Regulation of Recombinant and Native Hyperpolarization-Activated Cation Channels

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Abstract

Ionic currents generated by hyperpolarization-activated cation-nonselective (HCN) channels have been principally known as pacemaker h-currents (I_h), because they allow cardiac and neuronal cells to be rhythmically active over precise intervals of time. Presently, these currents are implicated in numerous additional cellular functions, including neuronal integration, synaptic transmission, and sensory reception. These roles are accomplished by virtue of the regulation of I_h by both voltage and ligands. The article summarizes recent developments on the properties and allosteric interactions of these two regulatory pathways in cloned and native channels. Additionally, it discusses how the expression and properties of native channels may be controlled via regulation of the transcription of the HCN channel gene family and the assembly of channel subunits. Recently, several cardiac and neurological diseases were found to be intimately associated with a dysregulation of HCN gene transcription, suggesting that HCN-mediated currents may be involved in the pathophysiology of excitable systems. As a starting point, we briefly review the general characteristics of I_h and the regulatory mechanisms identified in heterologously expressed HCN channels.

Index Entries: Pacemaker; rhythmogenesis; ion channel; allosteric; cyclic AMP; phosphorylation; transcriptional regulation; cardiopathy; epilepsy; injury.

Molecular Commonalities of Cellular Rhythms in Cardiac and Nervous Systems

Cardiac sinoatrial cells and some central neurons exhibit pacemaking properties, which render them capable of generating electric discharges on a defined time-scale, independently of external stimuli. Rhythmicity in the heart fulfills the need to drive the periodic contractions of cardiac muscle. In the mammalian brain, rhythmic neural activity controls not only motor functions but also higher cognitive functions, such as the state of arousal and the encoding and retrieval of information. Interestingly, despite their different functions, the ionic mechanisms underlying some of these

rhythms show strong molecular commonalities. Indeed the ionic channels that generate autonomous pacemaking capabilities in cardiac and nervous tissue are all members of the small family of voltage- and ligand-gated pacemaker channels.

Pacemaker channels belong to the superfamily of voltage-gated ion channels but form a distinct subgroup that is closely related to the voltage-independent, cyclic nucleotide-gated channels. The molecular structure of the four cloned channel subunits, which are termed hyperpolarization-activated cation-nonselective (HCN) channels 1–4, exhibits both voltage-sensing and ligand-binding domains (Fig. 1A; for recent reviews, *see* refs. *1–4*). It is increasingly clear that the four HCN channel subtypes give rise to ionic currents that are involved in an unusually broad range of neural functions reaching beyond single-cell rhythmogenesis. The wide physiological context in which HCN channels are active is based on the rich repertoire of modulatory pathways to which the channels can be subjected.

Not only are HCN channels gated by voltage but they also contain binding sites for intra- and extracellular ligands (for review, *see* refs. *2* and *4*). Furthermore, subunit heteromerization, glycosylation, and association with auxiliary subunits are important determinants of the functional properties of expressed channels *(5–9)*. In contrast to the rapidly expanding insight into the regulation of channel molecules in heterologous systems, the signaling pathways and the physiological context that determine the regulation of pacemaker currents in native cells is just beginning to be explored. In addition to regulatory properties reminiscent of those expressed HCN channels, channels in native tissue appear to be regulated via activity-dependent and -independent, short- and long-term alterations in HCN messenger RNA (mRNA) and protein expression *(10)*. This latter level of regulation contributes to the developmentally controlled I_h expression but may also account for the causes and/or consequences of some cardiac and neurological pathologies.

Basic Properties of Native I_h

This section highlights three peculiar properties of pacemaker currents that make them unique among the family of voltage-gated ionic currents and that earned them the name I_f for "funny current" in the heart *(11)*, or I_q, for "queer current" in the brain *(12)*, when they were originally discovered. First, the currents typically activate upon membrane hyperpolarization (below approx–60 mV) rather than depolarization, opposite to most voltage-gated ionic currents involved in shaping the neuronal response to excitatory input (Fig. 1B). This unusual voltage window of activation is reflected in the now widely used name h-current (I_h), where "h" stands for hyperpolarization. Upon hyperpolarization, the conductance activated is permeable to both Na^+ and K^+ ions (permeability ratio $Na^+ : K^+$ = 0.2–0.4). The current is carried mainly by Na^+ ions at the membrane voltages within its activation range and produces an elevation in the intracellular Na^+ concentration *(13)*. More recently, a small permeability to Ca^{2+} ions was also identified via imaging techniques *(14)*. The current is blocked by at least four distinct classes of agents. Extracellular blockage occurs by millimolar concentrations of Cs^+ or by capsazepine, a blocker of vanilloid receptors *(15, 15a)*; intracellular blockage occurs by the lidocaine derivative QX-314 *(16)* or by bradycardiac agents (e.g., ZD7288, *see* refs. *4* and *17–19*). However, some of these compounds show nonspecific effects: Cs^+ blocks neuronal K^+ channels *(20)* and interferes with K^+ uptake in glial cells *(21)*, whereas ZD7288 depresses synaptic transmission *(19)*. Therefore, unless more selective blockers are developed, the pharmacological identification of novel physiological roles of I_h should be based on the effects of several blockers that belong to different classes.

Second, activation of the current is fairly slow, with activation time constants ranging between hundreds of milliseconds and seconds, even at strongly hyperpolarized voltages around –100 mV. The few exceptions include
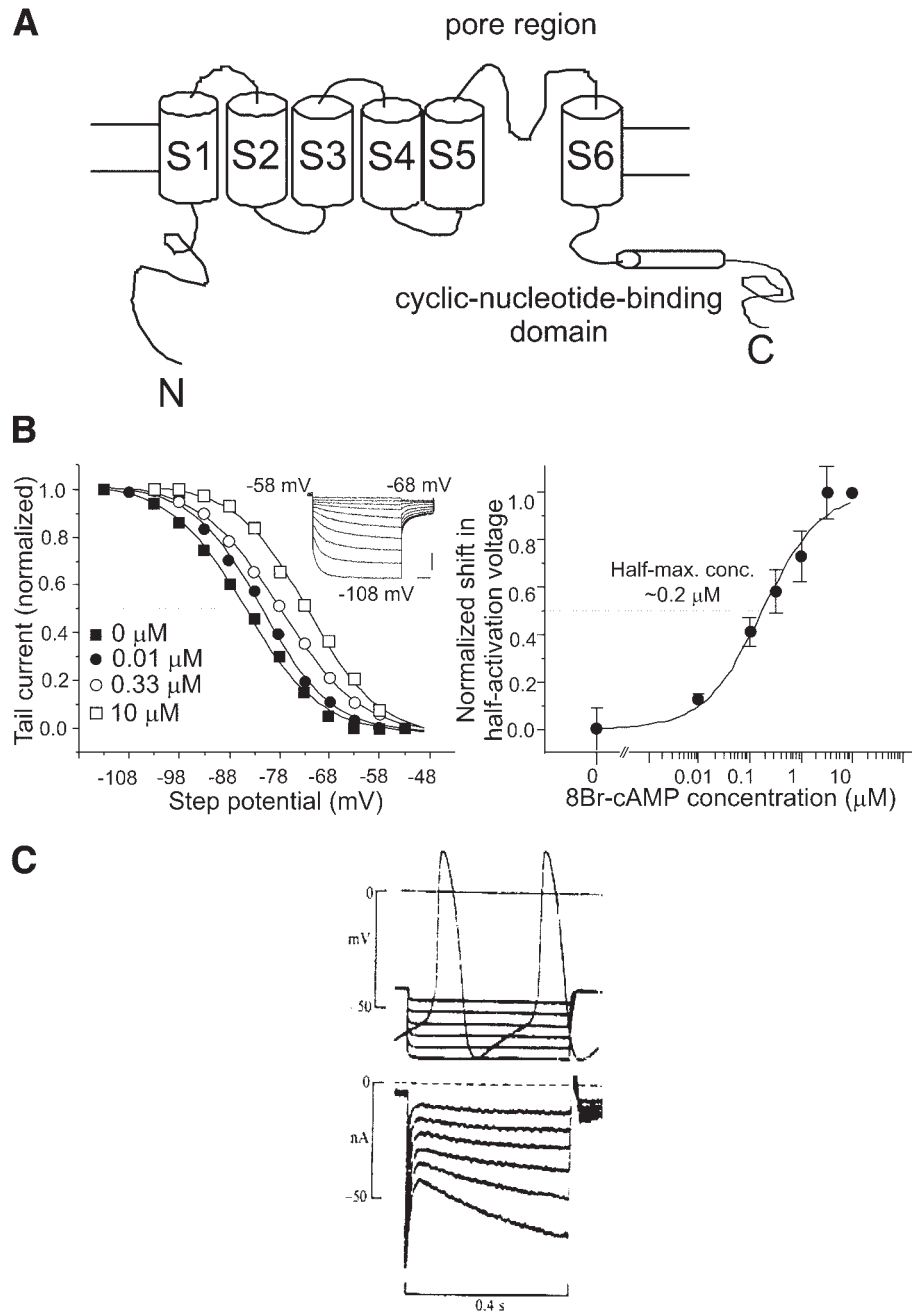
Fig. 1. Summary of basic functional and structural characteristics of I_h and the underlying HCN channels. **(A).** Transmembrane topology of the cloned HCN channels. S1–S6 symbolize the six transmembrane-spanning domains of the channels; N and C are the N- and C-terminus, respectively. The box at the C-terminus represents the cyclic nucleotide-binding domain, which is connected to the channel via a C-linker domain *(94)* that is important in coupling the binding of cyclic nucleotide to alterations in voltage-gating of the channel. The number of amino acids at both termini vary for the four HCN subunits. **(B).** *Left,* Activation curves of I_h recorded in ferret thalamocortical cells in the presence of incrementing concentrations of 8Br-cAMP, a weakly hydrolyzable analog of cAMP, in the whole-cell recording pipet. Note the progressive rightward shift of the activation curve with increasing levels of the cyclic nucleotide. *Inset* shows a family of current responses 0 µM 8Br-cAMP generated by steps to the voltages indicated. Vertical and horizontal scale bars indicate 400 pA and 400 ms, respectively. *Right,* Concentration–response curve of the shift in the half-activation voltage induced by 8Br-cAMP. The maximal shift corresponds to 10 mV. **(C).** Identification of the "funny" current, activating at voltage ranges covered by the diastolic potential. (Reproduced from ref. *11*; copyright permission from *Nature.*)

