

# IMPACT OF PROTEIN KINASE C ACTIVATION ON STATUS EPILEPTICUS AND EPILEPTOGENESIS: OH, WHAT A TANGLED WEB

**Deficits in Phosphorylation of GABA<sub>A</sub> Receptors by Intimately Associated Protein Kinase C Activity Underlie Compromised Synaptic Inhibition during Status Epilepticus.** Terunuma M, Xu J, Vithlani M, Sieghart W, Kittler J, Pangalos M, Haydon PG, Coulter DA, Moss SJ. *J Neurosci* 2008;28(2):376–384. Status epilepticus (SE) is a progressive and often lethal human disorder characterized by continuous or rapidly repeating seizures. Of major significance in the pathology of SE are deficits in the functional expression of GABA<sub>A</sub> receptors (GABA<sub>A</sub>Rs), the major sites of fast synaptic inhibition in the brain. We demonstrate that SE selectively decreases the phosphorylation of GABA<sub>A</sub>Rs on serine residues 408/9 (S408/9) in the  $\beta$ 3 subunit by intimately associated protein kinase C isoforms. Dephosphorylation of S408/9 unmasks a basic patch-binding motif for the clathrin adaptor AP2, enhancing the endocytosis of selected GABA<sub>A</sub>R subtypes from the plasma membrane during SE. In agreement with this, enhancing S408/9 phosphorylation or selectively blocking the binding of the  $\beta$ 3 subunit to AP2 increased GABA<sub>A</sub>R cell surface expression levels and restored the efficacy of synaptic inhibition in SE. Thus, enhancing phosphorylation of GABA<sub>A</sub>Rs or selectively blocking their interaction with AP2 may provide novel therapeutic strategies to ameliorate SE.

## COMMENTARY

Seizure discharges are typically self-limiting, yet the mechanism by which seizures usually terminate is not fully understood. A number of studies in recent years have demonstrated an association between diminished GABA<sub>A</sub> receptor expression and status epilepticus (SE)—that is, the state in which seizures fail to self-abort. When SE occurs, surface expression of GABA<sub>A</sub> receptors progressively decreases, resulting in increasing pharmacoresistance of the seizure to barbiturates and benzodiazepines, which are positive allosteric modulators of the GABA<sub>A</sub> receptors (1).

A combination of molecular and physiological experiments reported by Terunuma and colleagues examined the mechanisms underlying this GABA<sub>A</sub> receptor-deficient state accompanying SE. They used pilocarpine to induce unremitting grade 5 seizures in mice (a commonly used model of SE); they then compared the hippocampi of these animals with those of control animals that received saline and experienced no seizures. The investigators first documented a shift of GABA<sub>A</sub> receptor subunit composition following 1 hour of SE, with a reduction of beta ( $\beta$ ) and gamma ( $\gamma$ ) subunits and an increase in delta ( $\delta$ ). The functional implication is that synaptic inhibition should be impaired, since the benzodiazepine-sensitive GABA<sub>A</sub> receptors at inhibitory synapses most typically contain  $\gamma$  subunits and not  $\delta$ . Indeed, Terunuma et al. nicely documented correlating

electrophysiological evidence, revealing a postsynaptic defect of GABA<sub>A</sub> receptor responses. Additional data revealed that hypoactivity of conventional protein kinase C (PKC) isoforms is responsible for deficient GABA<sub>A</sub> receptor phosphorylation, resulting in endocytosis of these receptors. PKC normally binds to the  $\beta$ 3 subunit of the GABA<sub>A</sub> receptor and phosphorylates it, thereby preventing its association with the clathrin adaptor AP2—a process critical for receptor internalization. If phosphorylation fails to occur, the result is decreased GABA<sub>A</sub> receptor expression at the surface that is due to excessive endocytosis and results in defective GABAergic inhibition.

Cellular proteins, be they channels, receptors, or enzymes, are all under constant modulation and regulation by processes that drive their phosphorylation and dephosphorylation. Kinases will phosphorylate proteins by transferring a phosphate group from a nearby molecule of ATP, while phosphatases will remove the phosphate group, thereby dephosphorylating the protein in question. The presence or absence of a phosphate group on a critical amino acid within the protein will alter the protein's configuration and have functional consequences of either enhancing or impairing the activity of the channel, receptor, or enzyme. And, depending on which amino acids within which channels and receptors serve as the target of phosphorylation, the functional impact on the cell could be excitatory or inhibitory. PKC is one of the more prominent kinases, commonly activated by various G-protein-linked metabotropic receptors.

