

H-CHANNEL DYSFUNCTION IN GENERALIZED EPILEPSY: IT TAKES TWO

Impaired Regulation of Thalamic Pacemaker Channels Through an Imbalance of Subunit Expression in Absence Epilepsy

Budde T, Caputi L, Kanyshkova T, Staak R, Abrahamczik C, Munsch T, Pape HC

J Neurosci 2005;25(43):9871–9882

The role of hyperpolarization-activated, cyclic nucleotide-modulated (HCN) channel isoforms and hyperpolarization-activated cation current (I_h) for seizure-related burst firing in thalamocortical (TC) neurons was investigated in a rat genetic model of absence epilepsy [Wistar Albino Glaxo rats, bred in Rijswijk (WAG/Rij)]. Burst discharges in TC neurons locked to seizure activity *in vivo* were prolonged during blockade of I_h by Cs^+ and ZD7288 (4-ethylphenylamino-1,2-dimethyl-6-methylaminopyrimidinium chloride). *In vitro* analyses revealed a hyperpolarizing shift of half-maximal I_h activation (V_h) in WAG/Rij ($V_h = -93.2$ mV) compared with nonepileptic controls [August \times Copenhagen-Irish (ACI) ($V_h = -88.0$ mV)]. This effect is explained by a shift of the responsiveness of I_h to cAMP toward higher concentrations in TC neurons from WAG/Rij, as revealed by application of 8-

bromo-cAMP and the phosphodiesterase inhibitor IBMX. During blockade of adenylyl cyclase activity, I_h activation was similar in the two strains, whereas the difference in cAMP responsiveness persisted, thereby voting against different ambient cAMP levels between strains. Increasing the intracellular cAMP level and shifting I_h activation led to a change from burst to tonic firing mode in WAG/Rij but not in ACI rats. Furthermore, HCN1 expression was significantly increased on mRNA and protein levels, with no changes in HCN2–4 expression. In conclusion, there is an increase in HCN1 expression in the epileptic thalamus, associated with a decrease in cAMP responsiveness of I_h in TC neurons and resulting impairment to control the shift from burst to tonic firing, which, in turn, will prolong burst activity after recruitment of I_h during absence seizures.

COMMENTARY

The idea that defects in the function of hyperpolarization-activated cyclic nucleotide-gated cation channels (HCN or h-channels) may contribute to epilepsy continues to gain support (1). A genetic “h-channelopathy” has not yet been identified in humans as has been for other voltage-gated ion channels, such as KCNQ potassium channels or the SCN1A sodium channel (2). However, recent studies of an animal model of inherited generalized epilepsy have implicated h-channel dysfunction in the origin of epileptic discharges. However, the nature of this h-channel impairment was not what might have been predicted; instead, recent evidence has added another layer of complexity to the current understanding of the role of h-channels and thalamocortical function.

H-channels comprise four families of gene products, HCN1–4. Each HCN subtype has in common the properties of activation by hyperpolarization, passage of depolarizing, inward current (I_h), and fairly slow activation. The two most common subtypes in the brain, HCN1 and HCN2, possess distinctly different activation kinetics and second messenger regulation, thus performing quite different functions in the brain regions where they predominate. HCN1, the main isoform in neocortex and hippocampus, activates relatively rapidly (activation time constant is in the tens of milliseconds), has a comparatively depolarized activation voltage, and is insensitive to modulation by cAMP. The distribution of HCN1 to pyramidal neuron dendrites allows it to dampen excitatory synaptic inputs in these cells. Loss of HCN1 activity after provoked seizures and upregulation of HCN1 activity by certain antiepileptic drugs suggest that this h-channel isoform acts to limit epileptic discharges in neocortex and hippocampus and, thus, may play a role in focal epilepsies, such as temporal lobe epilepsy (3,4).