pyramidal neurons from hippocampus, cortex, and cerebellum, in which activation is complete within tens of milliseconds (*see* the section on Basic Properties and Regulation of Cloned HCN Channels). Once activated, the current does not inactivate, such that a steadily activated ("standing") I_h contributes to the resting membrane potential in many neurons, often by opposing the action of tonic outward currents *(22–25)*.

Third, in most cases, I_h is exceedingly sensitive to the presence of intracellular cyclic nucleotides. The nucleotides cyclic adenosine monophosphate (cAMP) and, to a much weaker extent, cyclic guanine monophosphate (cGMP; for review *see* refs. *3* and *4*) not only accelerate the kinetics of activation but also shift the voltage dependence of activation toward more depolarized values (Fig. 1B). In the presence of these ligands, the extent and duration of current activation at a given voltage is substantially increased.

In summary, I_h is generated by voltage-gated ionic channels that are also sensitive to intracellular ligands—the cyclic nucleotides. Thus, they are part of a small group of ionic channels that are dually gated by both ligands and voltage (Fig. 1B). The molecular correlates of I_h are phylogenetically related to the ether-à-go-go channels and plant inward rectifier currents, which are also gated by voltage and cyclic nucleotides *(26)*. This dual gating imparts an unprecedented level of flexibility to channel function that so far has been most impressively illustrated by studying the function of HCN channels.

The Multiple Roles of I_h

Oshima et al. *(27,28)* originally described deviations from ohmic behavior in the steady-state current–voltage relationships of motoneurons. These were termed anomalous or inward rectification, referring to the increase in slope conductance that occurs when neuronal membranes are hyperpolarized. A physiological role for the conductance underlying this abnormal

behavior was first reported in rod photoreceptor cells, in which a Cs^+-sensitive membrane conductance *(29)* permeable to both Na^+ and K^+ ions *(30)* caused a rebound depolarization during light-induced hyperpolarization. This conductance activated at potentials below –50 mV and manifested as a slow inward current that was capable of depolarizing the membrane over the time-course of seconds. Vertebrate rod photoreceptor cell membranes reached potentials below –50 mV upon light-induced hyperpolarization *(29,31)*. Thus, activation of this conductance opposes the cellular response to prolonged exposure to light and, therefore, is involved in adaptation to visual stimuli.

Interest in slowly activating cation currents gated by hyperpolarization grew considerably when it was discovered that in cardiac tissue, such a current could endow cells with an intrinsic propensity to generate oscillatory activity (Fig. 1C; for review, *see* ref. *32*). The diastolic phase of the heartbeat cycle is associated with membrane hyperpolarization that is large enough to allow the voltage-gating of this current *(11)*. The diastolic depolarization eventually reaches the threshold for Ca^{2+} current activation and action potential firing. Although the diastolic voltage waveform is generated by a combination of voltage-gated currents *(33,34;* for review, *see* ref. *35)*, I_h is essential for the generation of rhythmic cardiac output. Thus, genetic deletion or dominant-negative suppression of HCN channel subunits perturbs the rhythmic depolarizations in intact heart *(36)*, sinoatrial node cells *(37)*, and cultured neonatal cardiomyocytes *(38)*. Moreover, zebrafish that carry a mutation in the *slo mo* gene show a slowed heart rate associated with a decreased amplitude of I_h in cardiomyocytes, whereas other currents involved in cardiac pacemaking remain unchanged *(39,40)*.

H-currents with properties similar to the originally described cardiac I_f were later identified in a large number of electrically excitable cells, ranging from uterine smooth muscle cells *(41)* and enteric neurons *(42)* to the pyramidal neurons of the hippocampus *(12)* and cortex *(43)*. However, to date, convincing evidence for

Rhythmogenesis
Resting membrane potential/stability
Primary sensory reception

Synaptic transmission

Temporal summation of
excitative inputs

)(Voltage-mediated regulation

)(Cyclic-AMP-mediated regulation
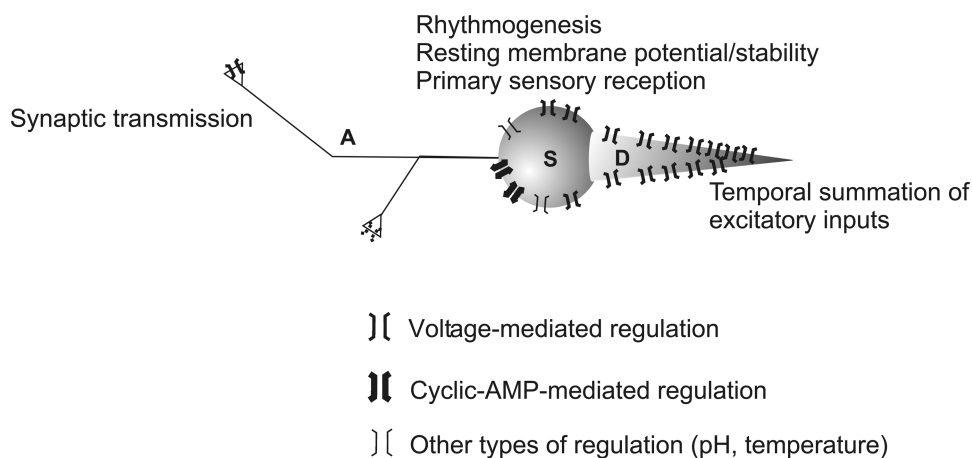
)(Other types of regulation (pH, temperature)

Fig. 2. Summary of old and novel physiological roles of I_h in neurons. A single cell is drawn schematically with an axonal (A), somatic (S), and dendritic (D) compartment. The axonal compartment can also represent a presynaptic specialization in retinal neurons. The roles of I_h are described with key words assigned to these compartments. The different regulatory pathways involved in these roles are symbolized by ionic channels with various line styles, as indicated in the legend.

a role of I_h as a pacemaker current—particularly the determination of its active pacemaker role independently of effects on resting membrane potential—is available for a fairly small number of neural rhythms. The most prominent among these include sleep-related rhythms generated by thalamocortical neurons *(44)* and thalamic networks *(45,46)*, γ-oscillations in the hippocampus *(47)*, synchronized oscillations in the inferior olive *(48)*, and subthreshold oscillations in the entorhinal cortex *(49)*. Furthermore, in slice preparations, spontaneous firing of hippocampal interneurons *(50)*, neostriatal interneurons *(51)*, substantia nigra *(52)*, and area postrema neurons *(53)* depends on activation of I_h between individual action potentials. However, in several rhythmically active systems, other currents are rhythmogenic with a minor role of I_h, such as in respiratory rhythms *(54)*, supraoptic neurons *(55)*, or paroxysmal discharges in neonatal hippocampus *(56)*.

