6. Silva AP, Lourenço J, Xapelli S, Ferreira R, Kristiansen H, Woldbye DPD, Oliveira CR, Malva JO. Protein kinase C activity blocks neuropeptide Y-mediated inhibition of glutamate release and contributes to excitability of the hippocampus in status epilepticus. *FASEB J* 2007;21:671–681.
7. Hsu KS, Ho WC, Huang CC, Tsai JJ. Transient removal of extracellular $Mg^{2+}$ elicits persistent suppression of LTP at hippocampal CA1 synapses via PKC activation. *J Neurophysiol* 2000;84:1279–1288.
8. Birnbaum SG, Yuan PX, Wang M, Vijayraghavan S, Bloom AK, Davis DJ, Gobeske KT, Sweatt JD, Manji HK, Arnsten AFT. Protein kinase C overactivity impairs prefrontal cortical regulation of working memory. *Science* 2004;306:882–884.
9. Toth M. The epsilon theory: a novel synthesis of the underlying molecular and electrophysiological mechanisms of primary generalized epilepsy and the possible mechanism of action of valproate. *Med Hypotheses* 2005;64:267–272.

# 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 nonselective, 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 messenger, 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

Epilepsy Currents, Vol. 8, No. 4 (July/August) 2008 pp. 103–105
Wiley Periodicals, Inc.

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 potently 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.

*by Edward C. Cooper, MD, PhD*

## References


1. Robinson RB, Siegelbaum SA. Hyperpolarization-activated cation currents: from molecules to physiological function. *Annu Rev Physiol* 2003;65:453–480.
2. Santoro B, Grant SG, Bartsch D, Kandel R. Interactive cloning with the SH3 domain of N-src identifies a new brain specific ion channel protein, with homology to eag and cyclic nucleotide-gated channels. *Proc Natl Acad Sci USA* 1997;94:14815–14820.
3. Herrmann S, Stieber J, Ludwig A. Pathophysiology of HCN channels. *Pflugers Arch* 2007;454:517–522.
4. Chen K, Aradi I, Thon N, Eghbal-Ahmadi M, Baram TZ, Soltesz I. Persistently modified h-channels after complex febrile seizures convert the seizure-induced enhancement of inhibition to hyperexcitability. *Nat Med* 2001;7:331–337.
5. Shah MM, Anderson AE, Leung V, Lin X, Johnston D. Seizure-induced plasticity of h channels in entorhinal cortical layer III pyramidal neurons. *Neuron* 2004;44:495–508.
6. Bender RA, Soleymani SV, Brewster AL, Nguyen ST, Beck H, Mathern GW, Baram TZ. 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. *J Neurosci* 2003;23:6826–6836.


# Not All Sweetness and Light: The Role of Glycogen in Hypoglycemic Seizures


**Factors Which Abolish Hypoglycemic Seizures Do Not Increase Cerebral Glycogen Content *In Vitro*.** Abdelmalik PA, Liang P, Weisspapir M, Samoilova M, Burnham WM, Carlen PL. *Neurobiol Dis* 2008;29(2):201–209. Epub 2007 Aug 29. The brain is heavily dependant on glucose for its function and survival. Hypoglycemia can have severe, irreversible consequences, including seizures, coma and death. However, the *in vivo* content of brain glycogen, the storage form of glucose, is meager and is a function of both neuronal activity and glucose concentration. In the intact *in vitro* hippocampus isolated from mice aged postnatal days 8–13, we have recently characterized a novel model of hypoglycemic seizures, wherein seizures were abolished by various neuroprotective strategies. We had hypothesized that these strategies might act, in part, by increasing cerebral glycogen content. In the present experiments, it was found that neither decreasing temperature nor increasing glucose concentrations (above 2 mM) significantly increased hippocampal glycogen content. Preparations of isolated frontal neocortex *in vitro* do not produce hypoglycemic seizures yet it was found they contained significantly lower glycogen content as compared to the isolated intact hippocampus. Further, the application of either TTX, or a cocktail containing APV, CNQX and gabazine, to block synaptic activity, did not increase, but paradoxically decreased, hippocampal glycogen content in the isolated intact hippocampus. Significant decreases in glycogen were noted when neuronal activity was increased via incubation with l-aspartate (500 $\mu$M) or low $Mg^{2+}$. Lastly, we examined the incidence of hypoglycemic seizures in hippocampi isolated from mice aged 15–19 and 22–24 days, and compared it to the incidence of hypoglycemic seizures of hippocampi isolated from mice aged 8–13 days described previously (Abdelmalik et al., 2007 *Neurobiol Dis* 26(3):646–660). It was noted that hypoglycemic seizures were generated less frequently, and had less impact on synaptic transmission in hippocmpi from PD 22–24 as compared to hippocampi from mice PD 15–19 or PD 8–13. However, hippocampi from 8- to 13-day-old mice had significantly more glycogen than the other two age groups. The present data suggest that none of the interventions which abolish hypoglycemic seizures increases glycogen content, and that low glycogen content, *per se*, may not predispose to the generation of hypoglycemic seizures.


## COMMENTARY

Matthew Arnold's 1869 essay, "Culture and Anarchy," posits that every culture strives for the ideals of sweetness (beauty) and light (truth, clarity). When applied to the role of glucose and glycogen in cerebral energy regulation, the sweetness is obvious but the light still eludes our grasp.

Seizures that are due to hypoglycemia can lead to permanent neuronal damage and even death. In the clinical setting, hypoglycemic seizures occur most commonly in individuals with poorly regulated type 1 diabetes, especially at times of altered insulin availability or function. Hypoglycemic seizures also occur frequently in infants of diabetic mothers and in newborns

Epilepsy Currents, Vol. 8, No. 4 (July/August) 2008 pp. 105–107
Wiley Periodicals, Inc.