

HYPERPOLARIZATION-ACTIVATED CATION CURRENTS: From Molecules to Physiological Function

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■ **Abstract** Hyperpolarization-activated cation currents, termed I_f , I_h , or I_Q , were initially discovered in heart and nerve cells over 20 years ago. These currents contribute to a wide range of physiological functions, including cardiac and neuronal pacemaker activity, the setting of resting potentials, input conductance and length constants, and dendritic integration. The hyperpolarization-activated, cation nonselective (HCN) gene family encodes the channels that underlie I_h . Here we review the relation between the biophysical properties of recombinant HCN channels and the pattern of HCN mRNA expression with the properties of native I_h in neurons and cardiac muscle. Moreover, we consider selected examples of the expanding physiological functions of I_h with a view toward understanding how the properties of HCN channels contribute to these diverse functional roles.

INTRODUCTION

Hyperpolarization-activated cation currents (I_h) have puzzled physiologists since their initial discovery over 25 years ago (1). Unlike most voltage-gated channels, I_h channels are activated by hyperpolarizing voltage steps to potentials negative to -60 mV, near the resting potentials of most cells. This property earned them the designation of I_f for “funny” (2) or I_Q for “queer” (3). Although these channels clearly contribute to determining the resting potential and resting membrane properties of cells in which they are expressed, their role in dynamic signaling events is less well agreed upon. I_h is perhaps most widely known for its proposed role in the generation of spontaneous pacemaker activity, both in the heart (4) and central nervous system (5). As a result, I_h is often referred to as the pacemaker current. However, it is clear that the spontaneous firing of certain cells, such as

Purkinje neurons in the cerebellum (6) and respiratory neurons in the brainstem (7), do not require the participation of I_h to generate automaticity. Even in the heart, the importance of I_h as the prime generator of depolarizing pacemaker current has been questioned (8–10). Why has it been so hard to define a function for these channels? What roles do they play in the many non-pacing cells in which they are localized?

The recent identification of a family of four mammalian genes that encode the hyperpolarization-activated, cation nonselective channels, HCN1–4, now presents the opportunity of addressing the physiological role of I_h at the molecular level. In this review we focus on the molecular bases for the physiological function of I_h , some surprising new roles this current plays in cellular activity, as well as some unresolved controversies. Several excellent recent reviews have focused on the structure-function relation of these channels (11–15), which therefore is not covered in depth herein.

HISTORICAL PERSPECTIVE AND BASIC PROPERTIES OF I_h

Noma & Irisawa (1) first reported the existence in sino-atrial node (SAN) tissue of a slow, time-dependent inward current that was activated by membrane hyperpolarization, later termed I_h (16). DiFrancesco and colleagues (2, 17–19) first provided a detailed characterization of this current, which they named I_f . Shortly thereafter, a similar hyperpolarization-activated cation current was identified in rod photoreceptors (20) and hippocampal pyramidal neurons (3), which was termed I_Q . Since these early studies, the biophysical properties of I_h (the nomenclature we use here) have been characterized in great detail (see Reference 5 for an excellent review). Here we only briefly summarize some of the more salient features of I_h that are important for an appreciation of its physiological function.

I_h is a mixed cation current that typically activates with hyperpolarizing steps to potentials negative to -50 to -60 mV. The kinetics of activation during a hyperpolarization, and deactivation following repolarization, are complex. Activation is usually preceded by a significant delay, resulting in a marked sigmoidal time course of onset for I_h . Following this delay, channel opening can be empirically described by either a single or double exponential function, depending on the cell type. These exponential kinetics also vary widely among different cells. In heart and thalamic relay neurons, the time course of activation is quite slow, requiring several seconds to reach a steady state. In hippocampal CA1 neurons by contrast, the kinetics of activation are quite rapid, with time constants of activation on the order of 30 to 60 ms.

The voltage-dependence of I_h activation is usually assessed by tail current activation curves, in which the amplitude of the tail current upon return to a fixed holding potential is plotted as a function of the hyperpolarizing potential step used to activate the current. These activation curves show a typical S-shaped dependence

on hyperpolarizing voltage and can be well fit by a Boltzmann function. Such fits provide estimates of the voltage at which the channels are half-maximally activated, $V_{1/2}$, and the steepness of the relation between voltage and fractional activation (measured by the slope factor s , in mV). $V_{1/2}$ values can vary widely among different cells and typically range from -60 to -90 mV, but in adult ventricle can be negative to -120 mV. The slope factor also varies significantly but can be as steep as an e -fold increase in channel opening for every 4 mV of hyperpolarization.

The I_h channel has unusual ion selectivity in that it conducts both Na^+ and K^+ ions but excludes Li^+ (see 5). Divalent cations neither permeate nor block the channel. The ratio of the K^+ to Na^+ permeability of the channel, $P_K:P_{\text{Na}}$, ranges from 3:1 to 5:1, yielding values for the reversal potential of -25 to -40 mV. As a result, activation of the channel at typical resting potentials results in a net inward current carried largely by Na^+ , which will depolarize the membrane toward threshold for firing an action potential. One other unusual feature of the channel is that its conductance is highly sensitive to external K^+ levels. Reduction of K^+ below normal extracellular levels (2–4 mM) can result in a dramatic decrease in current magnitude. I_h channels also have a very small single channel conductance. Even in elevated external K^+ , channel conductance is only 1 pS (21). The sensitivity of I_h to external K^+ might provide an important means for regulating I_h function; for example, external K^+ elevation during seizure activity or cardiac ischemia might enhance the magnitude of I_h and thus alter excitability.

The characterization of I_h has often been hampered by its relatively small magnitude, combined with the presence of overlapping ionic currents that activate over a similar range of potentials. These currents include inward rectifier K^+ currents, persistent voltage-gated Na^+ currents (I_{NaP} ; 22), hyperpolarization-activated Cl^- currents, and transient A-type K^+ currents. I_h has often been distinguished from these other currents by its sensitivity to relatively low concentrations of external Cs^+ , which produce substantial ($>50\%$) blockade at a concentration of 1–2 mM, and by its insensitivity to 1–2 mM external Ba^{2+} , a potent blocker of inward rectifier K^+ channels. However, Cs^+ has the drawback of blocking certain K^+ channels at this concentration. A number of organic compounds have been described that block I_h fairly specifically. These include ZD-7288 (23, 24), UL-FS49 (zatebradine) (25, 26), and S-16257 (ivabradine) (27), although zatebradine also blocks K^+ channels (27, 28), and ZD-7288 and DK-AH 269 (which is structurally similar to zatebradine) alter synaptic transmission, independently of blocking I_h (29).

One of the most interesting and important characteristics of I_h is its regulation by cyclic nucleotides. Neurotransmitters that elevate cAMP levels greatly facilitate the activation of I_h by shifting its voltage dependence of gating to more positive potentials, typically by 10 mV or more. As a result, during a hyperpolarizing step to a given voltage, I_h activates more completely and more rapidly (Figure 1A). Conversely, neurotransmitters that downregulate cAMP depress the activation of I_h , shifting its activation curve to more negative potentials. DiFrancesco & Tortora (30) made the surprising discovery that the regulation of I_h by cAMP does not require protein phosphorylation. Rather, because cAMP enhanced channel

opening in cell-free membrane patches in the absence of MgATP, DiFrancesco & Tortora concluded that I_h gating is directly regulated by cAMP binding (with an apparent $K_d < 1 \mu\text{M}$) to a site on the cytoplasmic surface of the channel. cGMP also binds to this site and enhances channel activation, although the affinity for cGMP is an order of magnitude less than the affinity for cAMP (30, 31).

