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Structure and Function of Cardiac Pacemaker Channels

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Key Words

Hyperpolarization-activated current · Pacemaker current · HCN channel

Abstract

Cardiac pacemaking is controlled by a mixed Na^+/K^+ current named I_f, which is activated by hyperpolarized membrane potentials. Recently, a family of hyperpolarization-activated cyclic nucleotide-gated cation (HCN) channels has been cloned. The members of this family exhibit the general features of I_f channels. This review describes the molecular diversity of the HCN channel family and the structural determinants of channel function including activation by voltage, modulation by cylic nucleotides and ion permeation. The relationships beween cloned HCN channel types and native cardiac I_f currents are explored.

Introduction

Cardiac pacemaking determines heart rate and rhythm and is generated by the slow membrane depolarization phase occurring between action potentials. A main part of the conductance underlying the cardiac pacemaker depolarization was identified nearly 20 years ago [1–3] and called I_f (f for funny) or synonymously I_h (h for hyperpolarization activated). I_f is activated on membrane hyperpolarization and is carried by both Na^+ and K^+. Hence, the activation of I_f at the end of the action potential induces a slow membrane depolarization towards the threshold at which calcium channels activate. Sympathetic stimulation leads to activation of β-adrenergic receptors, activation of G_s protein and in turn adenylyl cyclase. The elevation of intracellular cAMP shifts the voltage dependence of I_f activation by around 10 mV in the positive direction resulting in increased inward current at a given negative membrane potential. This mechanism is largely responsible for the

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acceleration of the heart rate during sympathetic stimulation [4]. Muscarinic stimulation slows the heart rate, partly due to G-protein mediated activation of the muscarinic potassium channel (I_{KACh}) and partly due to a decrease in intracellular cAMP and a resulting reduction of the I_f current [5, 6]. The modulation of I_f by cAMP does not depend on the action of protein kinase A but is mediated by the direct binding of cAMP to the channel [7]. Thus, the channel underlying I_f combines features common to both voltage-gated channels (activation by hyperpolarization) and ligand-dependent channels (modulation by cAMP).

The I_f current has also been detected in a variety of neurons [8, 9]. In the brain, a major function of the current is to control the rate of rhythmic oscillations of single neurons and neuronal networks ('neuronal pacemaking'). In addition, the current is involved in determining the resting membrane potential of neurons and the response to hyperpolarizing currents. The recent isolation of five different cDNA clones encoding pacemaker channels made it possible to gain first clues about the molecular mechanisms governing I_f channel function.

Cloning of Pacemaker Channels

A large number of ion channel cDNAs have been isolated and characterized, but attempts to clone pacemaker channels were unsuccessful until recently. In 1998, three groups reported independently the cloning of several cDNAs encoding pacemaker channels. A yeast two-hybrid screen with the SH3 domain of a neural specific isoform of Src detected a brain cDNA clone (BCNG-1) which was supposed to encode a cyclic nucleotide-modulated K^+ channel based on sequence alignments [10]. Functional expression of BCNG-1 demonstrated that it formed a member of a family of hyperpolarization-activated cation channels [11]. A more direct approach to clone pacemaker channels was based on the idea that I_f channels like cyclic nucleotide-gated (CNG) channels [12, 13] or cyclic nucleotide-dependent protein kinases [14] would contain a cyclic nucleotide-binding domain (CNBD). Screening the expressed sequence tag (EST) database for sequences related to the CNBD of CNG channels identified an EST sequence which was then used to isolate three homologous full-length cDNAs (HAC1-3) from mouse brain [15]. A third group reported the cloning of a nonmammalian hyperpolarization-activated cation channel (SPIH) from the testis of the sea urchin *Strongylocentrotus purpuratus* [16]. Recently, a fourth full-length cDNA encoding a hyperpolarization-activated cation channel (HAC4 [17], HCN4 [18]) was isolated. A unifying nomenclature for these pacemaker channel cDNAs was introduced lately [19, 20], the suggested new name is *h*yperpolarization-activated and *c*yclic *n*ucleotide-gated (HCN) channel. Original names and proposed new designations for all family members isolated to date are listed in table 1. The species from which the cDNA was cloned is indicated by a preceding lowercase letter, e.g. hHCN1 and mHCN1 for the human and mouse HCN1 channels, respectively.