One might expect neuronal excitation underlying seizures to be associated with increased PKC activity. Although most

papers indeed report elevated PKC activation with both seizure activity and SE, Terunuma and colleagues describe a decrease in PKC, with no comment addressing the conflicting data. Interpretation of this diminished PKC activation is unclear: it is possible that defective activation of PKC underlies the development or progression of SE; alternatively, prolonged excitation associated with SE may trigger autoregulatory processes, such as desensitization, preventing excessive continued PKC activation. But, let the reader beware: the accurate explanation for the discrepancy most likely lies in the details. For example, PKC responses to the same event may yield different responses among cell types. Guglielmetti et al. reported kainate-induced seizures elicit reduced PKC mRNA in hippocampus but increased levels in dentate gyrus (2). Furthermore, PKC comes in various isoforms, each isoform having distinct physiological roles and potentially responding differently to the same trigger. The current paper by Terunuma et al. focused on the conventional isoforms (i.e.,  $\alpha$ ,  $\beta$ , and  $\gamma$ ) and found all three to be reduced in both level and activity in hippocampal slices after 1 hour of SE. By contrast, the reduction of PKC mRNA in CA1 and CA3 that Guglielmetti and coworkers reported was limited to the  $\gamma$  isoform, and lasted 1 to 2 days. Furthermore, the increased PKC levels in the dentate granule cells and their mossy fiber axons were limited to the epsilon ( $\epsilon$ ) isoform and persisted for months following kainate-induced seizures, which may contribute to the epileptogenic mossy fiber sprouting and synaptic reorganization induced by SE. Additional factors should be considered as well, including: the stage of brain development; brain region or subregion being analyzed; precise timing of analysis relative to seizure onset, length, and termination; and whether the PKC measurement was based on total level versus activated PKC (also called phosphorylated or membrane bound) and protein versus mRNA—all these factors can influence results and need to be carefully assessed to interpret the findings accurately.

The authors boldly conclude their report by suggesting that enhancing phosphorylation of the GABA receptor  $\beta 3$  subunit may have therapeutic value in SE; one could achieve this end with agents that activate PKC. Activation of PKC also prevents the induction of group I metabotropic glutamate receptor (mGluR)-driven epileptogenesis in vitro (3), suggesting that agents that activate PKC may be clinically useful in the suppression of both SE and epileptogenesis. Yet, recent data indicate that PKC activation elicits ictal discharges in vitro, sustained in part by enhanced mGluR5 responses (4). PKC-induced phosphorylation has additional seizure-promoting effects: phosphorylation of NMDA receptor subunits promotes trafficking of the NMDA receptors to the cell surface (5); and phosphorylation of presynaptic voltage-gated calcium channels enhances calcium entry, thereby boosting glutamate release (6). Overactivation of PKC may have negative consequences on cogni-

tion as well: seizures driven by hyperstimulated NMDA receptors produce sustained PKC elevation associated with reduced hippocampal long-term potentiation, which could account for memory impairment postictally and beyond (7). Furthermore, in prefrontal cortex, increased PKC activation, driven by excessive noradrenergic activation, has been associated with impaired cognitive functioning, with possible relevance to the cognitive dysfunction seen in bipolar disorder and schizophrenia (8).