The regulation of I_h through cyclic nucleotides contributes to the speeding of the heart rate in response to β -adrenergic agonists (which raise levels of cAMP) and to the slowing of the heart rate in response to muscarinic acetylcholine receptor agonists (which reduce levels of cAMP). In the brain, a number of transmitters have been shown to regulate I_h in different neurons through either enhancing or diminishing cAMP levels. I_h can also be regulated in both brain (32) and heart (33) by nitric oxide, which stimulates soluble guanylate cyclase and elevates cGMP levels. Subsequent studies have suggested that the activity of I_h may also be regulated by protein phosphorylation and dephosphorylation (34). A number of protein kinases (PKs) have been implicated, including PKA (35, 36), PKC (36) and tyrosine kinases (37–40). However, direct evidence for I_h channel phosphorylation is so far lacking. Perhaps the most striking evidence for some regulatory factor is the pronounced “run-down” of I_h in excised patches or during prolonged whole-cell recordings, which cause a large 40–50 mV hyperpolarizing shift in the voltage dependence of activation (41). An effect of basal levels of cAMP to tonically shift activation to more positive potentials may account for up to 15 to 20 mV of this shift (42). The mechanism responsible for the large remainder of the voltage shift remains unknown.

PHYSIOLOGICAL ROLE OF I_h : GENERAL PRINCIPLES

At least four physiological roles have been ascribed to I_h : (a) control of pacemaker activity (in both heart and brain), (b) control and limitation of resting potential, (c) control of membrane resistance and dendritic integration, and (d) regulation of synaptic transmission. We address these various functions in specific examples detailed below. First, however, we consider some general consequences of the biophysical properties of I_h for regulation of membrane voltage.

As mentioned above, a defining feature of I_h is its activation by hyperpolarization to potentials at or negative to typical neuronal and muscle cell resting potentials, generating an inward excitatory current. In a cell at rest, tonic activation of I_h helps set the level of resting potential at a somewhat depolarized level. In addition, activation of I_h also contributes to the resting membrane conductance. Thus the presence of I_h tends to decrease a cell's input resistance, membrane time constant, and length constant.

Unlike a voltage-gated Na^+ channel, however, the depolarization produced by I_h is not by itself regenerative or destabilizing, rather it is self-limiting and stabilizing. As the membrane depolarizes through influx of Na^+ via I_h , the positive voltage will cause I_h to turn off, decreasing Na^+ influx and limiting the extent of the

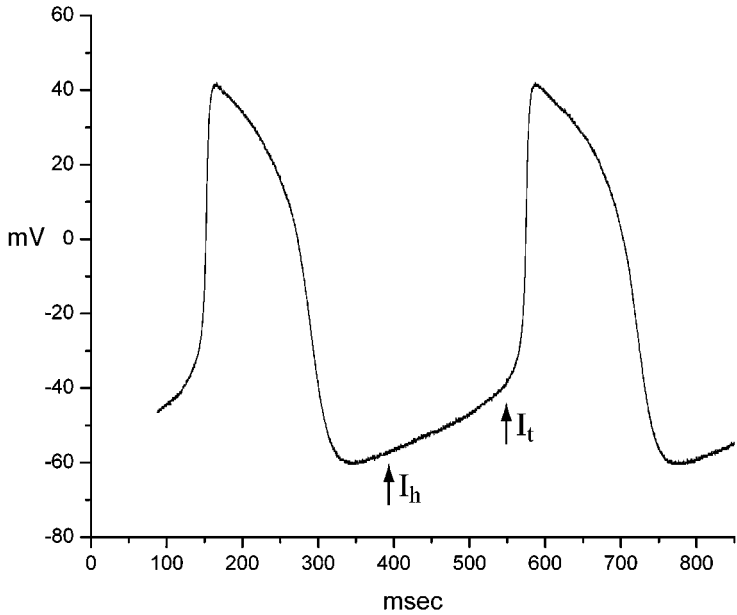
depolarization. The stabilizing effect of I_h can be readily seen in a current clamp experiment (Figure 1B).

In cells that lack I_h , a negative current step causes the membrane to hyperpolarize along an approximately exponential time course governed by the passive membrane time constant. When the current step is terminated, the membrane voltage smoothly decays back to its original resting potential. However, in cells that express I_h , there is a pronounced depolarizing sag in the hyperpolarizing voltage response owing to the activation of I_h channels (Figure 1B). Thus I_h drives the membrane back toward its initial resting potential. Upon termination of the inward current, there is a rebound depolarization caused by the extra inward Na^+ flux through I_h channels that were activated by hyperpolarization. In some cells, this rebound depolarization can trigger an action potential. As the I_h channels deactivate at the positive potential, the membrane voltage returns to rest. An opposite effect of I_h is seen in response to a depolarizing current pulse. Here, any I_h that is active at the resting potential will be turned off by the depolarization. The decrease in inward current leads to a hyperpolarizing sag in the membrane potential. Upon termination of the depolarizing current step, there is a hyperpolarizing undershoot (afterhyperpolarization), owing to the initial deficit in I_h . These actions of I_h help limit the extent of membrane hyperpolarization during a burst of inhibitory postsynaptic potentials (IPSPs) and can contribute to the firing of rebound action potentials once the burst terminates. Similarly, in heart, I_h has been suggested to protect the peripheral SAN from excess hyperpolarization by the surrounding atrial muscle (43) and to contribute to anode break excitation in the ventricle (44).

These basic functions of I_h provide the leitmotif upon which the complement of ion channels expressed in a particular cell acts to orchestrate that cell's electrical signature. Thus I_h is thought to serve as a primary pacemaker current in some cells, including SAN cells and Purkinje fibers in the heart (4), thalamocortical relay neurons (45; see also 46), inferior olive neurons in the brainstem (47), and hippocampal stratum oriens interneurons (48). The activation of I_h at negative voltages upon repolarization of the action potential provides inward current that drives the membrane to threshold for firing a subsequent action potential (Figure 2). The actual triggering of an action potential by the pacemaker depolarization usually requires the activation of a more rapidly activating excitatory current, such as the low-voltage-activated T-type Ca^{2+} current or L-type Ca^{2+} current, which can provide a sufficiently rapid increase in inward current to elicit a spike.

In other spontaneously active cells, I_h does not serve as a primary pacemaker current. Thus in such cells blockade of I_h does not reduce action potential firing rates. Cells that do not rely on I_h for pacemaker activity, including cerebellar Purkinje neurons (6), hippocampal stratum lucidum interneurons (49), and neurons of the respiratory brainstem nucleus (7), often rely on a persistent inward Na^+ current (I_{NaP}) to generate rhythmic firing. I_h is thought to function in these cells as a safety net that helps prevent the membrane from hyperpolarizing out of the range in which the maintained Na^+ current is able to drive spontaneous firing (50).