Besides being involved in rhythmicities and control of membrane potential, I_h is now known to contribute to additional central neuronal functions, such as dendritic integration,

synaptic release, and two types of primary sensory reception (Fig. 2; for further review, *see* ref. *4)*. The novel roles of I_h predominantly rely on its partial steady activation at the resting membrane potential and its modulation by intra- and extracellular signaling molecules. In dendrites of hippocampal CA1 *(57)* and neocortical layer V pyramidal *(58,59)* neurons, a standing I_h contributes to the resting membrane potential of dendrites by up to 11 mV *(58)*. The rapid deactivation of I_h during excitatory inputs produces a hyperpolarization that accelerates the decay of synaptic potentials. During repetitive presynaptic discharges at intervals of up to less than 5 ms, the temporal summation of postsynaptic responses is further attenuated by the accruing deactivation of I_h. Interestingly, the density of I_h augments by several-fold along the somatodendritic axis of apical dendrites *(60)*. Therefore, its effects on the temporal summation of distal synaptic inputs is increasingly pronounced. Indeed, the density of I_h appears to be tuned so that it exactly compensates the incrementing electrotonic filtering of the dendritic cables, indicating that I_h is a major factor

in normalizing temporal summation in CA1 and cortical pyramidal cells. As a physiological consequence, temporal summation of sub-threshold excitatory inputs in principal hip-pocampal and cortical neurons (and hence the eventual timing of action potential generation as well) are independent of the location of synaptic input (for review, *see* refs. *61* and *62*). Therefore, the subcellular expression of I_h helps excitatory inputs into the dendritic trees to con-vey the same temporal information indepen-dently of where they were generated.

By virtue of its voltage dependence, the h-current also dampens cellular responses to inhibitory synaptic input and allows a rapid resumption of tonic firing, as is shown by recording the response of Purkinje cells to ramp- or pulse-like injections of currents when I_h is either blocked *(63)* or the *HCN1* gene is knocked out *(64).* HCN1-deficient Purkinje cells completely lack an h-current and show a retarded generation of action potentials during the transition from subthreshold to supra-threshold current injections *(64).* Furthermore, sinusoidal current injections into the dendrites of cortical and hippocampal principal cells has revealed that I_h controls the temporal relation-ship between the phase of the current injection and the timing of action potentials *(65,66).* The involvement of I_h in the control of the phase relationship between a periodic stimulus and repetitive action potential generation may have direct consequences at the behavioral level. Thus, HCN1-knockout mice are selec-tively compromised in learning repetitive motor tasks that involve the phasic excitation and inhibition of cerebellar Purkinje cells. To explain this deficit, it has been proposed that I_h of Purkinje cells may be involved in the plastic events that lead to motor learning, perhaps by facilitating the coincidence of pre- and postsy-naptic activity in the time window required for synaptic plasticity of afferents to Purkinje fibers *(64).*

Interestingly, HCN1 protein is also expressed in presynaptic terminals of cerebellar basket cells *(67)* and—together with HCN2 and HCN4—in the presynaptic zones of retinal

bipolar cells *(68).* Clustering of HCN3 protein was found at the base of the pedicles generated by cone photoreceptor cells, *(68).* Electrophysi-ologically, the presence of I_h has been verified in the terminals of inhibitory cerebellar basket cells as well as in those of excitatory brainstem afferents, but the role it plays in neurotransmit-ter release appears to be minor *(69,70).* How-ever, if I_h is activated for prolonged periods of time (>10 s), the Ca^{2+} ions permeating through the channels can facilitate neurotransmitter release in response to repetitive action potential discharge in dorsal root ganglia neurons *(14).* This suggests that the elevation of basal Ca^{2+} levels during periods of hyperpolarization can modulate synaptic short-term plasticity *(14).* It has also been proposed that presynaptically expressed I_h may contribute to modulation of synaptic transmission; it may also underlie presynaptic forms of long-term potentiation via detection of the cAMP generated through either receptors for neuromodulatory transmit-ters or Ca^{2+}-sensitive adenylyl cyclases (ACs) *(71–73;* however, *see* ref. *19).*

Finally, primary sensory pathways exploit I_h for stimulus detection. Upon application of a low pH solution, a subset of cells that are sensitive to sour taste generates an inward current that is blocked by Cs^+ ions *(74).* The expression of HCN1 and HCN4 protein in these cells implicates I_h as a major component in the detection of sour stimuli *(74).* Reduc-tion of a standing I_h by lowering temperature also contributes to the control of the ther-mosensation of a subgroup of trigeminal neu-rons *(75).*

Basic Properties and Regulation of Cloned HCN Channels

Three groups have succeeded independently of each other in identifying the genes that encode HCN channel subunits *(76–79).* These subunits display the overall membrane topol-ogy of voltage-gated K^+ channels, with six transmembrane domains S1–S6 (Fig. 1A). The sequence motifs that are typical for voltage-

gated ion channels are present in these domains, including the amino acid sequence GYG (which is characteristic for the narrow portion of the selectivity filter in voltage-gated K^+ channels between S5 and S6) and the voltage-sensor motif (which has regularly spaced, positively charged amino acids in S4) *(80; for review, see* ref. *81)*. Thus far, HCN1, -2, and -4 genes that are transcribed in heterologous expression systems give rise to currents with the properties typical for I_h: activation upon hyperpolarization and modulation by intracellular cAMP. Currents that are mediated by HCN3 subunits have not been described *(see* ref. *9)*. The detailed characteristics of these properties are strikingly different between channels generated by homomeric assembly of HCN1, -2, or -4. Whereas channels composed of HCN1 subunits activate rapidly (within tens of milliseconds at voltages below –100 mV) and are weakly sensitive to cAMP, HCN2 and, especially, HCN4 subunits give rise to currents that activate slowly (hundreds of milliseconds to seconds below –100 mV) and are highly sensitive to cAMP. Besides generating hyperpolarization-activated inward currents, HCN1 and -2 homomers also give rise to an instantaneous current component that is Cs^+-insensitive and voltage-independent *(82,83)*.

The expression of HCN genes at the level of the mRNA distribution in the brain reveals complementary, yet partially overlapping, expression profiles, which correlate reasonably well with the characteristics of native I_h in diverse neuronal cell types. HCN1 expression predominantly occurs in cortical, hippocampal, and cerebellar regions, whereas HCN2 expression is widespread and, concomitantly with HCN4, is found in regions in which I_h functions as a pacemaker *(84–87)*. To a first approximation, the heterogeneous properties of native I_h are believed to arise from this differential expression of HCN1, -2 and -4.

