



Dephosphorylation Proves Detrimental to GABAergic Inhibition

NMDA Receptor Activity Downregulates KCC2 Resulting in Depolarizing GABA_A Receptor-Mediated Currents.

Lee HHC, Deeb TZ, Walker JA, Davies PA, Moss SJ. *Nat Neurosci* 2011;14:736–743.

KCC2 is a neuron-specific K⁺-Cl⁻ co-transporter that maintains a low intracellular Cl⁻ concentration that is essential for hyperpolarizing inhibition mediated by GABA_A receptors. Deficits in KCC2 activity occur in disease states associated with pathophysiological glutamate release. However, the mechanisms by which elevated glutamate alters KCC2 function are unknown. The phosphorylation of KCC2 residue Ser940 is known to regulate its surface activity. We found that NMDA receptor activity and Ca²⁺ influx caused the dephosphorylation of Ser940 in dissociated rat neurons, leading to a loss of KCC2 function that lasted longer than 20 min. Protein phosphatase 1 mediated the dephosphorylation events of Ser940 that coincided with a deficit in hyperpolarizing GABAergic inhibition resulting from the loss of KCC2 activity. Blocking dephosphorylation of Ser940 reduced the glutamate-induced downregulation of KCC2 and substantially improved the maintenance of hyperpolarizing GABAergic inhibition. Reducing the downregulation of KCC2 therefore has therapeutic potential in the treatment of neurological disorders.

Commentary

A good deal of attention has been focused in recent years on the role of chloride ion transporting pumps in epilepsy. This is because Cl⁻ cotransporters (other ions, depending on transporter type, are moved in concert with Cl⁻) set the level of intracellular Cl⁻ concentration ([Cl⁻]_i) in neurons and hence determine the effectiveness of GABAergic inhibition. The K⁺/Cl⁻ cotransporter KCC2 performs most of the work in setting intracellular Cl⁻ concentration, moving Cl⁻ out of the neuron against its electrochemical gradient to maintain [Cl⁻]_i that is lower than the [Cl⁻]_o in the extracellular space.

Much has been written about the developmental changes in KCC2 expression and the mirror-image changes in the Na⁺/K⁺/Cl⁻ cotransporter NKCC1 (1). NKCC1 essentially opposes the action of KCC2, establishing high levels of [Cl⁻]_i. NKCC1 expression is high during development while KCC2 levels are low, thus the resulting high [Cl⁻]_i leads to depolarizing GABAergic IPSPs in the immature nervous system, opposite to the hyperpolarizing effect of IPSPs in maturity. Because of this situation, antiepileptic drugs acting on GABA_A receptors might paradoxically increase excitation in infants with seizures (2).

Outside of the early developmental period when NKCC1 expression predominates, how KCC2 function and expression relate to epilepsy has been less well examined. Several studies have shown that KCC2 expression is decreased in both human temporal lobe epilepsy (3) and in animal models of epilepsy

following provoked status epilepticus (SE) (4). Understanding how KCC2 cotransporter expression is lost following SE might be important for clarifying both a key process during epileptogenesis and how GABAergic inhibition loses its efficacy during a prolonged seizure. The current study by Lee and colleagues establishes just such a mechanism for KCC2 downregulation during seizures, implicating loss of phosphorylation of the KCC2 molecule as a key mediator. Although this might seem an arcane topic of investigation, in fact their findings join a number of recent publications highlighting altered phosphorylation signaling as one of the important cellular processes underlying ion channel dysfunction in epilepsy (5–9).

The experiments in this study were conducted in a reduced system using cultured neurons. Lee and colleagues began by demonstrating that exposure of neurons to exogenous glutamate, at a concentration that might be seen during a seizure, converted the normally hyperpolarizing response to GABA application to a depolarizing or purely shunting (minimal voltage change despite an increase in conductance) response. This loss of GABAergic inhibition was associated with a change in the GABA reversal potential that was largely dependent on the loss of KCC2 function. (NKCC1, which is usually expressed at only low levels in mature neurons, did not seem to be involved in the generation of depolarizing GABA responses.) When the authors measured KCC2 expression following glutamate exposure, they found markedly reduced levels of the transporter protein within just minutes of glutamate application.

They then correlated this phenomenon with dephosphorylation of a specific amino acid residue (the serine at position 940 in the protein, or Ser940). Serine, along with threonine and tyrosine, are amino acids that can be modified by covalent attach-



ment of a phosphate group by protein kinases. Such "posttranslational" phosphorylation is a ubiquitous cellular mechanism for altering the properties of proteins. In this case, the loss of Ser940 phosphorylation (dephosphorylation) was attributed to the action of a phosphatase, protein phosphatase 1 (PP1). When PP1 was pharmacologically blocked, glutamate exposure produced a much smaller effect on GABAergic transmission. Thus, the authors put forward a model in which intense neuronal activity, such as occurs during a seizure or SE, leads to intracellular activation of PP1, which strips away the phosphate group on a single serine residue on KCC2. This in turn leads to the rapid degradation of KCC2 protein and partial loss of the all-important low $[Cl^-]$, that enables GABAergic inhibition.

These findings are compelling for several reasons. The first is that this manuscript represents a clear distillation of pathologically altered synaptic physiology to loss of phosphorylation at a single amino acid residue—an elegant demonstration of reductionist biology. But more importantly, these findings join a number of other recent studies (some by the same laboratory group) that identify altered phosphorylation signaling as a key pathologic process in epilepsy. For example, the stability of GABA_A receptors in the neuronal plasma membrane becomes altered during prolonged seizures, leading to their internalization and loss of function, a process mediated by dephosphorylation of specific GABA_A subunits (5). A similar process mediated by increased phosphorylation of A-type K⁺ channels follows SE and, as with GABA_A receptors, leads to their internalization and increased neuronal excitability (6). Chronic loss of phosphorylation signaling occurs in the pilocarpine model of epilepsy, accounting for the loss of function of the hyperpolarization-activated cation (HCN) channel, which also contributes to hyperexcitability (7). Kindling epileptogenesis has been shown to depend on the activation of the receptor tyrosine kinase TrkB (8). And it is hyperactivity of the phosphorylation pathway mTOR that underlies the manifestations of the genetic disorder tuberous sclerosis (9).

These numerous lines of evidence suggest that phosphorylation signaling can be altered on time scales ranging from minutes to months following neurologic insults, or permanently in the case of inherited syndromes, with significant effects on ion channel and transporter function. Given the importance of phosphorylation signaling in cellular regulation and the fact that there are more than 500 different protein kinases identified in humans, it should not be surprising that numerous altered pathways have been thus far described in epilepsy. Yet this emerging and somewhat complicated area of investigation offers hope for therapeutic interventions: protein kinases have proven to be fruitful targets for drug development, with about half of all current pharmaceutical new drug

development in this category (10). As new altered phosphorylation pathways in epilepsy are discovered, specific drugs to reverse those changes will be available. This will allow testing of whether the reversal of pathologic phosphorylation signaling brought on by neural insults will retard the development of epilepsy or possess antiepileptic properties. The findings of the current manuscript, of course, are a long way from that future promise: they simply represent a proof of concept in a reduced cellular system that has not been tested at the level of an animal model. Nevertheless, these results underscore the importance of phosphorylation signaling in neuronal excitability and its possible future relevance to epilepsy therapeutics.

by Nicholas P. Poolos, MD, PhD

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