A. Sinoatrial node cell



B. Thalamic relay neuron

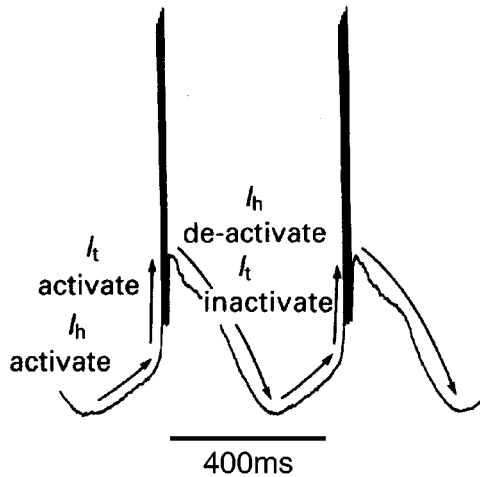


Figure 2 Pacemaking function of I_h in a cardiac sinoatrial node myocyte (A) and a thalamocortical relay neuron (B). In both cells, I_h activation following an action potential produces a slow depolarization that triggers an action potential through activation of calcium channels. During the action potential, I_h deactivates and the calcium current inactivates, causing repolarization. [(A) R.B. Robinson, unpublished data; (B) (148)].

Finally, I_h is also present in cells that are not normally spontaneously active, such as photoreceptors, hippocampal CA1 pyramidal neurons, and cardiac ventricular myocytes. I_h helps determine the resting and passive cable properties of such cells, including their resting potential, input resistance, membrane time constant, and length constant. In this manner, I_h regulates the response of a cell to excitatory or inhibitory inputs.

Given these diverse functions, it is perhaps not surprising that the properties of I_h vary considerably from cell type to cell type, in terms of kinetics and steady-state voltage dependence of activation, and sensitivity to cAMP. What accounts for the diversity of I_h ? How does cAMP binding regulate channel activity and why do different channels show different sized responses? How are the channels gated by hyperpolarization, rather than depolarization? Insights into these questions are beginning to emerge from the analysis of recombinant HCN channels.

MOLECULAR CHARACTERIZATION OF HCN CHANNEL FUNCTION

On the basis of amino acid sequence, the four mammalian HCN genes were shown to be members of the voltage-gated K^+ channel superfamily, with a single gene corresponding to a single K^+ channel subunit (31, 51, 52) (Figure 3). Related HCN genes have been cloned from a sea urchin sperm cDNA library (spHCN; 53) and from insect antenna (hvHCN; 54). *Caenorhabditis elegans* appears to lack the HCN genes.

HCN isoforms are highly conserved in their core transmembrane regions and cyclic nucleotide binding domains (80–90% identical) (see 11, 13, 15). However, the gene products diverge in their amino- and carboxy-terminal extreme cytoplasmic regions. Each HCN subunit is composed of six transmembrane segments, with a positively charged S4 voltage sensor, similar to the voltage sensors of depolarization-activated channels (Figure 3). In addition, the HCN channels contain a pore-forming P region, with the GYG signature sequence of the selectivity filter of K^+ -selective channels. Outside this GYG sequence, however, the amino acid sequence of the HCN channels and K^+ channels diverges, perhaps accounting for the low K^+ selectivity of I_h .

All four HCN isoforms have been expressed in heterologous cells and shown to form homomeric, hyperpolarization-activated, non-selective cation currents that are directly modulated by cAMP (31, 52, 53, 55–58). However, the four HCN isoforms give rise to channels that differ in their kinetics, steady-state voltage dependence and potency of modulation by cAMP. Given the diversity of native I_h in different cell types, a key goal is to relate the cellular heterogeneity to the molecular composition of the channel. This is complicated by the fact that native pacemaker currents exhibit greater diversity, for example with regard to voltage dependence, than the expressed isoforms.

A striking feature of the four HCN isoforms is the presence of a 120-amino acid cyclic nucleotide-binding domain (CNBD) in their cytoplasmic carboxy

terminus, which mediates the response to cAMP (42, 59). The CNBD is homologous to similar regions in other cyclic nucleotide-binding proteins, including the cAMP- and cGMP-dependent protein kinases, a bacterial cAMP binding protein, the catabolite-activating protein (or CAP), and the cyclic nucleotide-gated ion channels of olfactory neurons and photoreceptors (11).

Deletion of the CNBD mimics the effect of cAMP by shifting the voltage dependence of HCN gating to more positive voltages by an amount similar to the maximal shift seen with saturating concentrations of cAMP (59). This indicates that cAMP binding enhances gating by relieving a tonic inhibitory action of the CNBD, which shifts gating in the absence of cAMP to more negative potentials. Differences in the voltage dependence of gating and potency of cAMP modulation between HCN1 and HCN2 were shown to result from differences in the extent of inhibition produced by the CNBD, with HCN2 having a much greater inhibition than HCN1. As a result, the basal gating of HCN2 is shifted by 20 mV to more negative voltages compared with that of HCN1. Moreover, because of this greater tonic inhibition, HCN2 channels respond to cAMP with a greater positive voltage shift (+20 mV) than seen in HCN1 channels (+5 mV).

The finding that HCN channels contain a positively charged S4 voltage sensor, similar in structure to the S4 region of depolarization-activated Shaker K⁺ channels, raises the question as to the mechanism of hyperpolarization gating. Is S4 the HCN voltage sensor and does it move in response to changes in membrane potential similar to the movement of S4 in depolarization-activated channels? Two models have been suggested to account for the hyperpolarization gating of I_h: (a) Hyperpolarization leads to a removal of inactivation, which causes channel opening (60). According to this model the S4 segment, whose movement controls the activation gate in depolarization-activated channels, is thought to be locked in the activated state and thus cannot move during hyperpolarization gating. (b) Hyperpolarization causes an inward movement of S4, similar to its effect in depolarization-activated channels. However, the coupling between S4 movement and channel opening would be reversed in HCN channels, where the inward S4 movement would produce channel opening.

The fact that I_h activation is very steeply regulated by voltage, comparable to depolarization-activated channels, suggests that the S4 domain must act as the voltage sensor because it is the only transmembrane segment that contains the requisite charge necessary to produce such a high-voltage sensitivity. Recently, Larsson and colleagues (60a) used the substituted cysteine accessibility method to demonstrate that S4 of the sea urchin HCN channel does indeed move in response to hyperpolarization, just as it does in depolarization-activated Shaker K⁺ channels. Thus hyperpolarizing voltage steps lead to an inward movement of S4, and depolarizing voltage steps lead to an outward movement of S4, causing at least one S4 residue to translocate across the membrane from an external facing position at depolarized voltages to an internal facing position at hyperpolarized voltages. Moreover, this HCN residue lies in a homologous position to an S4 residue in Shaker K⁺ channels that undergoes a similar translocation across the membrane.

Because S4 appears to respond similarly to voltage changes in HCN and Shaker channels, the differences between hyperpolarization-activation and depolarization-activation must lie downstream of S4 movements, in the mechanism coupling S4 movement to the gate.

However, key aspects of this coupling mechanism also appear to be conserved between HCN and Shaker channels. Mutagenesis experiments demonstrate that the internal S4-S5 loop has an important influence on HCN gating (62), similar to its role in depolarization-gated K^+ channels (63). Furthermore, similar to Shaker, the HCN channel gate lies near the internal surface of the membrane, where it regulates the accessibility of residues lining the S6 transmembrane segment to internally applied ZD-7288 (24) and cysteine-reactive reagents (61). Although there must be some fundamental differences between how the HCN and Shaker gates respond to inward S4 movements, the details of these differences remain unknown.

MOLECULAR BASIS FOR I_h IN BRAIN

All four HCN isoforms are expressed in the mammalian brain (64–66). Of these four, HCN3 shows the weakest expression. HCN2 shows a broad pattern of strong mRNA expression and is present in most brain regions. HCN1 is more selectively expressed. For example, it is prominent in layer 5 pyramidal neurons of the neocortex, but not in other cortical layers. This mRNA expression pattern is consistent with the high density of I_h current recorded in the layer 5 neurons.

