



Not RESTing on Its Laurels: Timing and Mechanisms of HCN Channel Dysfunction in Epilepsy

Rapid Loss of Dendritic HCN Channel Expression in Hippocampal Pyramidal Neurons Following Status Epilepticus.

Jung S, Warner LN, Pitsch J, Becker AJ, Poolos NP. *J Neurosci* 2011;31(40):14291–4295.

Epilepsy is associated with loss of expression and function of hyperpolarization-activated, cyclic nucleotide-gated (HCN) ion channels. Previously, we showed that loss of HCN channel-mediated current (I_h), occurred in the dendrites of CA1 hippocampal pyramidal neurons after pilocarpine-induced status epilepticus (SE), accompanied by loss of HCN1 channel protein expression. However, the precise onset and mechanistic basis of HCN1 channel loss post-SE was unclear, particularly whether it preceded the onset of spontaneous recurrent seizures and could contribute to epileptogenesis or development of the epileptic state. Here, we found that loss of I_h and HCN1 channel expression began within an hour after SE and involved sequential processes of dendritic HCN1 channel internalization, delayed loss of protein expression, and later down regulation of mRNA expression. We also found that an in vitro SE model reproduced the rapid loss of dendritic I_h , demonstrating that this phenomenon was not specific to in vivo SE. Together, these results show that HCN1 channelopathy begins rapidly and persists after SE, involves both transcriptional and nontranscriptional mechanisms, and may be an early contributor to epileptogenesis.

Neuron-Restrictive Silencer Factor-Mediated Hyperpolarization-Activated Cyclic Nucleotide Gated Channelopathy in Experimental Temporal Lobe Epilepsy.

McClelland S, Flynn C, Dubé C, Richichi C, Zha Q, Ghestem A, Esclapez M, Bernard C, Baram TZ. *Ann Neurol* 2011;70(3):454–464.

OBJECTIVE: Enduring, abnormal expression and function of the ion channel hyperpolarization-activated cyclic adenosine monophosphate gated channel type 1 (HCN1) occurs in temporal lobe epilepsy (TLE). We examined the underlying mechanisms, and investigated whether interfering with these mechanisms could modify disease course. **METHODS:** Experimental TLE was provoked by kainic acid-induced status epilepticus (SE). HCN1 channel repression was examined at mRNA, protein, and functional levels. Chromatin immunoprecipitation was employed to identify the transcriptional mechanism of repressed HCN1 expression, and the basis for their endurance. Physical interaction of the repressor, NRSF, was abolished using decoy oligodeoxynucleotides (ODNs). Video/electroencephalographic recordings were performed to assess the onset and initial pattern of spontaneous seizures. **RESULTS:** Levels of NRSF and its physical binding to the *Hcn1* gene were augmented after SE, resulting in repression of HCN1 expression and HCN1-mediated currents (I_h), and reduced I_h -dependent resonance in hippocampal CA1 pyramidal cell dendrites. Chromatin changes typical of enduring, epigenetic gene repression were apparent at the *Hcn1* gene within a week after SE. Administration of decoy ODNs comprising the NRSF DNA-binding sequence (neuron restrictive silencer element [NRSE]), in vitro and in vivo, reduced NRSF binding to *Hcn1*, prevented its repression, and restored I_h function. In vivo, decoy NRSE ODN treatment restored theta rhythm and altered the initial pattern of spontaneous seizures. **INTERPRETATION:** Acquired HCN1 channelopathy derives from NRSF-mediated transcriptional repression that endures via chromatin modification and may provide insight into the mechanisms of a number of channelopathies that coexist with, and may contribute to, the conversion of a normal brain into an epileptic one.

Commentary

Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels are unique among ion channels with regard to their activity-dependent regulation of neuronal excitabil-



ity (1–3). HCN channels open upon hyperpolarization and are also directly regulated by cAMP binding in an isoform-specific manner. The ionic current mediated by HCN channels (I_h) opposes membrane potential deviations in either direction from resting potential— I_h is activated by hyperpolarization, generating an inward current that tends to return membrane potential toward resting potential, and deactivated by depolarization, again forcing membrane potential toward resting potential. This negative feedback property, augmented by cAMP-induced gating of HCN2, underlies the role of I_h in some HCN2-mediated rhythmic neuronal activities such as thalamocortical oscillations. In contrast, HCN1 is highly expressed in hippocampus and neocortex and functions mainly in the subthreshold voltage range, lowering a neuron's input resistance and acting as a filter of incoming excitatory activity. The localization, expression, and trafficking of HCN1 channels in distal dendrites makes them exquisitely poised to attenuate excitatory synaptic input and undergo regulation by neuronal activity (4–6).

The physiological properties of HCN channels are well suited for a role in epilepsy. I_h often keeps excessive excitation in check, and I_h and the expression and trafficking of HCN channels are altered in a number of models of experimental epilepsy, including febrile seizures (7), temporal lobe epilepsy (8), and generalized epilepsy (9). Knock-out mice lacking HCN channels develop susceptibility to epilepsy (10). Of importance, HCN channel expression is altered in human temporal lobe epilepsy (11), and mutations in HCN channels underlie some human idiopathic generalized epilepsies (12). Thus, exacerbation of seizures caused by loss or dysfunction of HCN channel expression comprises a form of acquired epileptic channelopathy.

