

## HYPERPOLARIZED VIEWS ON THE ROLES OF THE HYPERPOLARIZATION-ACTIVATED CHANNELS IN NEURONAL EXCITABILITY

**Upregulated H-Current in Hyperexcitable CA1 Dendrites after Febrile Seizures.** Dyhrfeld-Johnsen J, Morgan RJ, Csaba Foldy, Soltesz I., *Front Cell Neurosci.* 2008;2:2. doi:10.3389/neuro.03.002.2008. Somatic recordings from CA1 pyramidal cells indicated a persistent upregulation of the h-current ( $I_h$ ) after experimental febrile seizures. Here, we examined febrile seizure-induced long-term changes in  $I_h$  and neuronal excitability in CA1 dendrites. Cell-attached recordings showed that dendritic  $I_h$  was significantly upregulated, with a depolarized half-activation potential and increased maximal current. Although enhanced  $I_h$  is typically thought to be associated with decreased dendritic excitability, whole-cell dendritic recordings revealed a robust increase in action potential firing after febrile seizures. We turned to computational simulations to understand how the experimentally observed changes in  $I_h$  influence dendritic excitability. Unexpectedly, the simulations, performed in three previously published CA1 pyramidal cell models, showed that the experimentally observed increases in  $I_h$  resulted in a general enhancement of dendritic excitability, primarily due to the increased  $I_h$ -induced depolarization of the resting membrane potential overcoming the excitability-depressing effects of decreased dendritic input resistance. Taken together, these experimental and modeling results reveal that, contrary to the exclusively anti-convulsive role often attributed to increased  $I_h$  in epilepsy, the enhanced  $I_h$  can co-exist with, and possibly even contribute to, persistent dendritic hyperexcitability following febrile seizures in the developing hippocampus.

**HCN Hyperpolarization-Activated Cation Channels Inhibit EPSPs by Interactions with M-type  $K^+$  Channels.** George MS, Abbott LF, Siegelbaum SA. *Nat Neurosci* 2009;12(5):577–584. The processing of synaptic potentials by neuronal dendrites depends on both their passive cable properties and active voltage-gated channels, which can generate complex effects as a result of their nonlinear properties. We characterized the actions of HCN (hyperpolarization-activated cyclic nucleotide-gated cation) channels on dendritic processing of subthreshold excitatory postsynaptic potentials (EPSPs) in mouse CA1 hippocampal neurons. The HCN channels generated an excitatory inward current ( $I_h$ ) that exerted a direct depolarizing effect on the peak voltage of weak EPSPs, but produced a paradoxical hyperpolarizing effect on the peak voltage of stronger, but still subthreshold, EPSPs. Using a combined modeling and experimental approach, we found that the inhibitory action of  $I_h$  was caused by its interaction with the delayed-rectifier M-type  $K^+$  current. In this manner,  $I_h$  can enhance spike firing in response to an EPSP when spike threshold is low and can inhibit firing when spike threshold is high.

### COMMENTARY

Regulation of neuronal excitability involves the coordinated function of numerous ion channels. Many ion channels coexist in an individual neuron, and their orchestrated function, influenced by their abundance, subcellular locations, and cellular molecules, modulates channel properties that govern neuronal excitability (1–3). The role of the hyperpolarization-activated cyclic nucleotide-gated (HCN) channels in regulating neuronal excitability has long been a subject of debate (3). These unusual channels open in response to membrane hyperpolarization and mediate the noninactivating cationic, depolarizing current  $I_h$ . The depolarizing current conducted by HCN channels drives the membrane potential closer to the action-potential firing threshold; thus, it follows intuitively that these channels may serve an *excitatory* role. However, by being open at subthreshold membrane potentials, HCN channels also reduce the input resistance of the membrane ( $R_{in}$ ), which results in a shunt-

ing *inhibitory* effect that diminishes the efficiency of incoming EPSPs.

Both upregulation and downregulation of  $I_h$  and of HCN channel expression have been reported in several animal models of epilepsy (4–7) as well as in human epileptic hippocampus (8). However, the complex, apparently contrasting, effects of  $I_h$  have impeded further understanding of the physiological consequences of  $I_h$  regulation. Two recent studies have addressed directly these issues by studying the contribution of the opposing effects of  $I_h$  on dendritic and neuronal excitability. Whereas several previous studies associated *down*regulation of  $I_h$  with increased excitability (6,7), Dyhrfeld-Johnsen et al. asked whether *up*regulation of dendritic  $I_h$ , which they found after experimental prolonged febrile seizures, could coincide with or even account for hyperexcitability. Importantly, the experimental setup used by Dyhrfeld-Johnsen et al. allowed for free fluctuations in resting membrane potential, permitting the study of the depolarizing effect of  $I_h$  along with its shunting properties.