HCN2, in contrast, is localized predominantly to the thalamus, where its slow activation (in hundreds of milliseconds) enables it to shape thalamocortical oscillations. Previous work has demonstrated that HCN2-mediated current acts to limit burst discharges in thalamocortical neurons. Unlike HCN1, HCN2 is regulated by cAMP, which provides a mechanism to link burst firing and I_h ; Ca^{2+} influx during thalamocortical bursting stimulates cAMP production through adenylate cyclase, which in turn upregulates HCN2 gating (5). Given the role of thalamocortical bursting in generalized epilepsies, such as absence epilepsy, it would be expected that downregulation of HCN2 function might lead to generalized epileptic discharges. This prediction was confirmed by the characterization of a mouse HCN2 knockout that, in fact, displayed generalized 5 Hz spike-wave discharges and behavioral arrest, consistent with generalized epilepsy (6).

The differing biophysical properties and anatomical localization of HCN subtypes might lead to the simple correlations of HCN1 with focal epilepsy and HCN2 with generalized epilepsy. Inbred animal models of generalized epilepsy provide an opportunity to test the validity of these assignments. The Genetic Absence Epilepsy Rats from Strasbourg (GAERS) and Wistar Albino Glaxo/Rijswijk (WAG/Rij) rats both display generalized spike-wave discharges and behavioral arrest that mimic many of the clinical features of human absence epilepsy. As in human absence, no single genetic locus has been identified as underlying the epileptic phenotype of either rodent model. The hypothesis that naturally emerges with respect to h-channels is that some dysfunction of HCN2 in the inbred rat models mirrors the correlation of generalized spike-wave discharge and loss of HCN2 in the knockout mouse.

The first study to investigate this hypothesis surprisingly found a loss of neocortical HCN1 function in WAG/Rij animals, manifested by a significant decrease in total I_h and loss of HCN1 protein expression (7). No loss of HCN2 expression was seen. A preliminary report substantiated this finding at a subcellular level, with a reduction of I_h in the dendrites of neocortical pyramidal neurons (8). These results suggested that like HCN2, HCN1 may act to limit generalized epileptic discharges—by influencing neocortical rather than thalamic excitability, however. This hypothesis is consistent with reports that in the WAG/Rij rat, spike-wave discharges initiate in the neocortex and secondarily recruit synchronized thalamocortical firing (9).

The present study extends this line of investigation to ask whether h-channel dysfunction is also present in the thalamocortical neurons of epileptic WAG/Rij rats. The authors' findings were again unexpected. There was no loss of HCN2 protein expression, nor any decrease in overall I_h . HCN1 expression, however, was significantly elevated. This was accompanied by a 7 mV hyperpolarizing shift in I_h activation and reduced sensi-

tivity to cAMP. These latter two changes would be expected to reduce h-channel activity and its upregulation during thalamocortical burst firing. Since HCN2 remained the predominant thalamic isoform in WAG/Rij rats, one must assume that alterations in HCN2 behavior underlie the thalamocortical I_h change seen. But what is the molecular basis of the change, and does HCN1 upregulation have anything to do with it?

The answers to both questions are unclear. No information is available regarding whether mutations in the *HCN2* gene exist that might affect HCN2 gating in WAG/Rij compared with nonepileptic Wistar strains. Of note, Strauss et al. claimed to find no mutations in the coding region of HCN1 of WAG/Rij animals (7). The present study carefully controlled for the possibility that changes in ambient cAMP levels might account for the difference in HCN2 gating. Aside from cAMP, relatively little is known about modulatory influences on I_h , such as the role of accessory proteins or protein kinase regulation.

These findings leave open the possibility that there is an abnormal heteromerization of HCN subunits. It is widely assumed that HCN subunits of different isoforms may freely associate to form heteromeric tetramers that underlie the functional ion channel. It is possible that ion channels with differing biophysical properties might result, depending on the ratios of HCN subunits. Furthermore, it is attractive to hypothesize that where dysfunctional I_h is found—say in epileptic phenotypes—an aberrant ratio of HCN subunits forming heteromeric h-channels may be the cause. If in a nonepileptic animal, thalamocortical neurons possessed h-channels with a 1:3 HCN1:HCN2 composition, might not a 2:2 mix produce a channel with pathological behavior? The finding of elevated HCN1 expression in the present study suggests something like this scenario.

