

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

behavior functions in the heart and brain. In pacemaking sinoatrial cardiomyocytes, HCN channel openings drive the membrane depolarization at the beginning of each heartbeat. Thalamic neurons that fire spontaneously use these channels to create the depolarization at the foot of action potentials. In both settings, action potential peaks mediated by sodium and calcium channels lead to potassium channel activation and membrane hyperpolarization. Such hyperpolarization then opens HCN channels again, and the cycle resumes.

Antibodies against HCN channels strongly stain the distal dendrites of hippocampal and neocortical pyramidal cells (2). This finding initially seems surprising, both because these locations are distant from the sites where action potentials begin and because the neurons are not spontaneously active. Physiological studies have revealed that HCN channels help these neurons balance the relative strength of excitatory inputs received at distal and proximal dendritic locations. To contribute to action potential initiation, a dendritic EPSP must travel from its site of origin toward the soma and axon, where action potentials arise. Just as a wave moving across water widens and diminishes in height over distance, EPSP signals can degrade with distance along the neuronal membrane. Without remedies, such broadening would increase the tendency of EPSPs arising almost simultaneously from distal locations in the dendritic arbor to overlap in time or summate. This effect is less of a problem when inputs are received proximally, since such inputs have less opportunity to broaden before arriving at the soma and axon. Placing HCN channels in a gradient along the length of the dendrite, with more channels distally and fewer proximally, ingeniously corrects this problem and makes the transmission of synaptic inputs toward the soma more distance independent. How? In the distal dendrite, the depolarization resulting from an excitatory input causes neighboring high densities of HCN channels to close, after a slight delay. HCN channel closure hastens the rate at which the membrane returns to the resting potential after the EPSP peak. Therefore, in pyramidal cells with large dendrites extending over hundreds of micrometers, an EPSP arising distally is more brief and sharp in its profile at its site of origin than one arising at a proximal site (i.e., where HCN channels are more sparse). By beginning its journey to the soma with this shortened time course, distal dendritic inputs are able to arrive at final sites of action potential initiation without becoming excessively broad, thereby preventing unwanted summation of excitatory inputs.

Given these important roles in oscillatory behavior, spontaneous firing, and dendritic signaling, HCN channels have been studied in animal models of epilepsy. Knockout of one HCN subunit (HCN2) in mutant mice was shown to lead to absence epilepsy; in addition, altered HCN currents have been implicated in hyperexcitability associated with febrile seizures (3,4). Previously, Shah et al. showed that kainic acid-induced SE in

adult rats resulted in an early loss of HCN currents in the dendrites of entorhinal cortical pyramidal cell dendrites. This loss of HCN currents caused increased neuronal firing responses to dendritic inputs and contributed to increased cellular excitability by several other measures (5). Because kainate-induced SE is potentially epileptogenic, Shah and colleagues' findings opened up the possibility that early loss of HCN channel activity might contribute to the development and/or expression of spontaneous seizures in this widely used model.

Jung et al. combined careful video-electroencephalographic monitoring after pilocarpine-induced SE with patch-clamp recording of HCN channels in hippocampal CA1 neurons. Like Shah et al., they found that dendritic HCN current loss begins early, when epileptogenesis is beginning. They also showed that HCN channel loss persists and is significantly more severe by 3 to 5 weeks after SE, when epileptogenesis is well established and the majority of animals exhibit spontaneous seizures. They further demonstrated that after SE, HCN currents are both reduced in size and altered in their voltage-dependence, so that the channels require greater hyperpolarization to be activated compared with those from control animals. Animals that experienced pilocarpine-induced SE, but were subsequently treated with phenobarbital to prevent seizures, exhibited reduced HCN current density equal to that of animals not given phenobarbital. However, phenobarbital prevented the shift in HCN voltage dependence, suggesting that this second effect may be due to seizures, *per se*. The data presented are quite convincing and significantly extend the findings of Shah et al., both by demonstrating HCN current reduction in a second model of SE-induced epileptogenesis and clarifying that this reduction persists throughout the period when epilepsy becomes established.

Although strengths of this article include careful characterization of both HCN currents and associated seizures, the links between HCN channel loss and cellular and network hyperexcitability remain relatively unexplored. The effects of HCN current loss on dendritic EPSP shape and dendritic responsiveness are topics systematically investigated by Shah et al., but not yet addressed by Jung et al. As Jung et al. themselves conclude, the relative importance of pyramidal cell HCN channel plasticity for epileptogenesis remains uncertain. It is likely that additional experiments will allow this issue to be further clarified. For example, it would be interesting to use inducible CA1 expression of an HCN transgene to restore HCN activity after endogenous channels are suppressed by pilocarpine-induced SE. It is not known whether the HCN current reductions noted in CA1 dendrites will be found in all cell types contributing to epileptic networks. Indeed, elevated levels of HCN mRNA and protein have been observed in dentate granule cells from both epileptic rodent and human brains (6). Novel approaches will be required before the mechanisms underlying

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.

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