Review

The h-channel: A potential channelopathy in epilepsy?

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Abstract

Ion channelopathy is a proven cause of inherited human epilepsy, and may play a role in acquired epileptic syndromes as well. Of the many ion channel causes of epilepsy, the h-channel is a potential new addition. H-channels are voltage-gated ion channels with unique biophysical properties. The h-channel exerts a significant modulatory influence on neuronal excitability, and is a target of antiepileptic drugs. Further, its activity is influenced by seizures, raising the question of whether it may play a role in epileptogenesis as well. This review summarizes the evidence for the contribution of h-channels to seizures and epilepsy, and outlines hypotheses concerning the existence of an “h-channelopathy” in human epilepsy.

Keywords: H-channel; I_h; Channelopathy; Epilepsy; HCN; Epileptogenesis

1. Introduction

That ion channels are involved in the pathogenesis of epilepsy has seemed a self-evident truth: voltage- and ligand-gated channels are the molecular mediators of neuronal activity, and pharmacological blockade of some channels, especially γ-aminobutyric acid (GABA) receptors, produces seizures in animal and in vitro models. Yet until the last 10 years, there was little proof that alteration of ion channel function caused any human epilepsy syndrome. However, the discovery of inherited “channelopathies” as a basis of epilepsy has restored the focus on the ion channel as a prime mover in epileptogenesis. Of the pure human epilepsy syndromes (i.e., lacking neurodegeneration or other neurologic phenotypes) with an identified genetic cause, at present all but one are due to ion channel mutations [1,2]. Considering the voltage-gated channels in this group, K+ and Na+ channels are prominent members—this is not surprising given their central role in generation of the action potential. Yet intense interest has developed in another ion channel not yet shown to cause human epilepsy, which plays no role in action potential firing, and which was first identified in the heart: the h-channel.

Since the discovery of a significant h-channel presence in the CNS, it is now understood that the h-channel (or I_h, the current produced by h-channels) modulates excitability in the regions of the brain most involved in seizure initiation, is a target of antiepileptic drugs, and is in turn regulated by seizure activity. In this review, we survey the evidence that this unique ion channel may soon join the elite club of epilepsy channelopathies.

2. Structure and function of h-channels

H-channels are voltage-gated ion channels that structurally resemble K+ channels. In evolutionary terms, they are a relatively recent addition to the ion channel repertoire, appearing along with voltage-gated sodium channels after the phylogenetic development of flies and worms (from which humans otherwise derived their entire ion channel complement) [3]. H-channels have a number of unusual features that set their function apart from all other ion channels [4]. First, despite structural similarity...
to K\(^+\) channels, they conduct primarily Na\(^+\) current under physiological conditions, thus depolarizing neuronal membrane potential. Second, h-channels are partly gated by intracellular levels of adenosine 3\(^\prime\)-5\(^\prime\)-monophosphate (cAMP), allowing channel activity to be modulated by both voltage and intracellular second messengers. Third, they open remarkably sluggishly, several orders of magnitude more slowly than most ion channels. And finally, the most unusual feature of the h-channel is that it opens in response to membrane hyperpolarization instead of depolarization (hence the “h” in h-channel), unlike virtually all other voltage-gated ion channels.

All these features together produce a channel that is activated by membrane hyperpolarization but then causes depolarization (through passage of Na\(^+\) current) over a time course of hundreds of milliseconds. This biophysical behavior constitutes an inherent negative feedback mechanism that tends to stabilize neuronal membrane potential against any perturbations. Thus, the h-channel tends to exert an inhibitory influence on neuronal excitability: a hyperpolarizing input (such as an inhibitory postsynaptic potential (IPSP)) will turn on h-currents, depolarizing the neuron back toward rest, while a depolarizing input will turn off h-channels open at rest, removing a net depolarizing current that again returns membrane potential to rest. In some neurons, pairing of this negative feedback behavior with a transient inward current (such as the T-type Ca\(^{2+}\) channel in thalamocortical neurons) produces neuronal oscillation [5]. The modulatory effect of intracellular cAMP on h-channel gating allows fine tuning of neuronal excitability or of oscillatory frequency [6].

H-channels are encoded by four separate genes, HCN1 to HCN4 [7]. Ion channels encoded by each of the isoforms have differing biophysical properties (such as speed of gating and sensitivity to cAMP), and are also differentially distributed throughout the brain. HCN1 and HCN2 are the main brain isoforms, with HCN1 predominant in neocortex and hippocampus, and HCN2 predominant in thalamus. The biophysical differences among HCN subtypes lead them to play varying functional roles in the brain regions in which they are found. How these biophysical features of h-channels contribute to the cellular hyperexcitability of epilepsy is discussed below.