The problem with the heteromerization hypothesis is, however, theoretically compelling, at present, it lacks evidence. Exogenous expression of recombinant HCN subunits in varying combinations produces an aggregate I_h with biophysical properties that are essentially an average of its constituent channels (10). Thus, an increasing HCN1:HCN2 ratio, as seen in WAG/Rij thalamocortical neurons, would be expected to produce overall I_h that had a decreased cAMP response (as was found) but a depolarized activation voltage (not as found). Of course, exogenous expression systems often do not replicate the behavior of ion channels in native tissues because of the lack of accessory subunits and second messenger systems, so it is unknown how heteromeric channels might function *in vivo*.

Proof that altered HCN1:HCN2 ratios produce aberrant h-channel heteromers will depend on single-channel patch clamp recordings. These recordings have been notoriously difficult to accomplish for h-channels because of their small single-channel conductance. A recent report, however, suggests that it can be done (11). Application of single-channel recordings

to h-channels under pathological conditions will help narrow down the mechanisms underlying their dysfunction. It is already known that loss of HCN2 can cause epilepsy. Does association of HCN2 with excess HCN1 produce a similarly dysfunctional mix? Or, will unanticipated defects in modulatory mechanisms be implicated? Whatever the answers to these questions, it appears that both HCN1 and HCN2 h-channel subtypes contribute to generalized epilepsy, at least in experimental animal models. And, while a recent report described an *HCN4* mutation in inherited human cardiac arrhythmia (12), evidence of a human h-channelopathy in epilepsy thus far remains elusive.

by Nicholas P. Poolos, MD, PhD

References

1. Poolos NP. The h-channel: a potential channelopathy in epilepsy? *Epilepsy Behav* 2005;7:51–56.
2. Noebels JL. The biology of epilepsy genes. *Annu Rev Neurosci* 2003;26:599–625.
3. Poolos NP, Migliore M, Johnston D. Pharmacological upregulation of h-channels reduces the excitability of pyramidal neuron dendrites. *Nat Neurosci* 2002;5:767–774.
4. Shah M, Anderson A, Leung V, Lin X, Johnston D. Seizure-induced plasticity of h channels in entorhinal cortical layer III pyramidal neurons. *Neuron* 2004;44:495–508.
5. Luthi A, McCormick DA. Modulation of a pacemaker current through Ca^{2+} -induced stimulation of cAMP production. *Nat Neurosci* 1999;2:634–641.
6. Ludwig A, Budde T, Stieber J, Moosmang S, Wahl C, Holthoff K, Langebartels A, Wotjak C, Munsch T, Zong X, Feil S, Feil R, Lancel M, Chien KR, Konnerth A, Pape HC, Biel M, Hofmann F. Absence epilepsy and sinus dysrhythmia in mice lacking the pacemaker channel HCN2. *EMBO J* 2003;22:216–224.
7. Strauss U, Kole MH, Brauer AU, Pahnke J, Bajorat R, Rolfs A, Nitsch R, Deisz RA. An impaired neocortical I_h is associated with enhanced excitability and absence epilepsy. *Eur J Neurosci* 2004;19:3048–3058.
8. Kole MHP, Brauer AU, Stuart GJ. Distance-dependent changes in dendritic I_h of layer 5 pyramidal neurons in a rat model of absence epilepsy. *Soc Neurosci Abstr* 2004;52:14.
9. Meeren HK, Pijn JP, Van Luijtelaar EL, Coenen AM, Lopes da Silva FH. Cortical focus drives widespread corticothalamic networks during spontaneous absence seizures in rats. *J Neurosci* 2002;22:1480–1495.
10. Ulens C, Tytgat J. Functional heteromerization of HCN1 and HCN2 pacemaker channels. *J Biol Chem* 2001;276:6069–6072.
11. Simeone TA, Rho JM, Baram TZ. Single channel properties of hyperpolarization-activated cation currents in acutely dissociated rat hippocampal neurones. *J Physiol* 2005;568:371–380.
12. Milanesi R, Baruscotti M, Gneschi-Ruscione T, DiFrancesco D. Familial sinus bradycardia associated with a mutation in the cardiac pacemaker channel. *N Engl J Med* 2006;354:151–157.