The experiments by Dyhrfeld-Johnsen et al. revealed a complex picture: using dendritic recording techniques in hippocampal CA1 pyramidal cells, the investigators observed an upregulated  $I_h$  in dendrites of rats that had experienced

febrile seizures approximately 4 weeks earlier. In comparison to control animals, this effect was associated with a depolarized membrane potential that was a result of the increased  $I_h$ , because it could be abolished by pharmacologically blocking the conductance. Injections of current to these neurons resulted in an increased firing rate, compared with control neurons, demonstrating the hyperexcitability of the neurons with augmented dendritic  $I_h$ . When the depolarizing effects of the augmented  $I_h$  on membrane potential were blocked (i.e., membrane potential was kept constant), the hyperexcitability was only partially reversed, indicating the involvement of additional, as yet unknown, factors in the modification of neuronal excitability following febrile seizures.

Thus, the results of Dyhrfeld-Johnsen et al. demonstrated that hippocampal, pyramidal CA1 neurons are hyperexcitable following febrile seizures and that this hyperexcitability was, at least in part, a result of a depolarized membrane potential that was because of upregulated  $I_h$ . To isolate the potential role of increased  $I_h$  in febrile seizure-induced hyperexcitability from other influences, the authors then turned to computational models in which the physiological consequences of  $I_h$  can be studied independently of uncontrolled changes that may occur in vivo. They applied their empirically measured  $I_h$  values obtained following febrile seizures to three models based on published work, yet allowed membrane potential to fluctuate rather than fixing it at a given value. Under these conditions, the investigators found that the depolarizing effect of  $I_h$  outweighed its shunting effect in each of the models; in other words, the overall effect of  $I_h$  was excitatory.

The study by Dyhrfeld-Johnsen et al. provides an elegant demonstration of how (under certain conditions)  $I_h$  may play a proexcitatory role and implies that additional channels/conductances may contribute to the hyperexcitability observed in the febrile seizure model. Their findings raise several questions: in which conditions or contexts does  $I_h$  play an excitatory role? Can the balance between the inhibitory and excitatory effects of  $I_h$  be dynamically regulated? These questions form the basis for the work by George et al. First, consistent with previous studies, George et al. found that selective pharmacological blocking of  $I_h$  resulted in the expected hyperpolarized membrane potential in CA1 pyramidal neurons and increased input resistance. A novel and interesting finding involved the biphasic effects of  $I_h$ : recordings of somatic responses to synaptic stimulation, in the presence or absence of  $I_h$ , revealed a relationship between the strength of the synaptic stimulus and the function of  $I_h$ . While the current had a proexcitatory influence on weak synaptic stimuli, it had an inhibitory effect when stronger, yet still subthreshold, stimuli were applied, as measured by reduced peak EPSP. The biphasic relationship between stimulus strength and  $I_h$  could not be reproduced in a simple computational model in which  $I_h$  was the only active conduc-

tance, because in such a model,  $I_h$  always exerted an excitatory effect on subthreshold EPSPs (i.e., the depolarizing effect of  $I_h$  was greater than its shunting properties), indicating that the parameters included in the model were not sufficient to represent the real life neuron. The discrepancy between the experimental observations and the computational prediction was resolved when the authors introduced a new player to their computational model—the subthreshold, slowly activating potassium conductance, known as the M-current ( $I_M$ ). Not only did the presence of  $I_M$  restore the biphasic relationship between stimulus strength and the effect of  $I_h$  on excitability, but changes in  $I_M$  levels could also shift the crossover point at which  $I_h$  turned from excitatory to inhibitory. Thus, the computational data predicted that increased  $I_M$  would promote the inhibitory effects of  $I_h$  on somatic EPSPs, whereas low levels of  $I_M$  would result in a more excitatory  $I_h$ . These predictions were tested by measuring the effect of  $I_h$  on somatic EPSPs in pyramidal CA1 neurons while pharmacologically blocking  $I_M$ . Indeed, in the absence of  $I_M$ ,  $I_h$  had a pure excitatory effect on both weak and strong stimuli.

Both studies reviewed here challenge the traditional notion of a single role for dendritic  $I_h$  in regulating neuronal excitability. They suggest the alternative concept that  $I_h$  may play either a pro- or anti-excitatory role, depending on physiological conditions, such as the regulation of other active currents and the nature of the neuronal input to the cell (3,9). While these studies provide experimental support for an important new perspective on  $I_h$ , they also point out a number of unexplored questions. For example, at the cellular level, HCN channels are regulated in numerous ways that influence not only the magnitude of  $I_h$ , but also its kinetics, voltage dependence, additional biophysical properties, and location within the neuron. Any of these factors can affect the function of HCN channels. Thus, existing studies have found exquisite transcriptional control of HCN channels (5–7), their heteromerization (10), and their interaction with accessory proteins that influence channel surface expression, subcellular localization, and channel properties (11–13). The elucidation of these different aspects of HCN channel regulation, especially in relationship to the coregulation of other ion channels and the physiological context, will further advance the understanding of the function this important class of ion channels. This information will help investigators and clinicians to better understand the pathologies associated with HCN channel dysregulation in the epileptic brain and ultimately will provide targets in the search for better therapies.

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