



Too Much of a Good Thing May Not Be Wonderful: GluR1 Phosphorylation and the Consequences of Early-Life Seizures

Glutamate Receptor 1 Phosphorylation at Serine 831 and 845 Modulates Seizure Susceptibility and Hippocampal Hyperexcitability After Early Life Seizures.

Rakhade SN, Fitzgerald EF, Klein PM, Zhou C, Sun H, Hanganir RL, Jensen FE. *J Neurosci* 2012;32:17800–17812.

Neonatal seizures can lead to later life epilepsy and neurobehavioral deficits, and there are no treatments to prevent these sequelae. We showed previously that hypoxia-induced seizures in a neonatal rat model induce rapid phosphorylation of serine-831 (S831) and Serine 845 (S845) sites of the AMPA receptor GluR1 subunit and later neuronal hyperexcitability and epilepsy, suggesting that seizure-induced posttranslational modifications may represent a novel therapeutic target. To unambiguously assess the contribution of these sites, we examined seizure susceptibility in wild-type mice versus transgenic knock-in mice with deficits in GluR1 S831 and S845 phosphorylation [GluR1 double-phospho-mutant (GluR1 DPM) mice]. Phosphorylation of the GluR1 S831 and S845 sites was significantly increased in the hippocampus and cortex after a single episode of pentylentetrazol-induced seizures in postnatal day 7 (P7) wild-type mouse pups and that transgenic knock-in mice have a higher threshold and longer latencies to seizures. Like the rat, hypoxic seizures in P9 C57BL/6N wild-type mice resulted in transient increases in GluR1 S831 and GluR1 S845 phosphorylation in cortex and were associated with enhanced seizure susceptibility to later-life kainic-acid-induced seizures. In contrast, later-life seizure susceptibility after hypoxia-induced seizures was attenuated in GluR1 DPM mice, supporting a role for posttranslational modifications in seizure-induced network excitability. Finally, human hippocampal samples from neonatal seizure autopsy cases also showed an increase in GluR1 S831 and S845, supporting the validation of this potential therapeutic target in human tissue.

Commentary

Mae West was undoubtedly right when she said, “Too much of a good thing can be wonderful” (1), but she never heard about the over-phosphorylation of glutamate receptors. Whether seizures beget seizures in the mature brain is often debated, but it is well established that seizures in early childhood can increase the risk of epilepsy and cognitive deficits later in life (2, 3). A recent study by Rakhade et al. (4) highlights a normal synaptic enhancement mechanism—phosphorylation of GluR1 subunits—that is intensified by neonatal seizures and may play a key role in the subsequent development of neurologic sequelae.

Neuronal networks of the immature brain are particularly sensitive to ongoing activity. The adverse effects of neonatal seizures are probably caused, in part, by hyperactivation of intrinsic physiological processes that normally modify the young brain in response to everyday experience. One of these mechanisms occurs at excitatory synapses, where AMPA-type

glutamate receptors are phosphorylated by protein kinases. Phosphorylation can lead to long-term potentiation (LTP) of synaptic currents as a result of modulation of existing receptors or the insertion of new ones (5, 6). Under normal conditions, this synaptic modification is essential for learning and the proper course of neurologic development. However, when seizures powerfully and synchronously activate a large number of glutamatergic synapses in the immature brain, the same process might become detrimental. Indeed, previous studies from the Jensen group demonstrated that in rats, hypoxia-induced seizures at postnatal day 10 (P10) cause a transient but substantial increase in phosphorylation of GluR1 subunits of the AMPA receptors (7), followed by a high incidence of spontaneous seizures later in life (8). This GluR1 phosphorylation occurs at two serine residues (S831 and S845). These are the same phosphorylation sites implicated in mechanisms of synaptic potentiation following physiological levels of activity.

Rakhade et al. have now extended this research and established a causative link between excessive GluR1 phosphorylation following a single seizure in pups and enhanced seizure susceptibility a month later. To that end, they exploited a mouse line that had been engineered to test the role of GluR1 phosphorylation in synaptic plasticity. In these mice, alanines



were substituted for serines at the S831 and S845 sites, resulting in GluR1 subunits that cannot be phosphorylated. The animals are termed GluR1–double-phosphomutants (DPM). GluR1-DPM mice have defective long-term synaptic plasticity *in vitro*, and reduced learning and retention abilities on several behavioral tasks (9, 10).