With the exception of HCN2 + HCN3, all dual combinations of channel subunits can give rise to heteromeric channel complexes inserted into membranes *(9)*. Electrophysiologically, the currents that are generated by at least some of the heteromeric channels show properties that are intermediate but that are distinct from those predicted through interpolation between the characteristics of the homomeric channels *(7,88)*. The demonstration that heteromerization generates unique forms of I_h, together with the overlapping mRNA expression patterns for HCN subunits in cardiac cells and in neurons *(77,78,84–87)*, indicates that formation of heteromers may contribute to the specification of native I_h. This issue was addressed by comparing the properties of native currents and of heteromers that consisted of the subunits expressed in the cells. In sinoatrial node, heteromers that were generated by HCN1–HCN4 reproduced the kinetics, but not cAMP sensitivity, of the native current *(7)*. In contrast, a linear superposition of the currents that were generated by HCN1 and HCN4 homomers accounted well for the kinetics of I_h that was found in subtypes of retinal bipolar cells *(68)*.

Heterologous expression experiments have also reported that accessory proteins may further determine native current properties. Currents generated by HCN1, -2, and -4 homomers are substantially larger in amplitude and are modulated in activation kinetics when coexpressed with the protein MinK-related peptide 1, which is an accessory protein of several K^+ channels (for a review, *see* ref. *89)*. These single transmembrane-spanning proteins could functionally interact with the C-terminal domain of HCN channels and contribute to the diversity in the whole-cell current *(6,8; however, see* ref. *7)*.

The molecular correlate of the observed sensitivity of I_h to cAMP resides in a cytosolic C-terminal cyclic nucleotide-binding domain (CNBD) that is highly homologous to the CNBD of kinases and to catabolite gene activator protein, which is a metabolic protein from *Escherichia coli (3,26)*. Removal of the CNBD or mutations of single amino acids abolishes the cyclic nucleotide sensitivity of the expressed channels *(88,90,91)*. The CNBDs of each subunit must be bound to cAMP to achieve a maximal effect on the voltage dependence *(91)*. An

exposed C-terminal domain (likely containing the CNBD) confers cAMP sensitivity in native currents as well. Cardiac cells, infusion of C-terminal-specific proteases into the intracellular compartment abolishes the cyclic nucleotide sensitivity of I_h *(92)*, yet leaves voltage sensitivity intact. Thus, the dual gating of both cloned and native I_h appears to be based on the modular composition of channel subunits by sequentially arranged voltage- and ligand-sensing domains.

A complicating aspect of the dual gating of HCN channels is the fact that the voltage- and ligand-sensing portions do not act independently of each other. This is particularly evident when recording currents generated by channels that are devoid of a CNBD. Such truncated channels show a dramatically accelerated activation comparable to that induced by exposure of the cytosolic face to maximal concentrations of cAMP *(90)*. This suggests that in the intact channel, the ligand-free CNBD influences the voltage-sensing transmembrane channel portions in a manner that retards the opening of the pore *(90,92)*, whereas binding of the cyclic nucleotides has an effect on activation kinetics that is equivalent to that of physically removing the CNBD from the protein. The interaction between ligand- and voltage-sensing domains accounts not only for the current acceleration in the presence of cAMP but also for the shift in the voltage dependence of I_h by cAMP *(90,93,94)*. In the case of HCN1 and -2, the differences in the kinetics and the variable cAMP response of the homomers arise, at least partly, from sequence differences within the CNBD and the C-linker domains that connect the CNBD to S6 *(94)*.

Dual gating by voltage and ligand has additional important implications for the dynamics of current activation: it allows for a prolonged activation of I_h that outlasts the presence of free ligands. Two computational studies approximated the gating of channels in cyclic allosteric gating models to explain the generation of such persistently activated I_h (Fig. 3A) *(93,95)*. In a Monod–Wyman–Changeux model, four distinct states of the entire channel were arranged

in a cycle *(95):* (a) the closed unliganded state; (b) the open unliganded state; (c) the closed liganded state; and (d) the open liganded state. The stabilized activation of I_h is explained by an 80-fold increase in the cAMP binding affinity to the open channel compared to the closed channel, such that voltage-gating of the unliganded channel facilitates binding of cAMP. A Hodgkin–Huxley model on cardiac I_h that incorporated two allosterically gated channel subunits produced a 6-fold decrease in the dissociation constant, thus yielding a 36-fold decrease for the dimer *(93)*. Both models demonstrate that the dually gated channels represent the channel configuration with the greatest free-energy decrease *(93)*. Therefore, these are the most reluctant to closure via an imposed depolarizing voltage and lead to a persistence of channels in the dually gated mode. These allosteric models show that by becoming "trapped" in the dually gated mode, channels can remain activated for prolonged periods of time—even if they have been only transiently exposed to both stimuli *(95)*. This results in the appearance of persistent, very slowly decaying current components.

In addition to being gated by voltage and cyclic nucleotides, HCN2 channel subunits are sensitive to changes in pH. Decreases in pH in the intracellular compartment, from 7.4 to 6.4, result in a downregulation of the current and in a slowing of the speed of activation. Conversely, alkalinization enhances current amplitude and activation rate. The sensitivity to pH_i allows shifts in voltage dependence of up to 20 mV and is mediated by a single His residue located within the linker between domains S4 and S5 *(96)*. While HCN2 is sensitive to changes in internal pH, the channel subunits HCN1 and -4 sense extracellular pH alterations—albeit with comparatively weak sensitivity. HCN1-mediated currents show a positive shift (up to 35 mV) in the voltage dependence when pH_e is decreased from 7.4 to 3.9; this is associated with a strong acceleration in the activation time course.

The primary sequence of HCN channels shows at least one potential consensus phospho-
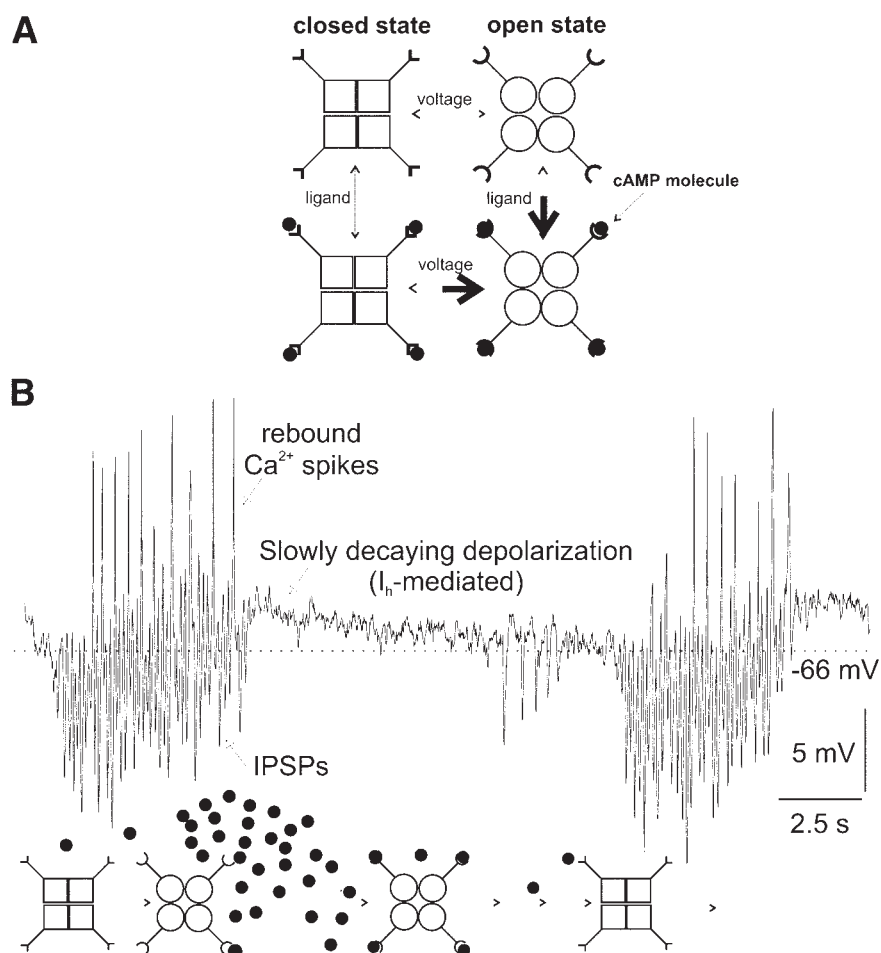
Fig. 3. The dual gating of I_h by voltage and cyclic nucleotides: model and its physiological consequences. **(A).** Model of a channel occupying four possible states following concerted voltage-induced transitions between the four closed (squares) and the four open (circles) subunits, as well as via ligand binding and unbinding. Binding sites for cyclic nucleotides are shown as half-squares or half-circles attached to the closed and open subunits by a line. Cyclic nucleotides are symbolized by filled circles. Thick arrows mark the preferred directions of transitions. For further details, *see* ref. *95.* **(B).** Dual gating of I_h by voltage and ligands results in a persistent activation of the channels that contributes to the timing of slow network rhythms. An intracellular recording from a ferret thalamocortical cell participating in spindle waves in vitro is shown, indicating the coincidental occurrence of repetitive hyperpolarizing inputs (inhibitory postsynaptic potentials [IPSPs]) and the rebound Ca^{2+} spikes, which trigger a CA^{2+}-dependent synthesis of cAMP (filled circles). Note the small depolarization (I_h-mediated) following each spindle wave. Presumed channel states occupied preferentially during the different phases of the network rhythms are shown at the bottom. Note the persistence of channels in the dually gated states even after the cAMP transient has mostly dissipated. For further details, *see* references in Regulation of Native I_h, Section 6.1.

