

HYPERPOLARIZATION-ACTIVATED CATION CHANNELS IN HUMAN AND EXPERIMENTAL EPILEPSY: DO THEY PLAY A ROLE IN EPILEPTOGENESIS?

Enhanced Expression of a Specific Hyperpolarization-activated Cyclic Nucleotide-gated Cation Channel (HCN) in Surviving Dentate Gyrus Granule Cells of Human and Experimental Epileptic Hippocampus

Bender RA, Soleymani SV, Brewster AL, Nguyen ST, Beck H, Mathern GW, Baram TZ

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Changes in the expression of ion channels, contributing to altered neuronal excitability, are emerging as possible mechanisms in the development of certain human epilepsies. In previous immature rodent studies of experimental prolonged febrile seizures, isoform-specific changes in the expression of hyperpolarization-activated cyclic nucleotide-gated cation channels (HCNs) correlated with long-lasting hippocampal hyperexcitability and enhanced seizure susceptibility. Prolonged early-life seizures commonly precede human temporal lobe epilepsy (TLE), suggesting that transcriptional dysregulation of HCNs might contribute to the epileptogenic process. Therefore we determined whether HCN isoform expression was modified in hippocampi of individuals with TLE. HCN1 and HCN2 expression were measured by using *in situ* hybridization and immunocytochemistry in hippocampi from three groups: TLE with hippocampal sclerosis (HS; $n = 17$), epileptic hippocampi without HS, or non-HS (NHS; $n = 10$), and autopsy material ($n = 10$). The results obtained in chronic human epilepsy were validated by examining hippocampi from the pilocarpine model of chronic TLE. In autopsy and most NHS hippocampi, HCN1 mRNA expression was substantial in pyramidal cell layers and lower in dentate gyrus granule cells (GCs). In contrast, HCN1 mRNA expression over the GC layer and in individual GCs from epileptic hippocampus was markedly increased once GC neuronal density was reduced by >50%. HCN1 mRNA changes were accompanied by enhanced immunoreactivity in the GC dendritic fields and more modest changes in HCN2 mRNA expression. Fur-

thermore, similar robust and isoform-selective augmentation of HCN1 mRNA expression was evident also in the pilocarpine animal model of TLE. These findings indicate that the expression of HCN isoforms is dynamically regulated in human as well as in experimental hippocampal epilepsy. After experimental febrile seizures (i.e., early in the epileptogenic process), the preserved and augmented inhibition onto principal cells may lead to reduced HCN1 expression. In contrast, in chronic epileptic HS hippocampus studied here, the profound loss of interneuronal and principal cell populations and consequent reduced inhibition, coupled with increased dendritic excitation of surviving GCs, might provoke a “compensatory” enhancement of HCN1 mRNA and protein expression.

COMMENTARY

Considerable debate in the literature has surrounded the possible relation between early febrile seizures, mesial temporal sclerosis, and temporal lobe epilepsy (1–3). Similarly, it has been unclear how brain insults during childhood and subsequent neuronal loss associated with epileptogenesis lead to electrophysiologic alterations that increase the propensity for seizures. Studies in immature animals subjected to febrile seizures have suggested that alterations in hyperpolarization-activated cation channels (HCNs), leading to changes in hyperpolarization-activated current (I_h), cause hyperexcitability (4–6). Data from the present study by Bender and colleagues, with tissue both from human surgical material and pilocarpine-treated rats, indicate that the extensive loss of neurons characteristic of severe mesial temporal sclerosis is associated with alterations in HCN1 channels. This finding is interesting, but how these data relate to possible mechanisms of epileptogenesis will require further electrophysiological studies.

As reviewed by Chen et al. (7), I_h contributes to pacemaker activity in the heart and brain. I_h has slow activation kinetics during hyperpolarization, is permeable to both Na^+ and K^+ ions, and is modulated by the binding of intracellular cyclic adenosine monophosphate (cAMP). The current has many

effects on the electrical properties of neurons in the hippocampal slice. For example, it influences resting membrane potential, the shape of excitatory postsynaptic potentials (8), and the propensity to generate rhythmic activity. The four genes that encode the HCN channels (i.e., HCN1-4) belong to the voltage-gated K^+ channel superfamily and have distinct overlapping patterns of mRNA expression, with significant expression of HCN1 and HCN2 in hippocampus and neocortex. The coassembly of HCN1 and HCN2 subunits generates heteromeric channels that have different properties from homomers expressed in heterologous cells, suggesting that there will be a diversity of I_h types in neurons and that channel properties will change when subunit expression is altered in cortical structures undergoing epileptogenesis.

The data provided in this report are based on *in situ* hybridization and immunohistochemical studies, and thus the results relate primarily to demonstrating which cells have increased expression of HCN1 mRNA and protein. Future research must focus on understanding the direct relation between the HCN channels in individual neurons and the electrophysiologic properties of those neurons, by using tissue that had undergone chronic epileptogenesis. Several factors complicate the analysis of this issue. For example, the coassembly of HCN1 and HCN2 subunits importantly affects the properties of I_h , as do resting levels of cAMP (7). Experiments conducted on I_h , particularly in the dendrites of hippocampal pyramidal cells, suggest that these ion channels may have complex effects on neural integration (8,9). Other studies provide evidence that pharmacologic upregulation of h-channels with the antiepileptic drug lamotrigine (LTG) reduces dendritic excitability of pyramidal cell dendrites (10). Because I_h is distributed in a nonuniform manner along the neuron and has a much higher density in the distal dendrites than in the soma, the LTG-induced enhancement of I_h reduces input resistance, length constant, and temporal summation. Thus LTG suppresses the effect of dendritic excitatory synaptic inputs and causes an overall reduction in excitability (10), which implies that an epileptogenesis-associated increase in I_h leads to reduction in excitability rather than hyperexcitability. Finally, recent experiments suggest that *downregulation* of I_h is associated with hyperexcitability shortly after kainate-induced status epilepticus (11). Thus gaining an understanding of how alterations in HCN channels and concomitant changes in I_h influence seizure susceptibility may not be a straightforward undertaking.

These results also lead to hypotheses concerning alterations in GABA_A-mediated inhibition and modifications of HCN1 channels and I_h . Previous studies on animal models

of febrile seizures have suggested a link between increased inhibitory postsynaptic potentials, I_h , and hyperexcitability (12). Because GABA_A-receptor-mediated synaptic potentials are typically hyperpolarizing in adults, the transient hyperpolarizations might activate I_h and thus secondarily change the excitability of hippocampal dendrites. In what ways these electrophysiologic mechanisms may interact, however, also will require further analysis. As the authors point out, it is unclear how the hypothetical alterations in the levels of HCN channels are involved in temporal lobe epilepsy, and in particular, whether they are compensatory or epileptogenic, remains to be determined.

by F. Edward Dudek, Ph.D.

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