So, while it is not yet sound to prescribe generalized PKC activators or inhibitors to patients based on any of these data, the article by Terunuma and coworkers opens the possibility that further detailed and carefully analyzed studies along these lines may permit greater knowledge; similarly, technological advances may open the way for more targeted approaches to intervening in PKC-relevant pathways. For now, there is probably but one antiepileptic agent currently in use that works in part by interfering with PKC: the broad-spectrum anticonvulsant valproate, which has been reported to have a modest inhibitory effect on PKC $\epsilon$ . This action has been credited with conferring part of the anticonvulsive and mood-stabilizing efficacy of valproate (9), and if the sustained elevation of PKC $\epsilon$  in the dentate is indeed necessary for SE-induced mossy fiber sprouting (2), it suggests a possibility that valproate may suppress epileptogenesis. While generalized PKC activation may be ictogenic (4), targeted localized PKC activation driven by selective activation of a specific mGluR subtype may have antiepileptogenic potential as well (3). Additional data are necessary to validate these suppositions, but it certainly encourages continued pursuit in this area of research to tease apart the tangled details relating PKC to epilepsy.

by Lisa R. Merlin, MD

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## FUNNY TEAM MEMBER MAKES KEY PLAYS, BUT LEAVES THE DENDRITIC FIELD WHEN HIT HARD

**Progressive Dendritic HCN Channelopathy during Epileptogenesis in the Rat Pilocarpine Model of Epilepsy.** Jung S, Jones TD, Lugo JN Jr, Sheerin AH, Miller JW, D'Ambrosio R, Anderson AE, Poolos NP. *J Neurosci* 2007;27(47):13012–13021. Ion channelopathy plays an important role in human epilepsy with a genetic cause and has been hypothesized to occur in epilepsy after acquired insults to the CNS as well. Acquired alterations of ion channel function occur after induction of status epilepticus (SE) in animal models of epilepsy, but it is unclear how they correlate with the onset of spontaneous seizures. We examined the properties of hyperpolarization-activated cation (HCN) channels in CA1 hippocampal pyramidal neurons in conjunction with video-EEG (VEEG) recordings to monitor the development of spontaneous seizures in the rat pilocarpine model of epilepsy. Our results showed that dendritic HCN channels were significantly downregulated at an acute time point 1 week postpilocarpine, with loss of channel expression and hyperpolarization of voltage-dependent activation. This downregulation progressively increased when epilepsy was established in the chronic period. Surprisingly, VEEG recordings during the acute period showed that a substantial fraction of animals were already experiencing recurrent seizures. Suppression of these seizures with phenobarbital reversed the change in the voltage dependence of  $I_h$ , the current produced by HCN channels, but did not affect the loss of HCN channel expression. These results suggest two mechanisms of HCN channel downregulation after SE, one dependent on and one independent of recurrent seizures. This early and progressive downregulation of dendritic HCN channel function increases neuronal excitability and may be associated with both the process of epileptogenesis and maintenance of the epileptic state.

### COMMENTARY

HCN stands for hyperpolarization-activated, cation non-selective, cyclic nucleotide modulated, a long but informative three-part moniker to describe a specific type of channel (1). The name begins with a reminder that HCN channels have uniquely modified pores, which are opened strongly by hyperpolarization, partially opened at resting potentials, and closed by depolarization. Because their voltage dependence is opposite that of other channels, they have sometimes been called “funny channels” by physiologists. “Cation nonselective” means that when open, HCN channels allow both sodium to enter the cell and potassium to leave through their pores. The combination of these current flows tends to drive the membrane potential to a weakly depolarized voltage. Finally, HCN channels possess a special intracellular pocket that can bind the second messen-

ger, cAMP, when its concentration rises to the upper end of the physiological range. When cAMP is elevated (e.g., by activation of certain receptors for norepinephrine, serotonin, and dopamine), HCN voltage gating speeds up, so that hyperpolarization opens and depolarization closes the HCN channels more rapidly. So, to summarize, HCN channels are partially open at the resting membrane potential and thereby exert a weakly depolarizing influence that is due mostly to inward sodium current. Strong membrane depolarization makes these channels close, eliminating this inward current. Hyperpolarization, from IPSPs or potassium channel activation following an action potential, causes HCN channels to open more strongly, which leads to a “rebound” of depolarization. Modulatory neurotransmitters can enhance these properties, allowing HCN channels to integrate intracellular chemical and membrane voltage signaling.

Because of their special ability to cause rebound depolarization after other channels cause hyperpolarization, HCN channels are important players in cells with intrinsic oscillatory

the connections between the intriguing changes in CA1 cells, as elucidated by Jung et al., and epileptogenic effects (ultimately expressed at the level of networks and behavior) are fully understood.

by Edward C. Cooper, MD, PhD

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