rylation site for protein kinase A (PKA), which resides within the CNBD *(78).* Furthermore, one of the successful approaches to clone the HCN channels was based on a yeast-two-hybrid screen that searched for proteins interacting with the SH3 domain of the neural-specific form of the protein tyrosine kinase (PTK) Src *(67).* This suggests that some HCN channels may molecularly

interact with protein kinases; this is comparable to the association of invertebrate cation channels *(97)* or human K$^+$ channels *(98)* with their regulatory kinases via Src homology 3-domains. However, direct evidence is currently lacking for a functional consequence of phosphorylation of HCN channels, although preliminary reports suggest a role for PKA and PTKs in controlling the maximal conductance and voltage dependence of HCN2 and -4 *(99,100)*. The finding that the cyclic nucleotide-gated channels, close relatives of HCN channels, can be phosphorylated by both Serine/Threonine (Ser/ Thr) as well as tyrosine kinase activity further supports potentially interesting roles of HCN channel phosphorylation (for review, *see* ref. *101*). These phosphorylations control channel-apparent affinity for cyclic nucleotides and could be important for circadian modulation of ligand sensitivity in cone photoreceptor cells *(102)*.

In summary, the cloning and functional expression of HCN channel subunits has revealed an array of modulatory capacities of the corresponding currents. We now discuss which of these are likely to be functionally exploited in native cells and how channel expression is regulated under pathological cardiac and neural conditions.

Regulation of Native I$_h$

Regulation by Ligands and Phosphorylation

Direct Regulation By cAMP

The recognition of I$_h$ regulation by cyclic nucleotides was intimately associated with the identification of the currents themselves. The quest for the ionic mechanism underlying the acceleration of the heartbeat via adrenaline revealed an enhancement of I$_h$ amplitude upon exposure to adrenaline *(11)*. Noradrenaline had long been associated with increases in the concentration of intracellular cyclic nucleotides in cardiac cells *(103,104)*. It was then demonstrated that the amplitude of I$_h$ in sinoatrial node cells could be enhanced by cAMP that was directly applied to the cytosolic portion of

cell-free patches, and neither constitutively active PKA nor PKA blockade interfered with this modulation *(105)*. Furthermore, a β-adrenergically mediated membrane depolarization in CA1 pyramidal cells was not affected by PKA inhibitors but was blocked by Cs$^+$ ions *(106)*. Since then, several neurons in slice preparations have shown a modulation of I$_h$ by neurotransmitter receptors, in a manner that implicates a direct action of cAMP. β-adrenergic receptors in thalamocortical neurons *(107–109)*, 5-HT receptors in hypoglossal neurons *(110)*, and neonatal rat motoneurons *(111,112)* have been prominent among these receptors. Furthermore, endogenous neuropeptides, such as vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide, potentiate I$_h$ via a mechanism that involves cAMP *(113,114)*, whereas substance P-mediated activation of neurokinin-1 receptors inhibits I$_h$ in sensory neurons through a messenger pathway that is yet to be determined *(115)*. I$_h$ also detects the increase of cAMP produced by prostaglandins in sensory neurons *(116)*. The application of PKA inhibitors did not affect the action of the agonists for at least some G$_s$-coupled neurotransmitter receptors *(106,112, 116)*, which further supports the idea that the effects of cAMP were also direct in cellular preparations.

In contrast to the G$_s$-dependent stimulatory effects, G$_i$-dependent inhibitory actions on cAMP-dependent regulation of I$_h$ have been documented in only a few cases, and a complete demonstration that these are mediated by inhibition of endogenous AC activity has been more difficult. In nodose ganglion neurons, opiod receptor activation inhibited forskalin-stimulated, but not basal AC activity *(117)*. In contrast, in thalamocortical and cholinergic mesopontine neurons, activation of adenosine A1 receptors inhibited I$_h$ in a manner that was consistent with inhibition of basal AC activity *(108,118)*, although an aderosine-induced decrease in cAMP sensitivity of the channels was not excluded in these studies. Using a combined assessment of both basal AC activity and channel sensitivity, we

recently provided more complete evidence that, in the case of the $G_{i/o}$-coupled γ-aminobutyric acid (GABA)$_B$ receptors, a substantial portion of the reduction in I_h amplitude is attributable to the inhibition of comparatively high basal AC activity in thalamocortical neurons *(109).*

An additional pathway of cAMP-dependent regulation was uncovered by addressing the issue of how positive and negative stimuli on cAMP synthesis summated when activated simultaneously. Contrary to a linear summation of the effects induced by agonists for β-adrenergic and GABA$_B$ receptors (and thus to a cancellation of these two stimuli), a marked potentiation of I_h amplitude was revealed that appeared to be induced by a distinct, powerful synthesis of cAMP *(109).* Furthermore, I_h can also be regulated by cAMP following increases in intracellular Ca^{2+} levels *(119)* or by cGMP via the nitric oxide pathway *(120).* Therefore, HCN channels are targeted by multiple pathways of cyclic nucleotide synthesis, suggesting that the channels may be surrounded by several—perhaps molecularly distinct—ACs.

Little is known about the molecular organization and the subtypes of ACs and associated regulatory enzymes that target I_h downstream from the neurotransmitter receptors. To date, at least nine different subtypes of ACs are characterized molecularly (for review, *see* refs. *121* and *122*), many of them with distributions that overlap the areas in which HCN channels are expressed *(123,124).* Based on the findings of native current regulation, it is conceivable that several molecularly distinct types of ACs generate cAMP that is detected by HCN channels. Regulation of I_h in thalamocortical neurons is a good example. In these cells, the influence of Ca^{2+} in the regulation of I_h points to an involvement of the Ca^{2+}-sensitive type I and/or type VIII ACs, both of which are expressed in thalamocortical neurons *(123,125).* Furthermore, the synergistic effect found by coactivation of G_s- and G_i-coupled neurotransmitter receptors strongly suggests a functional association of I_h with type II or IV ACs, which require binding

of both G_s- and $G_{βγ}$-subunits for activation (for review, *see* ref. *122*).

Finally, guanylyl cyclase also modulates I_h in thalamocortical cells *(120).* The fact that an ionic current is regulated by multiple enzymes that produce the same second messenger suggests that native membranes: (a) channels that give rise to I_h are localized in subpopulations or clusters, each of which is associated with a distinct cAMP synthesis pathway, perhaps similar to the association of Ca^{2+}-dependent K^+ channels with specific sources of Ca^{2+} (for review, *see* ref. *126*); or (b) channels that underlie I_h are colocalized with several types of ACs. Thus, a future goal in elucidating the regulation of I_h may focus on the characterization of the subcellular organization of channels with associated regulatory systems in a manner similar to that achieved for other ionic channels that are involved in cardiac *(127)* or neuronal *(126)* rhythmicity.