Structure of HCN Channels

Four members of the HCN family (HCN 1–4) have been isolated from mouse, rabbit and human cDNA libraries (table 1; fig. 1). The HCN family is distantly related to the CNG channel and the *eag* potassium channel family and belongs to the superfamily of voltage-gated cation channels. HCN channels contain six transmembrane helices (S1–S6) including a positively charged S4 segment and an ion-conducting pore between the fifth and sixth transmembrane segment. In the C-termi-

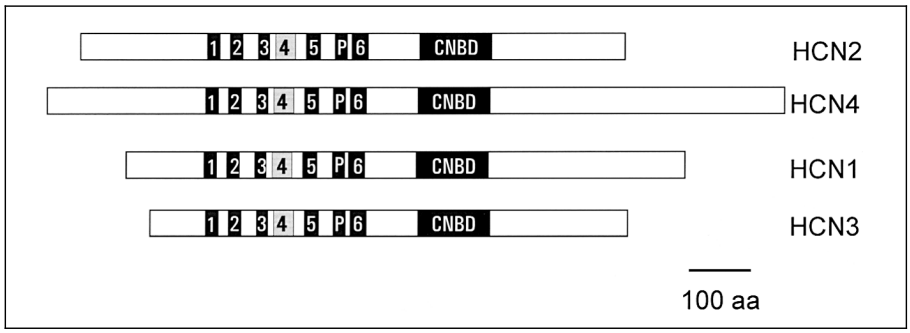

Fig. 1. Overall structure of the four HCN channel family members. The six transmembrane segments S1–S6 are numbered 1–6, P and CNBD indicate the pore region and the cyclic nucleotide-binding domain, respectively.

Table 1. HCN channel clones

HCN nomenclature	Clone (original name)	Species	Reference No.
HCN1	HAC2/mBCNG-1/hBCNG-1*	mouse/mouse/human	15/10/11
HCN2	HAC1/hHCN2/ mBCNG-2*/hBCNG-2*	mouse/human/ mouse/human	15/18/ 11/11
HCN3	HAC3/mBCNG-4*	mouse/mouse	15/11
HCN4	HAC4/hHCN4/mBCNG-3*	rabbit/human/mouse	17/18/11
spHCN	SPIH	sea urchin	16

* Partial cDNA sequence.

nal region they contain a CNBD (fig. 1, 2a). The four HCN channel types are closely related to each other having an overall amino acid sequence identity of about 60%. The homology is highest in the central core region (transmembrane segments plus CNBD) with a sequence identity of 80–90% at the amino acid level. In contrast, the N- and C-termini vary considerably in their length and share only modest homology. Analogous to CNG channels and potassium channels four HCN channel subunits probably assemble to a tetramer (fig. 2a). All four HCN channel types can be functionally expressed as homomers. However, since the expression pattern of the four HCN transcripts in heart and brain at least partially overlaps, the formation of heterotetramers cannot be excluded yet.

Structural Determinants of HCN Channel Properties

Activation by Hyperpolarization

The transmembrane core of HCN channels contains a positively charged segment that corresponds to the voltage-sensing S4 segment of voltage-gated cation channels. Notably, the

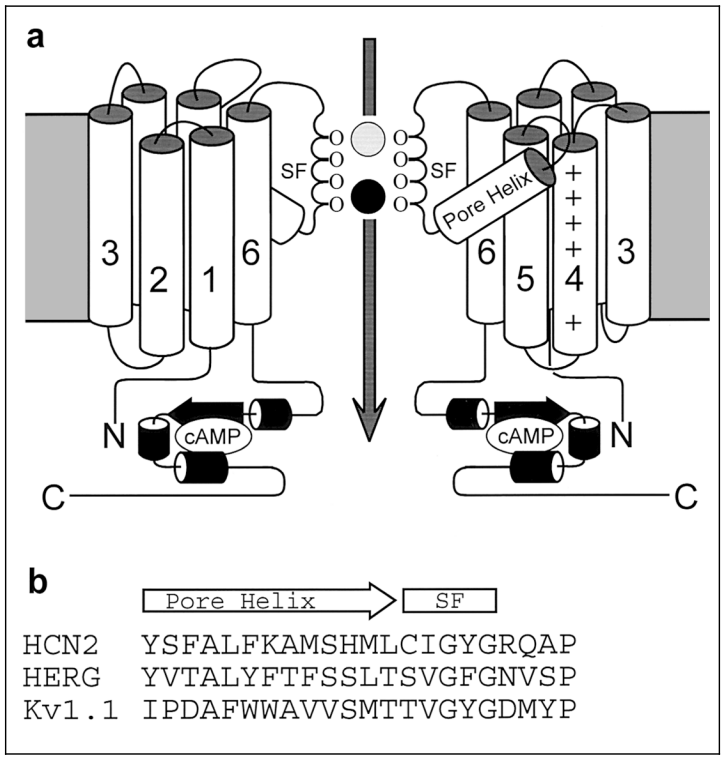

Fig. 2. a Three-dimensional model of the transmembrane topology of HCN channels. The pore region consisting of the pore helix and the selectivity filter (SF) is depicted according to the crystal structure of the *Streptomyces lividans* KcsA potassium channel [30]. The carbonyl oxygens from the main chain of the selectivity filter are indicated. For clarity of the drawing only two subunits of the putative tetramer are shown. **b** Comparison of the pore region of HCN2 with that of the HERG [50] and the human Kv1.1 [51] potassium channel. Pore helix and selectivity filter (SF) are delineated according to Doyle et al. [30].