HCN expression patterns have been particularly well characterized in the hippocampus. There, HCN1 is found in CA1 and CA3 pyramidal neurons, with somewhat higher levels of expression in CA1 neurons compared with that in CA3 neurons. HCN2, in contrast, is expressed at somewhat higher levels in CA3 than in CA1 pyramidal neurons. HCN1 and HCN2 are also expressed in scattered neurons in the stratum oriens and stratum lucidum regions of the hippocampus. Such cells most likely represent inhibitory interneurons, which have been shown to contain I_h . Expression of HCN1 in the dentate gyrus is quite low, especially in the mouse brain. HCN4 is only weakly expressed in hippocampus and neocortex.

HCN1 is also strongly expressed in the inhibitory basket cells and Purkinje neurons of the cerebellum. In the thalamus, high levels of expression of HCN2 and HCN4 are found in the excitatory thalamocortical relay neurons, whereas only HCN2 is found in the inhibitory thalamic reticular neurons. Thus the different HCN isoforms are differentially expressed in different regions of the brain.

Interestingly, the rapidly activating HCN1 is most strongly expressed in CA1 neurons of the hippocampus, which exhibit a very rapidly activating I_h . In contrast, thalamocortical relay neurons, which express the more slowly activating HCN2 and HCN4 isoforms, show a more slowly activating I_h . Thus within the brain, the functional heterogeneity of I_h is likely to result from the molecular heterogeneity of isoform expression. Finally, it is curious that certain brain regions that show high

levels of HCN mRNA, such as CA3 neurons and thalamic reticular neurons, show very little I_h . Recently, I_h formed from recombinant channels has been shown to exhibit a time- and voltage-independent instantaneous current component (under some conditions) that appears as part of the leak current (67). It has been suggested that the particularly large leakage current found in the reticular neurons may represent a contribution of the tonically active form of I_h .

MOLECULAR BASIS FOR I_h IN HEART

HCN1, HCN2, and HCN4 are expressed in heart. Their relative mRNA abundance varies with cardiac region, species, age, and, perhaps, disease state. Although there is good correlation between absolute level of HCN message and magnitude of measured I_h among cardiac regions and species, it has proven difficult to explain functional heterogeneity in the heart with specific isoform expression patterns. In this regard, heart differs from brain in that cardiac I_h exhibits a much wider range of regional voltage dependence.

The SAN, the normal pacemaking region of the heart, exhibits both the largest and most positively activating pacemaker current and expresses the highest message level. HCN4 is the most predominant isoform, accounting for >80% of the total HCN mRNA (58, 68, 69). Significant levels of HCN1 are present in rabbit SAN (20% of total HCN mRNA) (68) but only very low levels are detected in mouse (58). In dog SAN, low levels of HCN1 (7%) and HCN2 (8%) mRNA make up the remainder of HCN transcripts (69).

HCN4 is also the predominant HCN transcript in cardiac Purkinje fibers, specialized conducting tissue that can exhibit subsidiary pacemaker activity. Canine Purkinje fibers, which exhibit significant automaticity and display a large I_h , show the highest level of HCN mRNA expression in the heart outside of the SAN (35% of SAN) (69). HCN4 accounts for 90% of the transcripts, with the remainder contributed by HCN2. In contrast, rabbit Purkinje fibers, which tend not to be automatic and exhibit little I_h , show minimal levels of HCN message (4% of SAN) (68). The HCN mRNA that is present represents roughly equivalent levels of HCN1 and HCN4, with a minor contribution of HCN2 (10%).

In the rabbit ventricle only HCN2 is detected; however, overall levels are extremely low and no measurable I_h is found (68). Canine (69) and rat (68) ventricle exhibit greater I_h and higher HCN levels than rabbit. HCN2 is by far the predominant ventricular isoform, especially in adult animals, with the balance being HCN4.

The distinct biophysical properties of the HCN isoforms expressed in different regions of the heart cannot account for the marked differences in the time-dependence and voltage-dependence of activation of native I_h in these same regions. Thus, although I_h in the SAN activates more rapidly than I_h in other cardiac regions, the predominant isoform expressed in SAN, HCN4, is the most slowly activating isoform in heterologous expression systems. Moreover, whereas the $V_{1/2}$ values of the four HCN isoforms vary by no more than 20 mV, the $V_{1/2}$ values of

native I_h currents may vary by as much as 80 mV between SAN and ventricle. During development, the voltage dependence of I_h activation in ventricular muscle shifts by up to 40 mV toward more negative potentials, so that in adults the threshold for I_h activation is negative to the resting potential (70) [although there is some debate as to whether the developmental change is a shift in voltage dependence or a reduction in current magnitude (71)]. These results suggest that factors within a cardiac cell can influence the voltage dependence of an individual HCN isoform. In fact, when HCN2 is overexpressed in neonatal and adult rat ventricular myocytes, the voltage dependence is more positive in the neonatal cells, and this difference is independent of basal cAMP levels (72).

Although the biophysical properties of heterologously expressed HCN isoforms cannot fully account for the observed variation in native I_h , there is some correlation between which isoforms are expressed in a specific region and the voltage dependence of the native pacemaker current; regions with the most negative activation (e.g., ventricle) tend to express HCN2 predominantly, whereas regions with more positive voltage ranges of activation express HCN4. In addition, whereas HCN2 is the dominant ventricular isoform throughout development, the relative expression ratio of HCN2:HCN4 increases from 5:1 in the neonatal rat ventricle to 13:1 in the adult rat ventricle (68) at the same time that the voltage dependence of the native I_h is becoming more negative (70). The large phenotypic variation in I_h throughout the heart may reflect the differential modulation of HCN subunits by factors such as phosphorylation or auxiliary subunits, which may be turned on in distinct regions or at different developmental stages by the action of hormones, transmitters, or growth factors. Thus the rationale for the regional patterns of HCN isoform expression might be a differential susceptibility of these isoforms to such modulatory changes. In this respect, it is interesting that in a β_2 -adrenergic overexpressing mouse heart, ventricular HCN4 message is upregulated with no change in HCN2 (73).

SUBUNIT ASSEMBLY

The finding that different HCN isoforms can be expressed in a single cell raises the question as to whether different subunits can coassemble to form functional heteromers. Three groups recently approached this question for HCN1 and HCN2, isoforms that are co-expressed in both CA3 and CA1 regions of the hippocampus. Expression of a HCN1-HCN2 tandem dimer (74) or co-injection of recombinant HCN1 and HCN2 cRNA (42, 74) in *Xenopus* oocytes leads to the formation of I_h channels with novel properties that cannot be accounted for by the linear sum of independent populations of HCN1 and HCN2 homomers. Moreover, the HCN1 + HCN2 heteromeric channels more closely resemble the properties of native I_h in CA1 pyramidal neurons than do homomeric HCN1 or HCN2 channels. The demonstration that a dominant-negative HCN2 pore mutant was able to suppress the expression of both HCN2 and HCN1 wild-type channels provides further evidence that HCN1 and HCN2 coassemble (75). Xue et al., on the basis of the

dose-dependence of current suppression by a dominant-negative HCN1 cRNA, also provides the first direct evidence that the HCN channels are tetramers (75). Biochemical data suggest that channel assembly involves an interaction between the amino-terminal regions of HCN1 and HCN2 (76). Whether other HCN isoforms can coassemble to form heteromers remains unclear (cf 57 for HCN2 and HCN4).

