

## HYPOXIA RESULTS IN GABAERGIC CHANNELOPATHY

### AMPA/Kainate Receptor-mediated Downregulation of GABAergic Synaptic Transmission by Calcineurin after Seizures in the Developing Rat Brain

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Hypoxia is the most common cause of perinatal seizures and can be refractory to conventional anticonvulsant drugs, suggesting an age-specific form of epileptogenesis. A model of hypoxia-induced seizures in immature rats reveals that seizures result in immediate activation of the phosphatase calcineurin (CaN) in area CA1 of hippocampus. After seizures, CA1 pyramidal neurons exhibit a downregulation of GABA<sub>A</sub> receptor (GABA<sub>A</sub>R)-mediated inhibition that was reversed by CaN inhibitors. CaN activation appears to be dependent on seizure-induced activation of Ca<sup>2+</sup>-permeable  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (AMPA<sub>R</sub>), because the upregulation of CaN activation and GABA<sub>A</sub>R inhibition were attenuated by GYKI

52466 [1-(4-aminophenyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine hydrochloride] or Joro spider toxin. GABA<sub>A</sub>R  $\beta$ 2/3 subunit protein was dephosphorylated at 1 h after seizures, suggesting this subunit as a possible substrate of CaN in this model. Finally, in vivo administration of the CaN inhibitor FK-506 significantly suppressed hypoxic seizures, and post-treatment with NBQX (2,3-dihydroxy-6-nitro-7-sulfonylbenzo[*f*]quinoxaline) or FK-506 blocked the hypoxic seizure-induced increase in CaN expression. These data suggest that Ca<sup>2+</sup>-permeable AMPARs and CaN regulate inhibitory synaptic transmission in a novel plasticity pathway that may play a role in epileptogenesis in the immature brain.

### COMMENTARY

How early-life brain insults produce a lifelong susceptibility to seizures is a question that has preoccupied numerous investigators and has produced important insights into the mechanisms of acquired epilepsy. One model of this process, presented here by Sanchez and colleagues, uses hypoxia in immature rodent pups to simulate the perinatal “asphyxia” or in utero stroke that often produces cerebral palsy, developmental delay, or epilepsy in humans. What a growing body of work has shown is that a brief period of hypoxia in postnatal day 10–12 rat pups produces a transient period of acute seizures, which in later life predisposes to a lowered epileptic threshold (1). The provoked seizures are themselves dependent on activation of glutamatergic  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors. The properties of these AMPA receptors evolve developmentally such that during the period of the animal’s peak sensitivity to hypoxia-induced seizures, AMPA receptors lack a key subunit, GluR2, which limits Ca<sup>2+</sup> influx through the ionophore (2). Thus, unlike the neuronal death that follows excessive activation of *N*-methyl-D-aspartate (NMDA) receptors in mature animals, Ca<sup>2+</sup> influx

through GluR2-negative AMPA receptors in immature animals produces a more benign, but nonetheless pathologic outcome.

The significance of this line of investigation, aside from its relevance to a vexing clinical problem, is that the model has allowed the dissection of the pathologic process at a molecular level. The unexpected involvement of AMPA receptors, rather than NMDA receptors, offers the possibility of preventive treatment in humans. Although NMDA antagonists have been poorly tolerated in humans, topiramate (TPM)—a partial antagonist of both AMPA and kainate receptors—is a widely used and well-tolerated antiepileptic drug. TPM sufficiently blocks AMPA/kainate receptors to prevent hypoxia-induced seizures (3).

In the present study, Sanchez and colleagues follow this evolving story to the next mechanistic level, in essence asking, “What are the molecular consequences of neuronal Ca<sup>2+</sup> influx through AMPA receptors after provoked seizures?” They demonstrate that for immature rats with hypoxia-induced seizures, GABA<sub>A</sub>-receptor function is diminished within 1 hour of seizures, potentially contributing to the acute hyperexcitability caused by hypoxia. This altered GABA<sub>A</sub>-receptor function was dependent on the activation of calcineurin, a serine/threonine phosphatase induced by increased levels of intracellular Ca<sup>2+</sup>. The implication is that calcineurin activation strips away constitutive phosphorylation of GABA<sub>A</sub> receptors, altering their biophysical properties and diminishing their

function. The decreased GABA<sub>A</sub>-receptor function appeared to depend in part on altered presynaptic release as well (i.e., decreased frequency of spontaneous inhibitory currents), but this effect curiously also required postsynaptic calcineurin activity. Importantly, pretreatment of the animal with calcineurin inhibitors, such as the immunosuppressant drug FK-506, prevented the occurrence of hypoxia-induced seizures.