S4 segment of HCN channels contains a total of ten positively charged residues compared to only five to seven in the S4 segment of potassium channels. The ten basic residues are seperated in two segments of five positively charged residues at every third position by one regularly spaced serine. The presence of a positively charged voltage sensor in HCN channels is somewhat surprising since these channels are activated by hyperpolarization whereas other channels with positively charged S4 segments are activated by depolarized membrane potentials. A mechanism of the activation of HCN channels was proposed [11, 16, 20] based on studies on mutated Shaker channels [21] and on the HERG K^+ channel [22, 23]. According to this model, the three states of a voltage-gated channel, namely closed, open and inactive state are shifted in HCN channels pronouncedly to negative membrane voltages. Therefore, at resting potential, HCN channels are in the inactivated state even though the S4 segments forming the activation gates may be in the open configuration. Hyperpolarization would then open the channels by reversing the inactivation reaction. The structure of this 'inactivation gate' is not known at present. However, a first clue towards an understanding of HCN activation is provided by a recent study on the inwardly rectifying K^+ channel KAT1 from *Arabidopsis thaliana* [24]. KAT1 resembles HCN channels in that it is both activated by hyperpolarization and also contains a positively charged S4 segment. By investigating the effect of N-terminal deletions and a S4 mutation in the KAT1 channel it was concluded that in this channel the hyperpolarizing shift in the acti-

vation curve is due to the interaction of the cytoplasmic N-terminus with the S4 segment. It is not known if a similar interaction takes place in HCN channels.

Modulation by Cyclic Nucleotides

Pacemaker channels are gated not only by hyperpolarization but also by cyclic nucleotides. Cyclic nucleotides excert a dual effect on the channel, namely a 2- to 15-mV shift of the activation curve towards more positive membrane potentials and an acceleration of the channel activation. Cyclic nucleotides regulate HCN channels by a direct binding to a CNBD located in the C-terminus of the protein. The CNBD of HCN channels is homologous to the CNBDs of other cyclic nucleotide-regulated proteins like the catabolite activator protein (CAP) of *Escherichia coli*, the cAMP-regulated guanine nucleotide exchange factors cAMP-GEFs/Epac [25, 26], cAMP- and cGMP-dependent protein kinases and CNG channels. Amino acids which have been determined in the CAP crystal structure to lie close to the cAMP molecule [27] are well conserved in HCN channels. The activation of native [7] and the expressed HCN1 channel by cAMP reveals no cooperativity (Hill coefficient of about 1; table 2) in contrast to the highly cooperative activation of CNG channels by cyclic nucleotides.

Ion Selectivity

The I_f current is carried by both Na^+ and K^+ with the native I_f channel being fourfold selective for K^+ over Na^+ [28, 29]. The ion selectivity of heterologously expressed HCN channels agrees well with that of native channels (table 2). Sequence comparisons demonstrate that the pore region of HCN channels is related to that of K^+-selective channels [30, 31] (fig. 2b), despite the fact that K^+ channels are no less than hundredfold selective for K^+ versus Na^+. The pore region of K^+ channels is localized between the fifth and sixth putative transmembrane segment (fig. 2a, b) and consists of a pore helix and the selectivity filter [30]. The potassium channel signature sequence glycine-tyrosine-glycine (GYG) forms the main part of the selectivity filter [30, 32]. In some channels like the human ether-à-gogo-related (HERG) K^+ channel, the tyrosine residue is replaced by phenylalanine (fig. 2b). Surprisingly, HCN channels also contain a GYG sequence, although they pass both Na^+ and K^+. Hence, other amino acids in addition to the GYG sequence determine selectivity for K^+. In K^+-selective channels the narrowest part of the pore is formed by the carbonyl oxygens from the main chain of the signature sequence providing a ring of sites suitable for coordinating precisely a dehydrated K^+ ion. A sheet of aromatic amino acids formed between the tyrosine side chains of the signature sequence and aromatic amino acids of the pore helix works like a ring of springs holding the pore open at its correct diameter. The pore of HCN channels contains several amino acid substitutions compared to the pore of selective K^+ channels (fig. 2b). Therefore, in HCN channels the carbonyl backbone may have lost some of its structural rigidity, thus allowing both K^+ and Na^+ to pass the pore.