There is also evidence that HCN subunits can coassemble with an auxiliary subunit, the min-K related protein (MiRP1 or KCNE2), to form channels with modified properties (77). KCNE2 was originally identified as a possible β -subunit of the HERG delayed rectifier channel because coassembly of these subunits resulted in reconstitution of a current whose properties were more similar to the cardiac rapid delayed rectifier (I_{Kr}) than were the currents produced by HERG expression alone (78). However, the finding that KCNE2 is highly expressed in the SAN (77) suggested that it might also be an auxiliary subunit for HCN channels. Indeed, oocyte co-expression studies of KCNE2 and HCN isoforms demonstrated that KCNE2 increases I_h current density and HCN membrane protein expression and speeds the kinetics of activation (with little effect on voltage dependence) (77). The kinetic effect could help explain the relatively rapid activation of I_h in SAN compared with other cardiac regions. Another group (67), working in a mammalian expression system, observed that KCNE2 co-expression with HCN2 increased a time-independent, instantaneous component of I_h .

PHYSIOLOGICAL ROLE OF I_h : SPECIFIC EXAMPLES IN THE CENTRAL NERVOUS SYSTEM AND HEART

I_h as a Determinant of Resting Potential: Role in Sensory Signal Transduction

Because of its role in setting the resting potential, I_h can act both to generate sensory receptor potentials and modulate the response of sensory neurons to other stimulus-evoked currents. The inner segments of rod and cone photoreceptors were some of the first cells in which I_h was characterized (20, 79), and high levels of HCN1 mRNA have been detected in mouse photoreceptors, with no detectable expression of the other three isoforms (58). In addition, HCN1 protein has been found localized to the inner segments of rabbit rods (80). Activation of I_h during large hyperpolarizing responses to bright light is thought to contribute to visual adaptation by limiting the extent of hyperpolarization (20, 79, 81). Psychophysical studies of the effects of administration of an I_h antagonist to human subjects suggest that the depolarizing action of I_h is also required for high temporal resolution of rapidly changing visual signals (82).

Sensory stimuli-dependent changes in I_h serve a modulatory role in the somatic sensation of cold. I_h is known to have a high sensitivity to temperature, with Q_{10} values ranging from 4 to 6. Although members of the TRP family of ion channels are likely to be the primary mediators of cold sensation (see 83), in

certain trigeminal neurons that respond to cold temperatures, a slow decrease in the extent of I_h activation in the cold contributes to a membrane hyperpolarization that counteracts the depolarizing influence of the primary, cold-activated inward current response, thereby limiting the firing of action potentials (84).

HCN channels may serve as a primary generator of the sensory receptor potential in taste receptors to mediate the sour taste response (85). Electrophysiological recordings demonstrated the presence of I_h in a subset of ~20% of taste receptors. In situ hybridization and immunocytochemistry revealed that a similar fraction of taste receptors express HCN1 and HCN4. Moreover, I_h is selectively present in those taste receptors that respond to sour stimuli. Acidic solutions were shown to activate I_h leading to membrane depolarization by causing a strong (>30 mV) depolarizing shift in the voltage dependence of I_h activation.

I_h and Dendritic Integration

High levels of HCN1 protein are found in the dendrites of CA1 pyramidal neurons and layer 5 cortical pyramidal neurons (51). Patch-clamp recordings in both hippocampal CA1 (86) and layer 5 cortical (87) pyramidal neurons reveal a gradient of dendritic I_h , with current density increasing with increasing distance from the soma. Dendritic I_h is thought to be important in shaping the voltage response to excitatory synaptic inputs (88–90). Thus according to passive cable theory, a distal synaptic input should generate a slower depolarizing excitatory postsynaptic potential (EPSP) in the soma than a proximal synaptic input. Surprisingly, the time courses of distal and proximal EPSPs were found to be identical in pyramidal neurons from either layer 5 of the neocortex (88, 90) or the CA1 region of the hippocampus (89). The importance of I_h in the normalization of EPSP time course was demonstrated by the finding that inorganic or organic I_h antagonists caused a preferential slowing of distal EPSPs relative to proximal EPSPs (88–90).

The presence of I_h in the distal dendrites is thought to modify the EPSP time course by enhancing the local resting membrane conductance, thereby providing a leakage path for current flow that decreases the local membrane time constant and hence speeds the decay of the distal EPSP. During blockade of I_h , the slowing of the distal EPSP results in a greater temporal summation of successive EPSPs, which promotes action potential firing during a train of synaptic inputs (89). By normalizing the time course of the EPSP, I_h presumably increases the information processing capabilities of the dendritic tree, permitting distal and proximal EPSPs to carry similar temporal information content.

The high level of dendritic expression of I_h also leads to a short length constant of the distal dendrite, both in layer 5 neurons (91) and CA1 neurons (92). This causes a particularly large attenuation of the amplitude of a distal EPSP when it arrives in the soma. Despite the larger attenuation of a distal EPSP compared with a proximal EPSP, the amplitudes of distal and proximal EPSPs in the soma are quite similar. This equalization of EPSP size results from a higher density of AMPA-type glutamate receptors at distal dendritic synapses relative to proximal synapses (93).

One fascinating question is the physiological consequences of altered dendritic integration. A recent study of the action of a novel anticonvulsant drug, lamotrigine, has shed light on this issue (94). In CA1 pyramidal neurons, lamotrigine was found to inhibit action potential firing in dendrites with little effect on excitability in the soma. This effect of lamotrigine is the result of upregulation of I_h . The greater effect of the drug on action potential firing in the dendrite than in the soma is explained by the higher level of I_h expression in the dendrites. The decrease in action potential firing following upregulation of I_h is somewhat counterintuitive, given that I_h carries an inward excitatory current. Poolos et al. (94) argued that the inhibitory effect is a result of an increased membrane conductance at the resting potential owing to enhanced opening of I_h channels. This leads to both a decrease in the input resistance and a decrease in the length constant of the dendrite. As a result, distal excitatory synaptic potentials will be more greatly attenuated once they reach the soma, the site of impulse initiation. The upregulation of I_h by lamotrigine may contribute to the action of this drug to prevent seizures.

I_h not only regulates the passive cable properties of CA1 and layer 5 neurons, it also contributes to their resonance, a preferential response to inputs that occur at a certain oscillatory frequency. Many brain regions show prominent, network oscillations that consist of synchronous excitatory and inhibitory synaptic inputs. One well-characterized oscillation, the theta rhythm, occurs at a frequency of around 5 Hz and is particularly prominent in the hippocampus as an animal explores a novel environment (95). Stimulation of the Schaffer collateral inputs to CA1 neurons at a frequency of 5 Hz is a very common protocol used to induce long-term potentiation (LTP) of synaptic transmission (96). Although pyramidal neurons may not be a prime generator of theta rhythm, both layer 5 cortical neurons (97) and hippocampal CA1 pyramidal neurons (98, 99) are tuned to generate their greatest voltage response, or resonance, to inputs at a frequency of around 5 Hz. For layer 5 neurons, this effect is mediated by I_h as its blockade with ZD-7288 leads to a block of resonance (97). I_h also helps regulate the phase relation between sinusoidal excitatory, dendritic inputs and the firing of action potentials in the cell body (100).

