

GENETIC LOSS OF HCN1 CHANNELS IS EXCITING, BUT IS IT EPILEPTIC?

Loss of Dendritic HCN1 Subunits Enhances Cortical Excitability and Epileptogenesis. Huang Z, Walker MC, Shah MM. *J Neurosci* 2009;29(35):10979–10988. Hyperpolarization-activated cation nonselective 1 (HCN1) plasticity in entorhinal cortical (EC) and hippocampal pyramidal cell dendrites is a salient feature of temporal lobe epilepsy. However, the significance remains undetermined. We demonstrate that adult HCN1 null mice are more susceptible to kainic acid-induced seizures. After termination of these with an anticonvulsant, the mice also developed spontaneous behavioral seizures at a significantly more rapid rate than their wild-type littermates. This greater seizure susceptibility was accompanied by increased spontaneous activity in *HCN1*^{-/-} EC layer III neurons. Dendritic *I_h* in these neurons was ablated, too. Consequentially, *HCN1*^{-/-} dendrites were more excitable, despite having significantly more hyperpolarized resting membrane potentials (RMPs). In addition, the integration of EPSPs was enhanced considerably such that, at normal RMP, a 50 Hz train of EPSPs produced action potentials in *HCN1*^{-/-} neurons. As a result of this enhanced pyramidal cell excitability, spontaneous EPSC frequency onto *HCN1*^{-/-} neurons was considerably greater than that onto wild types, causing an imbalance between normal excitatory and inhibitory synaptic activity. These results suggest that dendritic HCN channels are likely to play a critical role in regulating cortical pyramidal cell excitability. Furthermore, these findings suggest that the reduction in dendritic HCN1 subunit expression during epileptogenesis is likely to facilitate the disorder.

COMMENTARY

The list of ion channels that may play causative roles in epilepsy seemingly grows monthly, with the identification of ion channel mutations in human inherited epilepsy syndromes as well as evidence linking ion channel dysfunction (or channelopathy) to the development of epilepsy in animal models of acquired syndromes (1). These latter acquired channelopathy candidates can be studied in transgenic mice in which expression of the underlying gene has been altered—a powerful technique to assess whether ion channel dysfunction produces an epileptic phenotype *in vivo*. The recent study by Huang et al. subjects the hyperpolarization-activated cyclic nucleotide-gated type 1, or HCN1, channel to this rigorous test using *HCN1* knockouts. The results are compelling and puzzling at the same time: as expected, mice with constitutive deletion of HCN1 subunits showed significant neuronal hyperexcitability, had an exaggerated response to a chemical convulsant, yet, in the absence of exposure to kainate, had no spontaneous seizures. What does this mean for the role of HCN1 channels—and for other channels whose genetic deletion produces similar results—in epilepsy?

The HCN channel is one of the most intriguing candidate epileptic channelopathies. A potassium channel by structure, it actually has poor selectivity for K⁺ ions and predominantly allows the flow of Na⁺ ions. Its slow activation kinetics means that the HCN channel does not contribute to the action potential waveform, as do sodium and potassium channels. However, it significantly affects neuronal excitability by virtue of its lack of inactivation: that is, by remaining open at neuronal resting potential when most other voltage-gated channels are closed, it sets the level of electrical leakiness of the cell and hence, the cell's response to synaptic inputs. Because HCN channels in the principal neurons of the cortex and hippocampus are localized mostly to the dendrites where excitatory synaptic inputs arise, HCN channels are particularly suited to controlling the synaptic excitation of those cells.

The contribution of HCN1 channels to neuronal excitability as well as to learning and memory has previously been assessed using knockout mice. A forebrain-specific deletion of *HCN1* yielded mice with enhanced long-term plasticity to excitatory synaptic inputs in hippocampal pyramidal neurons and, correspondingly, with a tendency to learn more quickly those tasks that were dependent on spatial memory (2). Knockout of the HCN2 channel (which is predominant in subcortical structures, such as the thalamus) produced a generalized epilepsy phenotype that is perhaps consistent with the role of the

thalamus in synchronizing cortical spike-wave discharges (3). In this study, Huang et al. asked how global *HCN1* deletion could affect the excitability of entorhinal cortical layer III pyramidal neurons in a parahippocampal region implicated in the genesis of temporal lobe epilepsy.