3. H-channels modulate neuronal activity

As discussed above, h-channels lend themselves to two main functions: stabilizing neuronal membrane potential against either excitatory or inhibitory inputs, and producing neuronal oscillation. The latter function was described for h-channels in the tissue of their first characterization, the sinoatrial (SA) node of the heart. In these cells, h-channels help set the frequency of firing that produces sinus rhythm, and modulation of \(I_h\) by changes in intracellular cAMP concentration contributes to the autonomic control of heart rate by \(\beta\)-adrenergic and cholinergic receptor activation [6].

A similar oscillatory function has been shown in thalamocortical projection neurons in the thalamus. These neurons underlie synchronization of cortical rhythms seen in sleep and in primarily generalized seizures such as absence [8]. H-channels play a critical role in the generation of rhythmic firing in these cells, together with T-type calcium channels [5]. An interesting feature of this interaction is that h-channels need to function in a narrow range of activity to mediate oscillation. Either downregulation or upregulation of steady-state \(I_h\) has the potential to abolish oscillation [9]. Similarly, blockade of the T-type Ca\(^{2+}\) channels will abolish the thalamocortical burst firing underlying absence seizures, a well-described mechanism of action of the antiepileptic drug (AED) ethosuximide [10]. It is reasonable to wonder whether h-channels might similarly act as a drug target in absence epilepsy, and in fact there is some evidence supporting this idea. This is discussed in more detail below.

How h-channels function in neocortical and hippocampal neurons has been intensely investigated in the last several years. In these brain regions, h-channels are found mainly in pyramidal neurons, the principal output neurons. Advances in single-cell electrophysiological recording techniques have disclosed a striking fact about h-channels in these pyramidal neurons: rather than being homogeneously distributed across the cell membrane, they are instead arrayed in gradient patterns along the somatodendritic axis, being present at low levels in the cell body, but at increasingly high density (7- to 10-fold compared with the soma) in the apical dendrites [11]. The high dendritic density of h-channels places them in proximity to excitatory inputs, the vast majority of which arrive in the dendrites. Because h-channel function tends to attenuate the effect of excitatory inputs, their location in dendrites makes them well suited to controlling the flow of excitation to the soma, where action potential firing initiates. Because h-channels activate slowly, they are most responsive to prolonged or repetitive synaptic inputs, thus influencing the temporal summation of synaptic activity [12].

4. Actions of antiepileptic drugs on h-channels

Because h-channels are ideally positioned, in terms of both subcellular localization and biophysical properties, to modulate flow of excitation in neocortex and hippocampus, one might view them as an attractive target for AED action. Indeed there is evidence of this effect. Lamotrigine (LTG), a broad-spectrum AED, has been found to upregulate the activity of h-channels by shifting the voltage dependence of channel activation [13].
upregulation has the effect of reducing the dendritic response to prolonged synaptic excitation, as would occur during seizures. This was an unexpected action of LTG, which had previously been described as reducing voltage-gated \(Na^+\) channel function, similarly to phenytoin (PHT) and carbamazepine (CBZ). Given its \(Na^+\) channel actions, it is unclear whether LTG’s h-channel effects are important in its antiepileptic action. Several lines of evidence, however, suggest that it is. First, it has been pointed out that action at \(Na^+\) channels could not explain LTG’s broad spectrum of antiepileptic action against primarily generalized seizures such as absence (which are often exacerbated by drugs like PHT and CBZ)[14]. In fact, in vitro evidence has shown that LTG can abolish thalamocortical epileptiform burst firing at therapeutic concentrations (John Huguenard, personal communication), consistent with an action on thalamic h-channels. Second, LTG’s remarkably benign side effect profile with respect to cognition, dizziness, and diplopia suggests that its mechanistic actions in the CNS might be quite different from those of PHT, CBZ, and other drugs with actions on \(Na^+\) channels. H-channels also appear to be upregulated by two AEDs, gabapentin and acetazolamide, whose antiepileptic mechanisms of action remain unclear [15,16].

5. Evidence linking h-channels and epilepsy

Aside from its possible role in mediating the actions of some AEDs, there is increasing evidence linking the h-channel with epilepsy. The first such association was established in studies by Soltesz and collaborators [17,18]. They used a novel model of provoked seizures in which immature rodent pups were exposed to hyperthermic conditions, producing a prolonged seizure. The intent of this model is to reproduce the conditions of human early childhood febrile seizures [19]. While animals undergoing hyperthermic seizures do not subsequently have spontaneous, recurrent seizures, an initial study established in studies by Soltesz and collaborators [17,18]. They used a novel model of provoked seizures in which immature rodent pups were exposed to hyperthermic conditions, producing a prolonged seizure. The intent of this model is to reproduce the conditions of human early childhood febrile seizures [19]. While animals undergoing hyperthermic seizures do not subsequently have spontaneous, recurrent seizures, an initial study demonstrated that GABAAergic inhibition was persistently heightened after the initial seizure, suggesting that even a single, prolonged early-life seizure—generally felt to be benign in human febrile seizures—could have lasting effects on neuronal excitability [18]. In a follow-on study, it was shown that \(I_h\), like GABAAergic currents, was persistently increased when measured at the soma of CA1 hippocampal pyramidal neurons. The investigators then demonstrated that under certain conditions, the combination of increased inhibitory currents and increased \(I_h\) could lead to increased action potential firing after an inhibitory postsynaptic potential, thus producing a net pro-excitatory effect. Although the experimental animals in this model have not been shown to have subsequent seizures, the implication of this result was that h-channels in hippocampal pyramidal neurons may potentially predispose to a lowering of seizure threshold.