Role of I_h in Cardiac Automaticity

The early studies by Brown & DiFrancesco (17) and Yanagihara & Irisawa (16) not only defined the initial biophysical properties of I_h , but also the nature of the debate that continues to this day concerning the contribution of this current to cardiac automaticity. Yanagihara & Irisawa concluded that because of the position of the I_h voltage threshold for activation relative to the SAN maximum diastolic potential (most negative voltage reached during the cardiac cycle), any current activated during diastole would be small and would exhibit little time-dependence during the cardiac cycle. They proposed that the main role of I_h was as a source of inward current to oppose the influence of the surrounding atrial muscle to hyperpolarize the SAN away from threshold. In this manner, I_h would provide only an

indirect contribution to automaticity. In contrast, Brown & DiFrancesco (2, 4) provided experimental evidence that, at voltages corresponding to the diastolic range of potentials, the magnitude and kinetics of I_h were consistent with a direct contribution to the pacemaker depolarization. They further demonstrated that norepinephrine, which increases SAN automaticity, increased I_h . Studies with selective I_h blockers have shown a significant slowing (although not a complete cessation) of heart rate, further supporting a role for I_h in SAN automaticity (101–104).

More recent studies have suggested that a number of additional currents may contribute to SAN automaticity, including a Na^+ -dependent background current (105), T-type Ca^{2+} current (106), a sustained inward current (thought to be a monovalent cation conducting variant of L-type Ca^{2+} current) (107, 108), and a $\text{Na}^+/\text{Ca}^{2+}$ exchange current (109–111). Moreover, the contributing currents may vary with development (112). Given the physiological importance of pacemaker activity and its profound modulatory control, it is not unreasonable to expect that multiple ionic mechanisms contribute, each perhaps tuned to a different voltage and frequency range of spontaneous firing.

In addition to any contribution of I_h to normal automaticity in the SAN, several additional potential contributions of this current have been proposed. First, the fact that I_h generates an inward current on hyperpolarization suggests a stabilizing effect that might minimize any inherent cycle length variability of the SAN. Indeed, overexpression of HCN2 in neonatal ventricular myocytes, which normally are automatic in culture, leads to not only a shorter cycle length but a reduced beat-to-beat variability (72). Thus even if I_h is not the sole initiator of pacemaking activity in the SAN, it may contribute to a more stable rhythm. In addition, the observation that I_h is greater in the periphery of the SAN, even though the central node serves as the primary pacemaking region, has led to the suggestion that it protects the SAN from excess hyperpolarization by surrounding atrial muscle (43). Finally, there is evidence that I_h is particularly important in the normal modulation of heart rate by the autonomic nervous system. I_h is more sensitive to acetylcholine than either $I_{K,ACh}$ or $I_{Ca,L}$, responding at markedly lower concentrations (113, 114). Whereas both I_h and $I_{Ca,L}$ exhibit a similar dose-response relation to β -adrenergic stimulation by isoproterenol, the I-V relation of $I_{Ca,L}$ suggests it contributes only to late diastolic depolarization and not to the isoproterenol-dependent increase in the slope of the early pacemaker potential (114).

Subsequent to the identification of I_h in the SAN, the current was found in other regions of the heart, beginning with Purkinje fibers (18). Initially, I_h was thought to be restricted to cardiac regions such as the SAN and the Purkinje fibers, which demonstrate automaticity. Although I_h is most apparent in those regions, it is in fact present throughout the heart, including the non-pacing cardiac tissues such as ventricles and atria (70, 115–119). However, its extreme negative voltage dependence in adult ventricle (with a threshold for activation as negative as -145 mV) (116), well outside the physiological range, raises questions as to its function in these tissues. It has been suggested that I_h serves as a source of depolarizing current during anode break excitation (44). In support of this argument, when

HCN2 is overexpressed in adult ventricular myocytes, the stimulus energy required to achieve anode break excitation is significantly reduced (72). Although this contribution to anode break excitation has potential implications for electrical pacemakers (120), its significance for normal physiology is not obvious.

I_h and Neuronal Automaticity

In the CNS, I_h plays three distinct roles in the generation of spontaneous activity. In some cells, I_h acts as a primary pacemaking current. In such cells, blockade of I_h with either Cs^+ or an organic antagonist either completely blocks pacemaker activity or substantially slows the rate of spontaneous firing. In other neurons, I_h is thought to act primarily to modulate the inherent spontaneous activity generated by other primary pacing currents. Blockade of I_h in such cells fails to alter the rate of spontaneous firing or enhances the rate of firing, or leads to bistable membrane potentials. Finally, in still other cells, I_h supports subthreshold oscillations. We focus on three well-characterized examples that illustrate these roles.

PRIMARY PACEMAKING IN THALAMOCORTICAL RELAY NEURONS In the absence of synaptic input, thalamocortical relay neurons show a pattern of rhythmic electrical activity consisting of bursts of action potentials at a frequency of 0.5–4 Hz (Figure 2B). This firing pattern is driven by slow, Ca^{2+} -dependent action potentials generated by T-type Ca^{2+} channels. The peak of the Ca^{2+} spike triggers a burst of rapid, Na^+ -dependent action potentials. The Ca^{2+} spike eventually repolarizes through inactivation of the T-type channels. As the membrane voltage returns to negative levels, activation of I_h generates a slow pacemaker depolarization, which triggers the next Ca^{2+} spike. A similar mechanism is proposed to underlie the spontaneous firing of neurons in the inferior olive of the brainstem. These neurons provide the excitatory climbing fiber input to cerebellar Purkinje neurons (47).

I_h not only controls oscillatory activity inherent to an individual thalamocortical cell, it also contributes to network oscillations observed in intact thalamic preparations in which these neurons participate. During slow-wave sleep, synchronized firing patterns called spindle waves appear in the EEG (121). Spindling, which results from reciprocal connections between GABAergic thalamic reticular neurons and glutamatergic excitatory thalamic relay neurons, consists of waxing and waning bursts of activity. During the active phase, thalamic neurons fire action potentials at 7–14 Hz for periods of 1–3 s. This phase of rapid spiking is followed by prolonged periods of silence, lasting 5–10 s, before the thalamic neurons become active again.

During the periods of activity, action potential firing in the excitatory thalamocortical relay neurons drives the firing of the reticular neurons, which generates a burst of hyperpolarizing IPSPs in the relay neurons. The IPSPs both cause firing of rebound action potentials in the thalamic neurons and produce a slow activation of I_h that generates a prolonged afterdepolarization. The depolarization of the relay neuron membrane eventually inactivates T-type Ca^{2+} channels, preventing relay

neurons from spiking and resulting in the long periods of silence during spindling. The prolonged afterdepolarization is not simply the result of hyperpolarization-dependent activation of I_h during the IPSPs; it requires Ca^{2+} entry into the relay neuron during activity (46), which is thought to activate a Ca^{2+} -stimulated adenylyl cyclase, leading to an elevation in cAMP that further enhances the activation of I_h (122).

A SAFETY NET FOR AUTOMATICITY: SPONTANEOUS FIRING IN CEREBELLAR PURKINJE CELLS These neurons normally fire spontaneously at high frequencies, around 40–50 Hz, via a complex interaction of K^+ currents, Ca^{2+} currents, and a maintained, voltage-gated Na^+ current (6, 123). Inhibition of I_h with antagonists has little or no effect on spontaneous firing frequency during periods of activity (6). However, upon blockade of I_h by ZD-7288, the Purkinje cell becomes bistable and undergoes spontaneous transitions between prolonged periods of spontaneous firing (at normal rates) and prolonged periods of quiescence (50). A brief burst of IPSPs, which normally produces a brief hyperpolarization that only transiently shuts off spontaneous firing, can now produce a much more stable, long-lasting hyperpolarization that inhibits spontaneous action potentials for tens of seconds. A similar bistable state can be induced in a physiologically meaningful way by inhibition of I_h through 5-HT. Thus in these cells, I_h is thought to help maintain membrane potential in a range that permits the primary pacemaking function of other channels to operate.

