclusions of the recently published joint American Epilepsy Society (AES) and International League Against Epilepsy (ILAE) report on optimization of preclinical epilepsy research (6). The potential benefit of AMPA receptor inhibitors in the setting of neonatal hypoxia is being replicated by studies showing neuroprotective effects, improved cognition, or reduced seizures in rats (7–10) and neuroprotective effects in piglets, although with a caution for the possibility of increased frontal white matter cell death (11); whereas in a small clinical study, topiramate was deemed safe in human neonates with hypoxic ischemic encephalopathy (12). Instead a few points that illustrate the challenges in preclinical drug discovery and the translation of findings to the clinical arena need to be discussed.

First, technical details may not mimic the in vivo state. The concept of silent synapses is being built through carefully studied AMPA receptors and NR physiology done, quite appropriately, under the influence of picrotoxin, a GABA<sub>B</sub> receptor inhibitor, so as to isolate glutamatergic sEPSCs. The role of GABA<sub>B</sub> receptors in the unsilencing of excitatory synapses in this model would be worth investigating, especially since many of the newborns with neonatal hypoxia seizures may have received antiseizure drugs that activate, rather than inhibit, GABA<sub>B</sub> receptors. At very early developmental stages, and before AMPA receptors populate the NR-containing synapses, depolarizing GABA<sub>B</sub> receptor responses provide the depolarization needed to release Mg<sup>2+</sup> block and activate NMDA receptors. PN12-13 male rats are just before the age when GABA<sub>B</sub> receptors shift from depolarizing to hyperpolarizing in CA1 pyramidal neurons (13). However, it is unclear how prior neonatal hypoxic seizures may affect the direction of GABA<sub>B</sub> receptor responses, since seizures and multiple other factors may change the direction of their responses, including strain, age, sex, type and time from insult, cell type, treatments, and life experiences (13).

Second, a paradox emerges when enhanced AMPA receptor signaling strengthens LTP at the immediate post-hypoxia period (PN10) but impairs it at the subsequent stages (PN12-13) (3). Conceptually, this may highlight the importance of balance in the system. When postsynaptic functional AMPA receptors are not enough and NR-containing synapses are less likely to be activated, events (including seizures) that recruit AMPA receptors at synaptic sites may improve the chances of activating NRs and will potentiate LTP. Yet when NR-containing synapses are overpopulated by AMPA receptors, and the synapse is already maximally stimulated by the afferent glutamatergic signals, such an additional benefit may not be obvious and LTP can be weak. Does this reflect a near-saturation functional state of the existing glutamatergic synapses, which could be anticipated in the setting of increased excitability? This possibility is suggested by the authors’ prior (4) and present work showing increased synaptic AMPA receptor expression and sEPSCs and possibly enhanced pre-tetanus field EPSCs (fEPSCs). In other words, is the hippocampus after hypoxic seizures too busy getting randomly excited and therefore not as responsive to specific stimuli that facilitate learning? Or, are there additional presynaptic or postsynaptic effects implicated? Most important, if finding the right balance of “normal” AMPA receptor activity is critical for normal hippocampal function, what is the optimal therapeutic range of AMPA receptor inhibitor doses and how can this be monitored in vivo?

Third, the response to treatments may be different in hippocampal regions that are involved in the pathogenesis of hypoxic seizures from those that are not. As described in Zhou et al., inhibition of AMPA receptors at PN10-11 counters the effects of hypoxic seizures and improves LTP at PN12-13 rats after hypoxic seizures. Yet, the AMPA receptor inhibitor NBQX has no such effect in controls. Nonetheless, in the rat hippocampus following hypoxic seizures, the natural enhancement of AMPA receptor activity at PN10-11 is needed to unsilence excitatory synapses, yet AMPA receptor activation in the normally developing rat PN7-13 hippocampus silences glutamatergic synapses (14). Furthermore, future studies should examine the effects of hypoxic seizures and proposed treatments on other brain regions, to fully determine the safety profile of candidate drugs. This might not be a critical issue when a systemic insult triggers the diseased state, such as hypoxic seizures. Indeed, the authors’ demonstration that systemic administration of AMPA receptor inhibitors improves in vivo the cognitive and epilepsy outcomes in their model is reassuring. Still, the applicability of this treatment protocol in the setting of a focal-onset neonatal seizure, when the rest of the brain may not be dancing in the same rhythms of synaptic physiology, is uncertain. The more we learn, the more obvious becomes the need for stage-specific treatments targeted to the epileptogenic focus. We need clinically relevant biomarkers for treatment selection, monitoring, and implementation to best transition such promising preclinical drug discovery advances to the clinical arena and reap the benefits but not the side effects (15). However, the realization of this goal for early life epilepsies may appear to be a Sisyphean task (16). But considering the advances collectively gained by the persisting efforts of preclinical epilepsy research investigators, it is a goal worth pursuing to achieve the cure.

by Aristea S. Galanopoulou, MD, PhD

References


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