The first step for Rakhade et al. in exploring seizure effects in mice was to determine the developmental expression profile of several membrane neurotransmitter receptors and ion transporters. The conversion rates from immature to mature patterns of AMPA receptor subunits were maximal around P7 to P9. Seizures were then induced in mice of these ages using either the chemical convulsant pentylenetetrazol (PTZ) or graded global hypoxia for 40 minutes. GluR1 expression and GluR1 phosphorylation were measured in the neocortex and hippocampus across time. As expected from previous work with rats, phosphorylation of both S831 and S845 was considerably increased within 1 hour after a single seizure; phosphorylation levels returned to baseline within about 24 hours.

The key experiments were to compare the seizure susceptibility and long-term outcome of GluR1-DPM mice and wild-type (WT) controls. When subjected to PTZ, both groups developed seizures of comparable severity, but the GluR1-DPM mice took longer to begin seizing; this suggests that the mutants are somewhat less sensitive to the convulsant. The difference between WT and GluR1-DPM mice was even greater when seizures were induced by hypoxia. Fewer mutant pups developed seizures immediately following hypoxia, and those that did seize were comparable to untreated controls in their vulnerability to kainate-induced seizures much later, at P40. By contrast, in WT mice the occurrence of early hypoxia-induced seizures was strongly correlated with the tendency to develop kainate-induced seizures as adults.

Since GluR1 phosphorylation enhances synaptic glutamate-mediated currents, the authors wondered whether the improved resilience of the GluR1-DPM mice was owing to the inability of their GluR1 subunits to phosphorylate, and hence for their AMPA receptor currents to increase. To test that theory, they recorded miniature EPSCs (mEPSCs) from hippocampal CA1 pyramidal neurons in acute *ex vivo* brain slices. As reported before (9), the baseline EPSCs from GluR1-DPM mice did not differ from mEPSCs of WT controls. One day after seizures, however, the mEPSCs differed greatly between the two mouse strains. The mean amplitude of mEPSCs was significantly larger in postseizure WT mice compared with untreated WT pups, whereas the mEPSCs from postseizure GluR1-DPM mice were actually smaller than those of naïve controls. Why seizures would reduce the size of synaptic events in mutants remains unexplained. Overall, the data suggest that phosphorylation of GluR1 at the S831 and S845 sites is an important step in mediating the acute response to seizures.

Alterations of GluR1 phosphorylation and mEPSC amplitudes are both consistent with changes on the postsynaptic side of glutamatergic synapses. Another sign of postsynaptic change was that expression of the scaffolding protein PSD-95, which anchors the AMPA receptors, was increased in control mice but not in GluR1-DPM mice following seizures. The

presynaptic release machinery received only cursory testing in this study. The afferent pathway onto CA1 cells was stimulated with pairs of shocks separated by intervals between 25 and 400 milliseconds. This type of synapse usually shows short-term potentiation (i.e., the second stimulus yields a larger response than the first). Changes of the paired-pulse ratio usually (though not always) reflect presynaptic modifications. Rakhade et al. found no postseizure alterations of paired-pulse ratios in WT or GluR1-DPM mice. Overall, the authors' results imply that excessive GluR1 phosphorylation following early-life seizures is responsible for modifications of the postsynaptic receptor-related apparatus.

Finally, Rakhade et al. established a tentative link to human disease by measuring the level of GluR1 phosphorylation at S831 and S845 in postmortem tissue. Hippocampi from infants with seizures that were apparently caused by hypoxic encephalopathy were compared with hippocampi from infants who died of other causes; indeed, phosphorylation levels of the two serine residues were considerably higher in postmortem tissue from the seizure patients.

Recall that GluR1 phosphorylation is a physiological process. What, then, are the effects of an overabundance of phosphorylation on normal activity-dependent plasticity during a critical period of postnatal development? A partial answer is provided by previous studies. Zhou et al. (11) found that seizures induced in rats on P10 led to larger evoked EPSCs in the hippocampus 48 to 72 hours later. The seizure-enhanced synapses were, however, less plastic. LTP was severely impaired, presumably because the postsynaptic membranes had been saturated with newly inserted AMPA receptors. The reduced propensity of synapses to potentiate lingered well into adulthood. Similarly, when rats subjected to a single seizure as pups were later tested as adults, they exhibited significant changes in the expression of glutamate receptors and PSD-95 and had reduced LTP *in vitro*. The neonatal seizure group also performed marginally worse on several hippocampus-dependent memory tasks (12). The mechanisms of long-lasting impairments of plasticity are unknown.

In the immature brain, seizures do beget seizures as well as other neurologic and psychiatric impairments. Even if the seizures themselves cannot be prevented, perhaps their long-term consequences can be derailed. Rakhade et al. make the interesting argument that blockade of GluR1 phosphorylation is a potential therapeutic target for neonates with seizures.

by Yael Amitai, MD, and Barry W. Connors, PhD

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