SUBTHRESHOLD OSCILLATIONS IN ENTORHINAL CORTEX NEURONS Certain cells that fail to fire action potentials spontaneously do show subthreshold oscillations, especially in response to depolarizing current injections. Subthreshold oscillations at a frequency of around 2–4 Hz are a prominent component of activity at potentials between -60 and -50 mV in entorhinal cortex layer 2 stellate neurons (124), which provide the main input to the hippocampus. These neurons also play an important role in the generation of hippocampal theta rhythm. I_h has recently been shown to be a critical factor in generating the stellate cell oscillations (125). Stellate neurons show a prominent I_h with rapid activation kinetics and a threshold voltage for activation of -45 mV, the region of the subthreshold oscillations. Moreover, blockade of I_h with ZD-7288 blocks the oscillations.

These oscillations were further shown to be maintained by an interplay between I_h and a rapid, persistent Na^+ current, I_{NaP} (125). During the oscillations, the magnitude of I_{NaP} tracks the voltage change precisely, owing to its rapid activation and deactivation kinetics. In contrast, I_h lags behind the voltage change by slightly more than half a cycle, because of its opposite voltage dependence and slower kinetics. As a result, the complementary activation and deactivation cycles of the two currents, together with the slight lag introduced by I_h kinetics, sustains the maintained oscillation. Although the function of these oscillations is not clear, they may be needed to integrate the many convergent inputs from association cortex to a synchronous output from an entorhinal stellate cell to the hippocampus.

Role of I_h in Synaptic Transmission

One of the most interesting and controversial roles of I_h in neuronal function is in the control of synaptic transmission. Electrophysiological recordings have demonstrated the presence of I_h in a number of presynaptic terminals, including chick ciliary ganglion neurons (126), crayfish neuromuscular junction (127), cerebellar basket cells (128), and the calyx of Held in auditory brainstem (129). HCN1 protein has also been shown to be expressed at high levels in basket cell terminals (51).

The functional role of I_h in regulating synaptic transmission is uncertain. At the calyx of Held, I_h has little or no effect on the amplitude of the synaptic response, even when the current is enhanced by cAMP (129). In cerebellar basket cells, blockade of I_h decreases the frequency of IPSPs in the postsynaptic cell (128). This effect is likely due to a reduction in spontaneous firing rate of the basket cells, because no effect was observed when I_h blockers were applied in the presence of TTX to block action potential firing.

Perhaps the most intriguing evidence for a regulatory role of I_h is in the cAMP-dependent, long-term facilitation of synaptic transmission in response to 5-HT at the crayfish neuromuscular junction (127). Beaumont & Zucker used intracellular recordings to demonstrate the presence of I_h in the crayfish motor nerve terminals. Moreover, these authors showed that blockade of I_h with ZD-7288 or Cs^+ inhibited the synaptic facilitation with 5-HT. Furthermore, hyperpolarizations that activated I_h could produce facilitation in the absence of 5-HT. At present, the mechanism whereby I_h activation enhances release remains unknown, although the enhancement is disrupted by actin depolymerization, raising the intriguing possibility that I_h channels may be directly coupled to the release process via the cytoskeleton (130).

In hippocampus, I_h has been proposed to mediate LTP at the mossy fiber synapse onto postsynaptic CA3 pyramidal neurons (131). The synaptic enhancement, induced by a brief, high-frequency tetanic stimulation of the mossy fibers, was blocked when the tetanic stimulation was applied in the presence of either ZD-7288 or DK-AH 269, two I_h antagonists. Previous studies showed that mossy fiber LTP was dependent on presynaptic cAMP and was likely mediated by an increase in transmitter release from the mossy fiber terminals (132). Mellor et al. suggested that the enhanced release is via a depolarization of the dentate granule cells and their mossy fiber terminals through activation of I_h .

However, the findings of Mellor et al. have been questioned by Chevaleyre & Castillo (133), who report a reduction in baseline synaptic transmission with either ZD-7288 or DK-AH 269. Moreover the inhibitory action of these compounds appears to be independent of any effect on I_h because the blockade of I_h , recorded in the cell soma, occurred much more rapidly than did the inhibition of synaptic transmission. When the authors used a normalization procedure to correct for any direct effect of the antagonists to depress transmission, they found that the tetanic stimulation was able to elicit a large, normal-sized LTP in the presence

of the antagonists. Thus the importance of I_h for mossy fiber LTP remains in question.

I_h AND DISEASE

Given the importance and widespread expression of I_h , both in neurons and cardiac muscle, there is surprisingly little direct evidence implicating these channels in either acquired or inherited disease. Perhaps the best-studied case is in cardiac ventricular muscle, where increases in I_h magnitude or shifts in voltage dependence have been associated with certain cardiovascular diseases (134–136). The cellular basis of this regulation is unknown, although studies on hormonal regulation suggest potential pathways for modulating HCN isoform expression. For example, in aged spontaneously hypertensive rats, where I_h is large, chronic treatment with the angiotensin II AT-1 receptor blocker losartan markedly reduces I_h maximal conductance (137). Also the β_2 -adrenergic overexpressing mouse, which is associated with cardiac hypertrophy in older mice, shows a fivefold increase in ventricular pacemaker current (73), as well as a preferential upregulation of HCN4 compared with HCN2. Finally, two separate studies report that HCN message level varies with thyroid hormone and/or thyroid hormone receptor level, perhaps contributing to the rapid heart rate associated with hyperthyroidism (138, 139).

In addition to these acquired diseases, a recent preliminary report (140) describes the first known human mutation in an HCN gene. The mutation, identified by genotyping a patient with sinus bradycardia (slow heart rate) and atrial ventricular fibrillation, results in truncation of the terminal portion of the HCN4 C-terminal, including the CNBD, which leads to a pronounced reduction in the magnitude of I_h .

I_h may also participate in neurological disease. I_h is upregulated during an experimental model of febrile seizures in rats, explaining an increased susceptibility of these animals to subsequent seizures (141). This change in I_h was associated with an increased level of expression of HCN2 mRNA in CA1 and CA3 hippocampal pyramidal neurons, together with a downregulation of HCN1 (142). An upregulation of I_h has also been reported following a lesion of the entorhinal cortex (143), which provides most of the synaptic inputs to the hippocampus and undergoes degenerative changes during Alzheimers disease (144). However, whether I_h is a causative agent in any human disease remains to be determined.

GENETIC DELETION EXPERIMENTS

Studies of the physiological and pathophysiological role of I_h in neuronal and cardiac function will be facilitated by the genetic manipulation of HCN isoforms.

To date only brief abstracts have appeared concerning deletion of HCN1 (145–147). Mice lacking HCN1 have been shown to be viable and fertile. They do show a motor deficit, as judged by performance on a rotarod, which is likely a result of the expression of HCN1 in the cerebellum because the general knockout shows altered firing rhythms in Purkinje neurons (147).

CONCLUSIONS

The hyperpolarization-activated cation current is clearly an important determinant of electrical activity in both the nervous and cardiovascular systems. Because of the unique properties of these channels—including activation by hyperpolarization, opening at resting potentials, and conduction of inward, excitatory current—they occupy a unique biophysical niche in a cell's complement of ion channels. However, the varied roles that these channels play and their complex interactions with other channels active at resting potentials often complicate the interpretation of their cellular functions.