There is molecular evidence supporting the idea of persistent changes in h-channel expression following febrile seizures, but some of it is at odds with the electrophysiologic studies. The Baram group quantified changes in HCN expression following hyperthermic seizures by examining HCN mRNA levels at varying time points postseizure [20]. In CA1 hippocampal neurons, they showed that the two main HCN isoforms, HCN1 and HCN2, had divergent responses to a prolonged seizure. The predominant HCN1 isoform showed a decrease, compared with control levels, which persisted at least 3 months; the minority HCN2 isoform showed a transient increase, which returned to normal or slightly depressed levels at the 3-month point. These results suggested that the increased \(I_h\) measured electrophysiologically at 1 week might reflect increased transcription of the HCN2 gene. However, the increased \(I_h\) at more chronic time points was not consistent with the overall loss of HCN subunits. The discrepancy between these two results might be explained by the fact that mRNA expression does not necessarily correlate with h-channel protein expression [21], and that additional posttranslational channel modifications may be at work, altering postseizure h-channels, although no such mechanisms have been elucidated to this point.

The concept that single seizures can lead to persistent changes in h-channel function has received additional support from the work by Shah et al. [22]. They used the kainate model of status epilepticus, but took the novel approach of studying h-channels in entorhinal cortical (EC) layer III pyramidal neurons. The entorhinal cortex is increasingly recognized as a vulnerable link in the limbic circuit involved in human temporal lobe epilepsy [23,24]. EC layer III neurons are particularly susceptible to damage and loss in chronic temporal lobe epilepsy, as are CA1 hippocampal pyramidal neurons. And because these cells project to the distal dendritic arbors of CA1 pyramidal neurons, hyperexcitability in EC neurons may drive similar behavior in hippocampal neurons in chronic epilepsy.

The findings of Shah et al. with respect to h-channels were more unequivocal than those in the febrile seizure model: kainate-induced status epilepticus caused an acute (24-hour poststatus) decrease in \(I_h\) in the dendrites of EC pyramidal neurons. This decrease in \(I_h\) was associated with dramatic neuronal hyperexcitability and epileptiform EEG abnormalities over the entorhinal cortex. Further, the acute electrophysiological change in \(I_h\) was accompanied by a downregulation of HCN protein expression, suggesting that functional ion channels had actually disappeared from neuronal membranes. The functional decrease in \(I_h\) persisted 1 week after status, although at that point HCN protein expression had returned to normal, suggesting that a posttranslational
change may also have been involved in the loss of h-channel function. The apparent discord between these results and those of the Soltesz group may be a function of seizure model, cell type, or electrophysiological recording location (soma vs dendrite), and merits further study.

The above studies have confirmed that seizure activity can lead to a modification of h-channel activity and expression. But what evidence is there that h-channel derangement by itself can lead to seizure activity? The most compelling data on this question have come from studies of HCN knockouts in mouse models. Ludwig et al. [25] generated a global knockout of the HCN2 gene. The phenotype of these mice correlated well with the known high density of the HCN2 molecule in the thalamus: the animals displayed spontaneous absence seizures, with generalized 5-Hz spike–wave discharges. Analysis of the firing properties of thalamic neurons (where HCN2 is predominant) showed heightened burst firing that could be suppressed by ethosuximide, a hallmark of absence seizures. Knockout mice also displayed a cardiac sinus arrhythmia, consistent with loss of HCN2 from sinoatrial node cells.