Thus, the authors add another link to their model of early-life seizures, showing that AMPA receptor-mediated Ca<sup>2+</sup> influx during hypoxia-induced seizures presumably leads to abnormal dephosphorylation of the GABA<sub>A</sub> receptors, diminishing their efficacy and predisposing to later-life seizures. Whereas increased intracellular Ca<sup>2+</sup> levels could trigger any number of second-messenger pathways, including protein kinases that would *increase* substrate phosphorylation, the experimental conditions studied led to a predominance of phosphatase over kinase activity. This result has some support from the literature, particularly for one of the best-studied Ca<sup>2+</sup>-activated kinases, Ca<sup>2+</sup>/calmodulin-dependent protein kinase II (CAMKII). McNamara and colleagues demonstrated, approximately 10 years ago, that mutant mice lacking activity of the CAMKII  $\alpha$ -subunit had spontaneous limbic seizures (4). Pharmacologic inhibition of this kinase *in vitro* caused similar hyperexcitability in hippocampal neurons (5). Most applicable to the current work, pilocarpine-induced status epilepticus in rodents caused an increase in calcineurin activity (6).

The limited animal-model evidence suggests that decreased phosphorylation, whether by loss of kinase activity or gain of phosphatase activity, promotes neuronal hyperexcitability and seizures. Despite this nice concordance of results, the human data, thus far, refuse to play along. Elger and colleagues found that hippocampal tissue resected from patients with temporal lobe epilepsy and mesial temporal sclerosis displayed exactly the opposite phosphorylation pattern (at least in dentate gyrus granule cells), with augmented CAMKII levels and a loss of calcineurin (7). In addition, another study using pilocarpine-induced epilepsy with rodents showed a loss of A-type K<sup>+</sup> currents, which was due to increased extracellular signal-related kinase (ERK) activity (8). Thus, it is unlikely that simple relations will exist for overall phosphorylation activity and human epilepsy; rather, it is likely that many individual links are to be found between kinase activity and ion-channel targets, some promoting epileptogenesis, and some retarding it.

Several questions arise from the present work that may stimulate further investigation. First, the present observations were largely made at 1 hour after seizure, whereas Sanchez and others previously demonstrated that epileptiform activity persists for at least a week and that a decreased seizure threshold lasts for no less than several months. It would be important to understand whether altered GABA<sub>A</sub>-receptor activity persists at some of these longer time scales. Second, although it is presumed that the blockade of epileptiform changes by calcineurin inhibitors

results from decreased dephosphorylation of the GABA<sub>A</sub> receptor, it would be useful to have direct evidence of such altered phosphorylation. These data might suggest which of the ~700 known kinases are having their constitutive activity reversed by pathologic dephosphorylation of the GABA<sub>A</sub> receptor. Such knowledge could yield another tool with which to intervene pharmacologically, by activating specific kinases rather than by blocking general dephosphorylation. Finally, before proceeding too quickly to clinical trials of calcineurin inhibitors in perinatal seizures, one wonders whether the current results are broadly applicable to other rodent epilepsy models, particularly those that produce spontaneous seizures, such as kainate or pilocarpine. These models share in common with the hypoxia model a provoking stimulus that produces a period of status epilepticus-like activity; thus, some commonality in mechanisms would augur well for potential relevance to the human epilepsy.

In short, the work by Sanchez et al. joins a handful of other studies demonstrating a kind of “acquired channelopathy” in animal models that, like human inherited channelopathies, may predispose to or actually be a direct cause of epilepsy. By beginning to tease apart the molecular links between the initial insult and later epilepsy, this work brings a little closer to reality the possibility of new pharmacologic tools to prevent epilepsy.

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## References

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