It is thus interesting to compare and contrast the biophysical characteristics and physiological function of I_h in brain and heart. Between brain regions, the range of biophysical characteristics of I_h is certainly measurable but not extreme. Yet the range of functions ascribed to the current is quite diverse. This functional diversity may be partly attributable to different isoform expression; for example, HCN1 is often the dominant isoforms in cells that do not rely on I_h as a primary pacemaker current, whereas HCN4 is often strongly expressed in cells in which I_h plays an important role in pacemaking. However, the functional diversity also must derive from the interplay with other currents within a particular type of neuronal cell, and the synaptic connections with other neurons in the brain. Thus the diversity in I_h physiological function is, to a large extent, an integrative characteristic in the brain.

In contrast, I_h in the heart exhibits a much greater range of biophysical characteristics, particularly with regard to voltage dependence. This biophysical diversity can only partially be attributable to HCN isoform distribution. Rather, I_h in cardiac cells seems particularly susceptible to regulation by the cellular environment. Some of these cellular modulators, such as the β -subunit MiRP1, are likely to exhibit distinct regional expression, whereas others seem to vary with development or disease. Despite the great range of biophysical diversity of I_h within the heart, the primary physiological function ascribed to I_h in this organ is automaticity. The biophysical diversity does not lead to additional physiological functions but rather determines the extent to which I_h contributes to automaticity.

Given these considerations, attempts to assign a single label for the role of these channels in excitable cells is probably not productive. Rather, their function can best be understood in terms of these complex interactions within and between cells, and the particular cellular context in which these channels participate.

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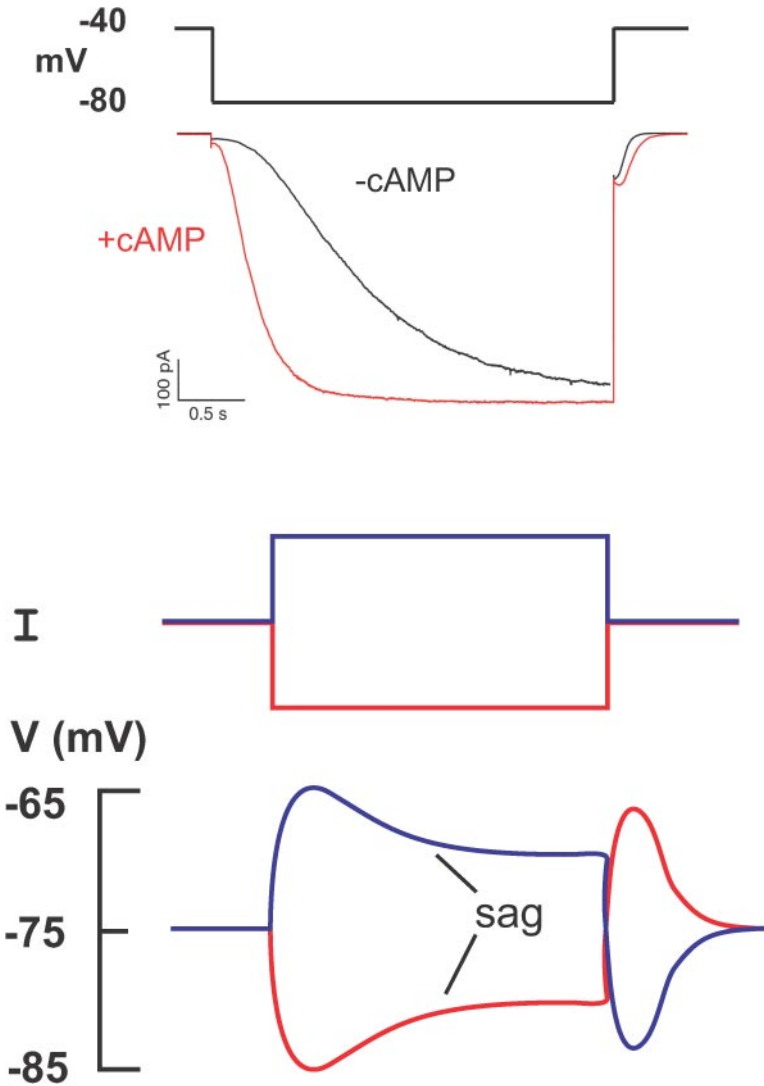
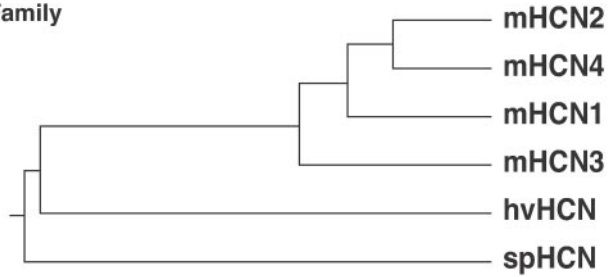


Figure 1 I_h and its role in stabilizing the resting potential. (A) Voltage-clamp experiment. Currents (*bottom*) in response to 3-s-long hyperpolarizing voltage steps (*top*), in absence and presence of cAMP. (B) Current clamp experiment. Hyperpolarizing (red) or depolarizing (blue) current steps generate a membrane potential response (*bottom*) that shows a sag back toward the resting potential owing to activation (red) or deactivation (blue) of I_h during current pulse. At end of pulse there is an afterdepolarization (red) following the hyperpolarizing current step and an afterhyperpolarization (blue) following the depolarizing step, reflecting the activation or deactivation of I_h during the preceding current steps, respectively.

HCN Channel Family



HCN Channel Domains

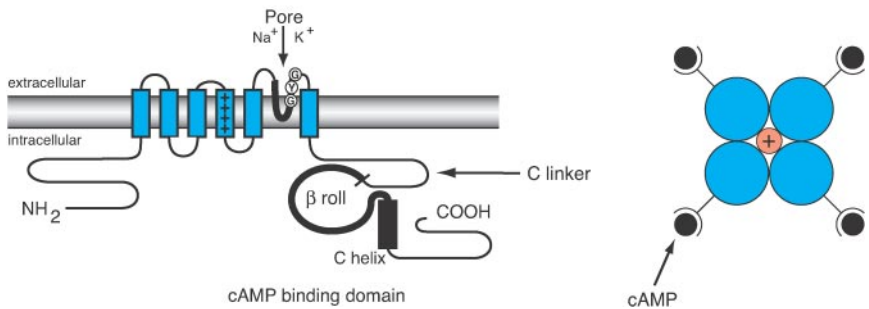


Figure 3 The HCN gene family. (*Top*) Dendrogram showing the relation between the four mammalian HCN isoforms (mHCN1–4), the insect channel from moth (hvHCN), and the sea urchin channel (spHCN). At right is a schematic of a tetrameric channel indicating four cyclic nucleotides (black circles) bound to channel, which promotes channel opening. (*Bottom, left*) Transmembrane topology of a single HCN subunit indicating S1–S6 transmembrane regions (blue rectangles), positive S4 segment, and pore-forming P-loop with GYG signature sequence of K⁺-selective channels. Also shown is carboxy-terminal C-linker, followed by a 120 amino acid CNBD, consisting of β-roll (thick black line) and C-helix (black box). Extreme amino and carboxy termini (thin black lines) are poorly conserved among HCN isoforms.