The extensive characterization of the multiple pathways of cyclic nucleotide-dependent regulation of I_h is quite different from the relative lack of understanding regarding the physiological conditions during which these types of regulation are induced. For example, it is still unclear whether the G protein-coupled neurotransmitter receptors (GPCRs) that lead to regulation of I_h can be synaptically activated. Alternatively, extrasynaptically located receptors may set a background level of ongoing G protein activity that determines a tonic exposure of I_h to cyclic nucleotides.

We recently studied the effects of GABA$_B$ receptor-mediated modulation of I_h and found that synaptically activated receptors can contribute to potentiate β-adrenergically mediated augmentation of the current *(109).* In contrast, synaptic activation of GABA$_B$ receptors alone did not result in a modulation, although addition of agonists for these receptors to the bath downregulated I_h *(109).* Therefore, at least some pathways of cAMP synthesis targeting I_h are coupled to GPCRs that can be activated following synaptic stimulation. Additionally, synaptically activated ionotropic glutamate receptors can contribute to the sources of Ca^{2+}

leading to acute regulation of I_h *(128)*. Therefore, currently available data clearly show that channels generating I_h belong to the family of ion channels that is regulated by synaptically activated neurotransmitter receptors.

Allosteric Regulation

A few years before the characterization of the dual allosteric gating of HCN channels, experiments that addressed the dynamics of I_h activation by cAMP were strongly suggestive of a preferred interaction of cAMP with the open state of the channels. For example, the duration of cAMP-mediated effects was found to depend on the voltage protocol used to activate the current *(119)*. If a transient cAMP stimulus was applied to a cell whose membrane potential was constantly held within the activation range of I_h, then current upregulation lasted longer than when the current was gated with brief hyperpolarizing steps from a holding potential outside the activation range. Voltage-gating at the channel therefore helps to produce a slowly developing, persistently activated current component in the presence at cAMP *(119)*. This prolonged activation of I_h is associated with a number of rhythmic network activities *(45,48,129)*. One illustrative example is the spindle waves, which arise predominantly during early periods of slow-wave sleep and are generated from a reciprocal synaptic interaction between thalamocortical neurons and nucleus reticularis neurons (for review, *see* ref. *130*). In vitro, spindle waves appear as 1- to 3-s periods of 6 to 14 Hz synchronized oscillatory activity that are interspersed with silent periods of 5 to 20 s (Fig. 3B). These silent periods are associated with a slowly decaying, I_h-dependent membrane depolarization that is maximal after the end of a phase of synchrony and fully disappears before the occurrence of the next spindle wave. It was initially proposed that this slow form of I_h enhancement could be explained by the slow kinetics of voltage-dependent deactivation of I_h *(45)*. However, closer inspection of the factors that induced the upregulation revealed a critical role for increases in intracellular Ca^{2+}, primarily triggered by the low-threshold Ca^{2+} bursts that occurred during spindling *(131)*. The Ca^{2+} ions are detected by a Ca^{2+}-sensitive AC, producing an increase in cAMP synthesis that enhances I_h *(119)*. The dual exposure of I_h to cAMP and to the repetitive inhibitory input during a spindle wave facilitates persistently activated I_h, which in turn prevents the next spindle wave until I_h is slowly decayed (Fig. 3B). Thus, persistent activation of I_h (based on allosterically stabilized ion channel configurations) is the electrophysiological consequence of activity-induced synaptic and biochemical events associated with synchronized network rhythms.

Regulation by Phosphorylation

In parallel to the identification of direct actions of cAMP on I_h, several studies have reported that in some preparations, cAMP-dependent actions on I_h could be completely blocked when inhibitors of protein kinases, including PKA, were present *(132,133)*. Support for a role of Ser/Thr protein kinase activity in tonically controlling the properties and regulation of cardiac I_h was found in studies that used selective Ser/Thr phosphatase inhibitors, which induced a positive shift in the activation curve and—at least in one preparation—an increase in the maximal conductance of the current *(134,135)*. In dorsal root ganglia and olfactory receptor neurons, the voltage dependence of basal I_h is subject to PKA-dependent phosphorylation, as assessed by specific inhibitors of this enzyme *(136,137)*. Stimulation of PKA can lead to a shift in the activation curve that is superimposed on that induced by a maximal dose of cAMP *(136)*. Additionally, activated PKA alters the dose–response curve of current activation to cAMP, rendering the channels preferentially sensitive to large changes in the concentration of this cyclic nucleotide *(135,136)*. Altogether, currently available data indicate that, at least for certain types of I_h, PKA activity is an additional parameter that determines the functionality of the ionic channels as well as the associated regulated systems. The level of action of the phosphorylation process may occur as a covalent

modification of the channel subunits or auxiliary subunits, and regulation of channel protein recycling to alter the maximal conductance may also be a potential target of kinases.

The phosphorylation of channel proteins has been repeatedly reported to play a pivotal role in the maintenance of current properties over time (for review, *see* refs. *138* and *139*), and phosphorylation-dependent processes may contribute to stabilize I_h. Curiously, I_h that is generated by either expressed or native channels shows a pronounced hyperpolarizing shift in voltage dependence ranging as high as 40 to 60 mV when maintained in cell-free patches, whereas cAMP sensitivity remains relatively unaltered *(88,140)*. This indicates the presence of essential regulatory factors besides cAMP that maintain the voltage dependence of the channels within a physiological range; adenosine triphosphate could be at least partly responsible *(136)*. In addition to PKA-mediated regulation of I_h, protein kinase C and PTKs may contribute to the control of current amplitude *(141,142)*. This regulation can be initiated by growth factors *(143)* and neurotransmitters *(144)* but may also contribute to the basal properties of the current.

Regulation By pH

By virtue of its sensitivity to strong extracellular pH changes, I_h may serve as a transducer for sour stimuli (pH 3.0–5.0) in a subset of taste cells by generating a depolarizing inward current in response to low pH *(74)*. Native currents may therefore not sense moderate (up to 1 unit) changes in extracellular pH, such as those occurring during transient ischemia in the brain *(145,146)*. Conversely, the high sensitivity of I_h to intracellular pH changes has been proposed to underlie the protective action of carbonic anhydrase inhibitors in generalized seizures *(147)*. Carbonic anhydrases catalyse the hydration of carbon dioxide, and their inhibition causes an increase in steady-state pH—presumably through an accumulation of intracellular hydrogen carbonate. In thalamocortical neurons, the resulting augmentation of I_h depolarizes neurons and prevents the generation of rebound calcium spikes, thus reducing their engagement in synchronized paroxysmal discharges, which are typical for some types of generalized seizures.

Regulation at the Level of mRNA and Protein Expression

In addition to the regulation of I_h and/or associated regulatory systems by voltage and ligands, differential up- and downregulation of individual HCN channel subunits occurs during development. These maturational processes at the mRNA and protein level correspond well to the developmental changes in current density and properties. Moreover, their temporal profile matches that of rhythmic synchronized electrical discharges appearing during circuit maturation, suggesting that age-specific network activity patterns may be promoted by regulated HCN channel transcription. Strikingly, it was recently observed that abnormal electrical activity (whether occurring in cardiac or neuronal cells) can profoundly disturb the properties of I_h in both immature and adult systems (*see* ref. *10*) and that this pathological modulation is often associated with an altered transcription of HCN channel subunits. Generally, an enhancement of neuronal or cardiac electric activity beyond normal seems to be a major cause in alterating in HCN gene expression, although the changes in each subunit (HCN1 through 4) seemingly occur independently of each other (*see* Table 1). The alterations in the level of transcripts develop on both short- and long-term timescales and often parallel those in the properties of functional channels, at least in a qualitative manner. Therefore, transcriptional regulation of HCN channels may be implicated not only in developmental processes and homeostasis of neuronal excitability but also in mechanisms of neurological and cardiac disease.