Potassium is not only a permeating ion of HCN channels, it also regulates the permeation of Na^+. Both the current amplitude and the P_{Na}/P_K ratio of HCN channels depend on the extracellular K^+ concentration, i.e. an increase in extracellular K^+ concentration results in a strongly increased current amplitude and in a slightly reduced selectivity for K^+ over Na^+ [28, 33, 34]. The interdependence of Na^+ and K^+ permeation in HCN channels is illustrated by the finding that the channels conduct little, if any, Na^+ in the absence of K^+ ions. Thus, K^+ is required for the channel to carry any current in spite of the fact that Na^+

Table 2. Functional characteristics of cloned HCN channels

	mHCN1	mHCN2	mHCN3	hHCN4
Amino acids	910	863	779	1,203
Tissue distribution	brain	brain, heart	brain	brain, heart
Activation constant at –140 mV, ms	τ_1 = 30 τ_2 = 171	241	n.d.	660
$V_{½}$*	–94 mV	–103 mV	n.d.	–109
P_{Na}/P_K	0.25	0.24	n.d	0.22
K_a for cAMP/Hill coefficient	n.d.	0.5 μ*M*/0.8	n.d.	n.d.
Shift by cAMP, mV	+1.8	+13	n.d.	+15

* Measured in whole-cell voltage clamp mode.

constitutes the major inward cation current in HCN channels at physiological membrane potentials. The structural basis of this phenomenon is presently not known. However, it seems reasonable to assume that the pore of HCN is, like that of CNG channels [35] and Ca^{2+} channels [36], a multi-ion pore possessing at least two cation binding sites: one at the external mouth of the channel having a higher affinity for K^+ and another having a higher affinity for Na^+ [37]. HCN channels are not only regulated by K^+ but also by external Cl^-. Substitution of external Cl^- by larger anions such as isothionate or gluconate results in a pronounced reduction of the current amplitude of both native [38] and expressed channels [11]. Thus, the pore of HCN channels is likely to contain an extracellular binding site for Cl^- which may be related to the presence of positively charged residues in this region (fig. 2b).

Native versus Cloned Cardiac Pacemaker Channels

Whereas all four HCN channel family members are expressed in brain, only HCN2 and HCN4 were detected in heart (table 2). RT-PCR [18] and in situ hybridization experiments [Moosmang et al., in preparation] demonstrated that both HCN2 and HCN4 are expressed at roughly equal levels in working myocardium. In contrast, HCN4 mRNA is strongly enriched in the sinoatrial node [17]. When expressed in HEK293 cells, HCN4 gives rise to a current with the typical features of native I_f but with a profoundly slower activation kinetic compared to the HCN2 current [18]. Interestingly, the I_f current in heart cells is composed of two kinetic components (fast and slow) [39–41] raising the possibility that native I_f may be generated by the activity of two distinct channel types. The above results suggest that HCN2 and HCN4 may underlie the fast and slow component of cardiac I_f, respectively.

The presence of HCN channels in diverse types of heart cells is compatible with reports describing I_f currents in ventricular [42–45] and atrial myocytes [46] as well as in different conduction tissues [47]. The I_f current from ventricular myocytes differs significantly from that of sinoatrial node cells in the voltage dependence of activation. Half-maximal activation potentials determined for I_f in ventricular myocytes range from –95 to –135 mV [42–45], wheras I_f in sinoatrial node cells acti-

vates at more positive potentials with $V_{½}$ ranging from −65 to −90 mV [7, 48, 49]. HCN2 and HCN4 exhibit half maximal activation at ~ −100 mV which is consistent with $V_{½}$ reported for ventricular myocytes. The reason for the differing $V_{½}$ values between I_f from ventricular and sinoatrial node myocytes is unknown.

Conclusions

Although a hyperpolarization-activated cation current is known for over 20 years, the cloning of the channels involved in this current was successful only recently. Four different genes encoding pacemaker channels have been identified demonstrating an unanticipated molecular diversity of this ion channel class. The differential expression of the genes may be of help in the development of therapeutic agents targeting specifically for instance cardiac pacemaker channels. Furthermore, it should now be possible to determine whether mutations in pacemaker channel genes may underlie certain congenital cardiac arrhythmias such as the inherited form of the sick sinus syndrome.

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