Another rodent model of absence epilepsy similarly appears to be associated with loss of HCN function. The WAG/Rij rat displays spontaneous spike–wave discharges associated with behavioral absence-like episodes [26]. These seizures have a developmental time course, with electrographic changes preceding the onset of seizures associated with behavioral arrest. Detailed analysis of seizure propagation in this model shows that seizures appear to begin from a cortical focus, then generalize via rapid intracortical spread [27]. The cortical origin of seizures has been found to correlate with loss of HCN1-mediated currents in neocortical neurons, accompanied by a loss of HCN1 protein expression [21]. Interestingly, HCN1 mRNA expression was unaffected, and protein expression of other HCN subtypes was unaffected. A separate study confirmed that the loss of HCN1-mediated currents was confined to the dendrites of neocortical neurons, and that this loss progressed during development, paralleling the clinical onset of seizures [28]. These results suggested that absence epilepsy could be associated with dysfunction of either HCN1 or HCN2 subunits. In both cases, epilepsy was associated with loss or downregulation of \( I_h \), adding weight to the concept that \( I_h \) exerts an inhibitory or antiepileptic function in the brain. Knockouts of the HCN1 subunit also exist and have shown both deficits in motor learning and (curiously) some enhancement of spatial memory [29,30]. There has been no description of behavioral seizures in these animals, but neither has there been any published studies of EEG recordings or detailed behavioral monitoring. Thus, it is unclear at present whether HCN1 knockouts have an epileptic phenotype.

Few human data exist at present linking h-channels and epilepsy. No human clinical disease has been associated to date with an HCN mutation. The one study that examined h-channel expression in human epilepsy produced intriguing results. Bender et al. [31] studied hippocampal tissue resected from patients with intractable epilepsy and measured HCN mRNA expression. In patients without evidence of hippocampal sclerosis, there was little change in HCN subunit expression compared with autopsy (nonepileptic control) patients. In patients with histologically proven hippocampal sclerosis, HCN signal was generally lower throughout the hippocampus, probably consistent with the degree of overall cell loss. In a subset of these patients with the most severe cell loss, there was a surprising upregulation of HCN1 mRNA which was confined to the dentate gyrus cells. It was suggested that this upregulation of HCN1 mRNA might be a “compensatory” response to ongoing seizure activity, that is, an attempt to attenuate excitation of the first link in the trisynaptic hippocampal circuit.

6. Areas of future investigation

The work described above makes it clear that altered h-channel function is associated with seizures and epilepsy. Especially for some animal models of primary generalized epilepsy, loss of HCN function appears to cause an epilepsy phenotype. This potentially places h-channels in the company of other inherited channelopathies, should a human genetic locus be described. Of possibly greater relevance to human epilepsy is the question of whether loss of h-channel function also occurs in localization-related syndromes, and whether an acquired or developmental “h-channelopathy” might underlie common focal seizure syndromes such as temporal lobe epilepsy. This is an area of intense investigation for several species of ion channels likely to be involved in epilepsy. For example, in the pilocarpine seizure model, chronic epilepsy is associated with an acquired loss of A-type K+ channels, producing hyperexcitability of hippocampal neurons [32].

The rationale for how an acquired alteration in ion channel function might contribute to epileptogenesis is illustrated in Fig. 1. The underlying mechanism is a cycle that basically recapitulates the logic of “seizures beget seizures”: an initial provoking insult, such as hyperthermia or a head injury, provokes a seizure in an otherwise normal individual. In animal models, the provoking insult may consist of chemically (with pilocarpine or kainate) or electrically (kindling) induced seizures, among others. Following the initial seizure, an alteration in ion channel function occurs (“seizure-induced channelopathy”). In this illustration, \( I_h \) is shown as the altered ion channel, but this could easily be any of a number
of ion channel species. This acquired channelopathy then produces an increase in the intrinsic excitability of individual neurons. In the last phase of the cycle, this altered excitability leads ultimately to network hyperexcitability, resulting in recurrent seizures. This last phase is the most difficult to understand, for while the first two phases proceed on a time scale of minutes to hours, the transition from acute seizure to chronic epilepsy generally requires days to weeks (in animal models) to months or even years (in humans). This phenomenon constitutes the “latent period” and is observed in virtually all forms of acquired epilepsy, both human and animal models, where an initial insult is clearly identifiable [33,34].

The evidence cited in this review establishes links between seizures and h-channel function for the first two steps of the cycle: it is clear that provoked seizures lead to altered h-channel function, predominantly a down-regulation of $I_h$, and the preponderance of evidence also shows that loss of h-channel activity produces intrinsic neuronal hyperexcitability. What is unclear, at least to date, is whether that acquired loss of $I_h$ leads to the establishment of epilepsy. Fortunately, the phenomenology of the latent period can help in this question. If h-channels, or any other ion channel, were to play a causative role in epileptogenesis, we would expect that alteration to be present immediately following the initial insult, to persist during chronic epilepsy, and to be independent of the recurrent seizures themselves (i.e., when treated with antiepileptic drugs). Further, we would expect that if h-channels or another channel were essential to epileptogenesis, then pharmacologically intervening to restore ion channel function during the latent period would prevent the progression to chronic epilepsy. These hypotheses are clearly testable in animal models, and we can expect some answers to these questions in the foreseeable future, potentially adding “h-channelopathy” to the roster of ion channels whose dysfunction leads to human epilepsy. And because h-channels are proven pharmacologic targets, the means for intervening against h-channelopathy may already be close at hand.

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References