Developmental Regulation

To date, the developmental regulation of HCN expression has been studied most extensively in mouse ventricular myocytes and in rodent hippocampus. Early embryonic myocytes show prominent regular beating and

express a large I_h, which is probably carried primarily by HCN1 and -4 channels *(148).* As spontaneous activity ceases perinatally, the amplitude of the current decreases by more than 80% and involves a strongly reduced expression of HCN1 and -4, whereas HCN2 is now the most predominant. The predominance of HCN2 over HCN4 grows even further during aging *(149).*

In pyramidal cells of the mouse CA1 and CA3 regions, the densities of I_h conductance undergo a transient increase over the course of the first 5 to 10 d postnatally; before smaller adult values are reached around d 20 postnatally *(150).* During this time, I_h activation rates increase up to 10-fold. The expression of HCN1 protein increases strongly in CA1 and CA3 regions and includes both somatic and dendritic layers, with a particularly strong signal in *stratum lacunosum.* HCN2 and -4 show a much weaker, but progressive and uniform increase. RNA transcripts that encode HCN genes also are detectable in developing interneurons, in which a differential expression arises around the fifth postnatal day *(151).* Transcripts for HCN1 are found predominantly in parvalbumin-reactive interneurons within the pyramidal cell layer and the *stratum radiatum.* Transcripts for HCN2 and -4 appear within *stratum oriens* and coexpress frequently with the neuropeptide somatostatin. These developmental expression patterns, which are specific for each HCN subunit, may relate to an age-specific role of I_h in the generation of slow network oscillations during the first postnatal weeks.

Cardiopathies

Cardiac myocytes undergo substantial electrical and structural remodeling to adapt to external stressful factors, such as pressure overload (e.g., hypertension), inflammation (myocarditis), and infarction (for review, *see* refs. *152* and *153*). These adaptations are beneficial to maintain cardiac function initially but can eventually lead to contractile abnormalities and sudden cardiac death. Hypertrophied ventricular myocytes from animal models of pressure overload and from the failing human

heart show a prolonged duration of the action potential associated with a decrease in repolarizing outward currents, thus principally enabling them to (a) increase Ca^{2+} entry, impair Ca^{2+} uptake, and retard relaxation and (b) contribute to the arrhythmias that are observed in cardiac disease (for review, *see* ref. *152*). Interestingly, in a more advanced stage of hypertrophy, recordings from ventricular myocytes of spontaneously hypertensive rats or of failing human heart revealed the increased appearance of a diastolic depolarization between the prolonged action potentials; this was associated with the presence of I_h activation at physiological voltages *(154–156).* Notably, in normal ventricular cells, I_h does not activate until hyperpolarizations below –100 mV in these nonpacing regions of the heart *(157),* suggesting that sustained hypertrophy led to an alteration in I_h properties. In the failing heart, remodeling of I_h was also found in sinoatrial node cells *(158).* The degree of myocardial hypertrophy was positively correlated with an increase in the density of I_h *(159),* whereas current voltage dependence, kinetics, and modulation by sympathetic stimulation remained unaltered *(159,160).* These electrophysiological changes were paralleled by an upregulation of the HCN2 and -4 mRNA levels *(160,161),* which are the predominant isoforms underlying ventricular I_h *(149).* The changes in expression levels were most pronounced in those cardiac regions that had highest pressure overload *(160).* The sequence of events leading from hypertrophy to enhancement of I_h in nonpacing regions of the heart appears to involve the activation of the type I angiotensin receptor, because its blockage not only prevents myocyte hypertrophy but also reverses I_h upregulation and overexpression of HCN2 and -4 mRNA *(161,162).* Moreover, given the similarities in the expression profile of fetal and hypertrophied myocytes, it has been speculated that cardiac hypertrophy provokes a reentry of cells into a fetal program and the reinitation of the corresponding gene expression patterns *(148,159).* In support of this idea, the density of I_h is higher in rat neonatal ven-

Table 1
Changes in mRNA Expression for HCN Subunits Found in Tissues Producing Abnormal Cardiac or Neural Activity

HCN subunit	Transcription enhanced	Transcription diminished	Transcription unchanged
HCN1	Chronic temporal lobe epilepsy (174) WAG/Rij rat, thalamocortical neurons (173)	Febrile seizures, CA1 (171) Kainate seizures in young animals, CA1+CA3 (171) Entorhinal cortex lesion, hilar neurons (180) Spinal nerve ligation, dorsal root ganglia cells (189)	Febrile seizures, CA3 (171)
HCN2	Febrile seizures, CA1 + CA3 (171) Kainate seizures in young animals, CA1+CA3 (171) Hypertrophy of cardiac ventricle (159,160)	Spinal nerve ligation, dorsal root ganglia cells (189)	Chronic temporal lobe epilepsy (174) Entorhinal cortex lesion, hilar neurons (180)
HCN4	Hypertrophy of cardiac ventricle (159,160)		Febrile seizures (171) Entorhinal cortex lesion, hilar neurons (180)

Numbers in parentheses indicate the reference. The correspondence between altered mRNA expression and functional current alterations are often incomplete and not presented in the table. The changes in mRNA expression were paralleled by similar alterations in protein expression in refs. 174,180, and 189.

tricular myocytes and progressively decreases postnatally *(148,163).* The studies on the consequences of myocardial hypertrophy presented the first evidence in favor of an altered I_h resulting from cellular mechanical stress and consequent abnormal electrical activity.

Epilepsies

Currently available data indicate that the expression of HCN channels is sensitively controlled by aberrant neuronal activity; even brief periods of seizures can be sufficient to persistently modify I_h function. These alterations can be neuroprotective or facilitate hyperexcitability, depending on the system. For example, in febrile seizures that occur during development, changes in the properties of I_h appear to facilitate, rather than to counteract, hyperexcitability. Seizures induced by fever are the most common type of seizure in the developing brain and affect up to 5% of small children (<5 yr old) during periods of high fever. Febrile-like seizures can be induced in a rat model in which 10-d-old rats are exposed to hyperthermia for a single period of approx 30 min. Such animals reliably (>98%) develop epileptic convulsions in the hippocampus that can be prevented by antiepileptic drugs *(164).* Moreover, these rats show an increased tendency to develop seizures in adulthood *(165),* suggesting that this type of early-life seizures may predispose to later epileptic susceptibility *(166,167).* Three persistent modifications of neuronal excitability were determined in hippocampal neurons from such rats. In addition to a long-lasting increase in the release of GABA *(168)* and increased retrograde signaling via endocannabinoids *(169),* I_h was persistently increased in CA1 cells, even if animals had experienced only a single seizure that lasted an average of 23 min *(170).* Other intrinsic currents that were important for hippocampal cell firing, as well as passive cell properties, remained unaffected. The enhanced expression of I_h induced an augmentation of a rebound sag potential and an increased probability of action potential generation. Interestingly, although the enhanced vesicular release of GABA was dependent on activation of PKA *(168),* the aug-

mentation of current amplitude was independent of this kinase *(170),* suggesting that multiple independent mechanisms controlling homeostasis of excitability were affected during the seizure. The functional changes in I_h were paralleled by an altered expression of HCN channel subunits *(171),* which could qualitatively explain the changes in current properties. These changes were not observed when seizures were prevented by antiepileptics. Therefore, hyperthermia-induced brief hyperexcitability led to a persistent functional modification of I_h, likely mediated to a large extent by modifications at the level of channel subunit transcription.

Functional changes in I_h have also been recently reported for animal models of generalized epilepsies, in which the thalamocortical system is primarily involved. In the stargazer mouse, cortical hyperexcitability was found to be associated with a threefold enhanced amplitude of I_h in cortical layer V pyramidal cells *(172).* In the WAG/Rij rat (an established model for human absence epilepsy), I_h activation was shifted negatively and cAMP sensitivity was reduced in thalamocortical cells *(173).* Although comparatively modest, these modifications hyperpolarize the membrane of thalamocortical cells and further their involvement in the burst discharges that are typical for spike-and-wave activity. Again, current changes may largely be explained by the observed enhanced expression of HCN1, the channel subunit with the lowest cAMP sensitivity. Both animal models show that in these generalized seizures, alterations in HCN channel expression appear to be maladaptive because they exacerbate the capacity of neurons to integrate in synchronized oscillations that are associated with absence seizures.