Two recent reports expand knowledge of the dynamic regulation of HCN1 channels in epilepsy. Jung and colleagues explore the timing of dendritic HCN protein loss in relationship to the onset of spontaneous seizures (epileptogenesis) using the pilocarpine model, while McClelland and coworkers investigate the molecular basis of *Hcn1* gene regulation in experimental temporal lobe epilepsy using the kainate model.

The loss of dendritic I_h and HCN1 protein following status epilepticus has been well documented (8), but a persisting question has been the time course and mechanism of those changes. That is, does the loss of channel protein precede the development of spontaneous seizures and thereby contribute to epileptogenesis? Jung and colleagues provide clear evidence that the loss of I_h and surface expression of HCN1 channel protein (as assessed by Western blots and biotinylation assays) occurs rapidly, within 1 hour after the onset of pilocarpine-status epilepticus, while total HCN1 protein is unchanged from baseline at 1 hour but is markedly reduced by 1 day after status epilepticus. These results suggest that membrane channels are internalized initially, causing I_h loss. The rapid time course disfavors a transcriptional mechanism to account for the initial I_h reduction, instead suggesting a post-translational modification of the channel protein. Later, by 1 week after status epilepticus, HCN1 mRNA levels are

reduced (transcriptional downregulation), as previously reported by several investigators (4, 8). This sequence of molecular changes, which was confirmed in an in vitro slice model of status epilepticus, suggests that the HCN channelopathy following status epilepticus has a rapid onset and precedes the development of spontaneous seizures, which in the pilocarpine model do not occur until at least 3 days after status epilepticus.

Because reduction of HCN1 expression has been found in several models of epileptogenesis, McClelland and colleagues attempted to uncover the mechanisms by which this reduction occurs. Since seizures alter the expression of numerous genes, such information could allow a broader understanding of how ion channel and other neuronal genes are dysregulated in the process of epileptogenesis. The expression of many neuronal genes is regulated by transcriptional repressors that bind to specific gene sequences; changes in levels of such transcriptional repressors or their binding to gene sequences could alter the epileptogenic process. McClelland and colleagues found that HCN1 repression involves the transcriptional repressor neuron-specific silencer factor (NRSF; also known as REST, repressor element 1-silencing transcription factor), which binds to neuron restrictive silencer elements (NRSE) on many neuronal genes. The *Hcn1* gene contains a highly conserved NRSE sequence to which NRSF can bind. Therefore, the investigators tested the hypothesis that NRSF binding to NRSE on *Hcn1* is altered after status epilepticus, thereby modifying the expression and function of HCN1 channels after an epileptic insult. Further, they tested the hypothesis that interfering with NRSF binding could attenuate the adverse epileptic consequences following status epilepticus.

Rats were treated with kainate to produce 30 minutes of status epilepticus. Three days later, I_h amplitude was reduced, I_h kinetics were slowed (as expected from a loss of the contribution of HCN1 to the total I_h current), and HCN protein expression was reduced in hippocampal CA1 pyramidal neurons, all consistent with an acquired HCN channelopathy. To test the hypothesis that NRSF binding to its cognate sequence on NRSE, could account for the decreased HCN protein, the investigators used hippocampal organotypic cultures to measure NRSF levels and found them to be elevated for more than a week following status epilepticus. This finding suggests an enduring modification of gene expression that could contribute to epileptogenesis. They then performed a clever experiment, interfering with NRSF binding by infusion of NRSE-sequence oligodeoxynucleotides (ODNs) to act as “decoys” for NRSF binding. In rats or organotypic hippocampal cultures pretreated with NRSE-ODNs, HCN was not reduced, and rats were protected from kainate-induced seizures (and, as a control, pilocarpine-induced seizures). Therefore, NRSE-ODNs, by blocking NRSF binding, effectively “rescued” HCN and I_h from the seizure-induced consequences. When kainate plus NRSE-ODNs were applied in vivo, there was a reduction in expected frequency of interictal bursts and spontaneous recurrent seizures, attesting to a role of NRSF in *Hcn1* gene repression after status epilepticus. As



another control, if the ODNs were “scrambled” (i.e., out of sequence for the NRSE), a rescue effect did not occur.

Taken together, these two reports advance our understanding of the role of HCN channels, and in particular HCN1, in epilepsy. The status epilepticus–induced HCN channelopathy following either pilocarpine or kainate predisposes neurons to both intrinsic and network hyperexcitability by virtue of loss of I_h . I_h may curtail membrane excitability and oppose the spread of excitatory synaptic input from distal dendrites; diminution of these constraints likely supports ongoing hyperexcitability and hypersynchronous neuronal firing. As demonstrated in these reports, discrete and specific gene alterations, involving both transcriptional and nontranscriptional mechanisms, likely promote a long-lasting epileptic state. Dissection of the molecular mechanisms involved in *Hcn1* gene expression could provide a novel avenue for therapeutics.

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