Using electrophysiological recordings in the dendrites of pyramidal neurons (a challenging task in mouse brain slices), the authors found that neurons lacking HCN1 channels were intrinsically hyperexcitable compared to those from wild-type animals. While this finding was not unexpected, as in previous work that had employed pharmacological means to decrease HCN channel function, it was a clear demonstration that HCN channels act to diminish principal neuron excitability—an issue that, at times, has been a subject of debate (4). More interesting was the authors' finding that *HCN1* knockouts showed increased spontaneous synaptic activity, both excitatory and inhibitory, with evoked synaptic stimulation causing prolonged plateau potentials in the dendrites of entorhinal cortical neurons, which is reminiscent of the paroxysmal depolarizing shift potentials seen in cortical neurons *in vivo* during interictal burst firing. These data support the idea that loss of HCN channels produces cortical hyperexcitability, both at single neuron and network levels.

But does neuronal hyperexcitability from HCN1 channel loss translate into epilepsy? It appears that it does not. Some 900 hours of EEG recording from *HCN1* knockout mice failed to detect a spontaneous seizure or even an interictal spike. Yet, when the authors challenged knockout mice with a standard dose of the convulsant kainate, the animals rapidly entered into status epilepticus and expired. Cutting the kainate dose in half allowed the knockouts to survive status epilepticus; interestingly, these mice showed a much shorter latency period to the development of spontaneous seizures than the wild-type controls, with a mean latency of about 60 hours in the knockouts compared to 368 hours in the controls. These data suggest that while the genetic deletion of HCN1 channels does not by itself produce an epileptic phenotype, HCN channels exert a powerful anticonvulsant action *in vivo* and may modulate the course of epileptogenesis after status epilepticus. The latter finding is in accord with recent work demonstrating that acquired loss of HCN channel function in wild-type animals following status epilepticus progressively worsens during the course of epileptogenesis, as does intrinsic neuronal hyperexcitability (5,6).

There are multiple possible explanations for why loss of HCN channel function might produce significant hyperexcitability when measured at levels ranging from the single neuron to the whole brain and yet still not produce epilepsy. The first and most mundane explanation is that mouse knockouts often produce unexpected results, which sometimes differ depending on the genetic strain employed. For example, mouse modeling of human mutations that produce the auto-

somal dominant disease, benign familial neonatal convulsions (BFNC), yields heterozygous animals with neuronal hyperexcitability and lowered threshold to provoked seizures but without spontaneous seizures (7). This example only reinforces the obvious conclusions that a mouse brain is not a human brain and that rodent models may not replicate the behavior of the far more complex primate brain. A second consideration concerns the consequences of constitutive deletion of a gene and the various compensatory mechanisms that may arise during development. An example of this phenomenon is seen with the deletion of the gene encoding the rapidly activating Kv4.2 channel that exerts profound inhibitory influences on neuronal excitability. Kv4.2 knockout animals, while showing neuronal hyperexcitability, also lack spontaneous seizures (8), and at least one study has demonstrated the upregulated expression of potassium channels unrelated to Kv4.2 that exert a compensatory effect on excitability, possibly preventing the development of epilepsy (9).

A third—and perhaps more intellectually compelling—explanation for the lack of epilepsy in animals with critical ion channels genetically deleted is that few ion channels are functionally unique and irreplaceable. This theory is supported by the rarity of human inherited epilepsy syndromes with single ion channel mutations and makes sense, given the 78 potassium channels, 11 calcium channels, and 10 sodium channels (but only 4 HCN channels) that so far have been identified in the human genome, not to mention the many subtypes of ligand-gated channels (10). Thus, it should not be surprising that genetic loss of any one channel subtype is unlikely to produce a functional deficit resulting in an epileptic phenotype. When considering the problem of epilepsy acquired from a neural insult, such as status epilepticus, stroke, or head trauma, it is almost certain that multiple ion channelopathies must result—some pathogenic, some compensatory; thus, it is unlikely that any one channel will be identified as a unique culprit in acquired epileptogenesis.

Despite these general caveats, the study by Huang et al. is important for clarifying the phenotype of HCN1 channel deletion. The investigators have confirmed the important role of this channel in limiting excitability in the cortex and hippocampus and have provided further new evidence that HCN channels may exert antiepileptic and antiepileptogenic functions. In addition, this study forces clinicians and researchers to think critically about the role of ion channelopathy and epilepsy, particularly in acquired models of the disease.

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