In contrast, in tissue from chronically epileptic human patients, a strong upregulation of HCN1 channel transcripts was found in the dentate gyrus *(174).* The survival of these granule cells in the sclerotic hippocampus *(175)* suggested that the augmented expression of HCN channels could act in a neuroprotective manner. Indeed, the degree of upregulation in surviving

cells was proportional to the extent of cell death in the granule cell layer. Further support for a neuroprotective role of enhanced I_h expression stems from the observation that the antiepileptic agents lamotrigine and gabapentin upregulate dendritic I_h in hippocampal pyramidal neurons *(176,177)*. The dampening effect on neuronal excitability in the hippocampus likely arises via an I_h-mediated decrease of neuronal input resistance and/or a reduction of the temporal summation of repetitive synaptic inputs (*see* section on The Multiple Roles of I_h). Consequently, the enhanced dendritic expression of HCN1 subunits in sclerotic tissue could represent an endogenous neuroprotective process that develops during prolonged hyperexcitability. This unique role of I_h shows that in designing antiepileptic drugs to selectively target molecular subtypes of channel subunits, the family of HCN channels should also be considered *(178,179)*.

In view of these various studies on animal models of epilepsy, it is clear that the control of HCN subunit expression in neurons is determined not only by the type of seizures but also by the cell type and the developmental stage. The latter factor appears to play a particularly important role, because the effects of single febrile seizures in young animals are persistent, whereas the effects of the stronger kainate seizures in adult animals show no consequence on HCN expression *(171,180 see* Note added in proof*)*. Generally, the alterations in the expression of channel message largely explain the functional alterations in current properties, although there is some disagreement regarding the persistence of the effects at these two levels (e.g., *see* ref. *171*). To date, the mechanisms that translate seizures into altered HCN channel expression remain unexplored but could range from acute influences, such as synaptic activity (e.g., *see* ref. *128*), to long-term, chronic modulation of channel expression (e.g., via hormones and inflammatory processes; *see* ref. *181*). Detailing the mechanisms that control transcription and expression of HCN channel genes would certainly be facilitated through cultured preparations that allow the induction of de-fined types of hyperexcitability for controlled periods of time (*see* ref. *182*).

Nerve Injuries

Abnormal spontaneous action potential discharge is a frequent consequence of peripheral nerve injury and is believed to be critical in the initiation and persistence of neuropathic pain syndromes such as tactile allodynia (strong sensation evoked by light mechanical stimuli) and spontaneous painful sensations *(183)*. Acute injury to the spinal cord can result in a hyperexcitability of not only nociceptive pathways but also of dorsal root ganglia cell bodies that give rise to large, myelinated $A\beta/\delta$-fibers that are normally not involved in the transmission of pain *(184,189)*. A resulting hypersensitivity of sensory pathways and a misrepresentation of sensory information is believed to contribute to the clinical symptoms of neuropathic pain, although central pain processing mechanisms are involved as well *(183)*. Several rat models of spinal cord injury, such as axotomy *(185)*, chronic constriction *(186)*, or ligation *(187)* of spinal nerves, show that an altered expression of several voltage-gated Na^+ channel subunits contributes to the persistence of neuronal firing in injured cells (for review, *see* ref. *188*). Interestingly, however, neuropathic pain behavior was reversed in a spinal nerve ligation model by the I_h blocker ZD7288, which also reversed the spontaneous discharges in injured large myelinated fibers *(189)*. In this as well as in a chronic compression model *(190)*, the maximal current density was enhanced 1.5 to 2.5-fold compared to control, with variable effects on voltage dependence and kinetics. These findings establish I_h upregulation, resulting from nerve injury, as an essential factor leading to the sensitization of spinal cord neurons and to neuropathic pain. The molecular identity of the HCN channels that contribute to these changes remains to be determined but appears to involve a decrease in the amount of HCN1 and -2 mRNA and protein in the case of nerve ligation *(189)*.

Besides neuronal injury, lesions in excitatory input can also cause an altered expression of HCN channels. Lesions of the entorhinal cortex

induced a downregulation of HCN1 mRNA and protein in several neuronal cell types of the hippocampus *(180)*. This decreased expression was paralleled by a strong (up to 19 mV) hyperpolarizing shift in current voltage dependence. These changes were partly reversed following reactive sprouting and replacement of entorhinal input by septal and associational afferents.

Conclusions

The past 5 yr have seen an explosion of information on the molecular basis of I_h and its role in normal and pathological processes. The crucial impetus arose with the identification of the molecular subunits that constitute the channels and is currently progressing with the arrival of the knockout animals, which now allow insight into possible behavioral roles of this current. Additionally, the misexpression of mRNA in diseased or injured tissue shows that HCN channels can promote channelopathies that arise at the transcriptional level *(188).* Although such properties are well-known for other channel types (for review, *see* ref. *188*), they appear to be particularly dramatic for the HCN channels, because these often occupy a unique physiological role in a cell's channel repertoire that cannot be easily complemented or substituted by other ionic channels. Dysregulated expression of HCN channels is complicated further by its variable appearance. It can be reversible or persistent in time and appears to be dependent on the precise cellular and developmental context in which it occurs. Furthermore, inflammation or ischemia that occurs under pathological situations may influence the current *(191,192).*

A few recent studies have highlighted that understanding the regulation of I_h functionality in intact systems also will require considerable attention in the near future. For example, in cardiac cells, the properties of I_h may be codetermined by sympathetic innervation *(193)* and by β_2-adrenergic receptor expression *(194).* In lobster stomatogastric neurons, I_h expression is coregulated with the expression of channels that give rise to transient K^+ currents. This regulation occurs in an activity-independent manner and appears to take place at the translational level, because it is not influenced by blockers of transcription *(195).* Altogether, these data strongly suggest that on-site regulation of the channels as well as many aspects of channel expression, including activation of promoter regions of HCN genes, channel synthesis, and trafficking, carefully control both density and properties of I_h. Understanding these pathways, in a manner such as that elaborated on the trafficking and homeostasis of synaptic glutamate receptors, may prove crucial in designing novel therapeutic targets for cardiac and neuronal pathologies. Moreover, they may contribute to the development of a conceptually novel therapeutic approach that is emerging in the HCN channel field: the *de novo* creation of biological pacemakers for the heart when the sinus node signal fails *(196)* and, perhaps, for diseases associated with rhythmicity in the brain.

Note

Since the acceptance of this manuscript, several publications have appeared in the field of HCN channel function and some of the most notable ones will be mentioned here. A detailed map of the localization of HCN protein in rat brain was established *(197)*. Since their original abstract *(128)*, Van Welie *et al.* have now demonstrated that I_h amplitude in CA1 pyramidal cells is upregulated by activation of glutamatergic receptors on the time scale of minutes. Enhancement of current amplitude requires an elevation of intracellular calcium levels and reduces intrinsic neuronal excitability *(198)*. Whether such a regulation occurs following synaptically induced activation of glutamatergic receptors and the induction of long-term plasticity in the CA1 area is currently under investigation.

Further investigations have corroborated mechanistic links between alterations in HCN

protein expression, I_h function and various types of experimental epilepsies. Interestingly, a downregulation of HCN proteins and I_h, and a concomitant increase in the dendritic excitability of entorhinal cortex layer III neurons develops within 24 hr after a single, kainate-induced seizure episode and persists for up to 1 week. This corresponds to a time period during which spontaneous electroencephalographic seizures have not yet started to appear *(199)*, suggesting that I_h may be modulated during the process of epileptogenesis. A decrease of HCN protein and I_h has now also been reported in cortex of the WAG/Rij rat model of chronic generalized absence epilepsy *(200)*. These studies show that the pharmacological alteration of HCN channel function should soon be evaluated in its effectiveness in anti-epileptic drug searches. However, a recent study indicates that *in vitro* studies characterizing the effects of anti-epileptic drugs on I_h and neuronal excitability have to be interpreted with caution *(176,201)*, as: a) they may depend on the concentration and on the neuronal cell type studied, and b) they may reflect the combined action of a drug on multiple types of ion channels.

Acknowledgments

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