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The Contribution of *HCN4* to Normal Sinus Node Function in Humans and Animal Models

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Abstract

Although sinus node bradycardia is a very common clinical condition, the cellular mechanisms contributing to abnormal sinus node function are not clearly delineated. In recent publications, mutations in the hyperpolarization-activated, cyclic nucleotide-gated (HCN) 4 channels have been associated with sinus bradycardia. These channels are thought to be crucial in generating the spontaneous sinus node action potential, in accelerating the heart rate during sympathetic drive, and decelerating heart rate during vagal stimulation. Humans carrying HCN4 mutations indeed display significant bradycardia. Recent studies generating HCN4 knock out mice suggested that although HCN4 is crucial in early development, other mechanisms may also play a role in the accelerated heart rate achieved during sympathetic drive. In this review, we focus on genotype–phenotype correlation of these mutations and discuss the relative contribution of various ion channels to sinus node function. We also discuss the importance of HCN in treating clinical conditions such as brady- and tachycardia.

Keywords

genetics; ion channels; bradycardia

Introduction

Sinus bradycardia is a common condition. However, in some individuals, such as athletes, bradycardia may be benign; in others it may have more serious consequences. In some, the bradycardia is associated with other myocardial conditions, such as congenital abnormalities, myocarditis, dystrophies, cardiomyopathies, and is often associated with structural remodeling and fibrosis of the sinoatrial (SA) node (SAN).^{1–8} Although there are many etiologies for slow heart rate, in terms of symptomatic bradycardia, the only current effective treatment is pacemaker implantation. The predominant ion currents, which contribute to pacemaker activity in the SA node, include currents flowing through hyperpolarization-activated, cyclic nucleotide-gated (HCN) channels⁹; L-type Ca, T-type Ca,¹⁰ delayed rectifier K^{11,12}; and acetylcholine-activated^{13,14} channels. However, their relative roles remain controversial and the cellular mechanisms contributing to abnormal sinus node function, such as bradycardia, remain unknown. In contrast to acquired bradycardia, familial diseases of the sinus node are rare. Sinus bradycardia syndromes are

commonly associated with other cardiac and extracardiac abnormalities.^{1–8} In most of the reported cases, permanent pacing was required. Some families with sinus bradycardia (usually without any extracardiac abnormalities) have a more benign course and may even be asymptomatic.^{1,2,15–18}

Mutations in the *HCN4* gene have been shown to be responsible for inherited sinus bradycardia.^{19–22} *HCN4* encodes the protein responsible for the channels that carry I_f , the hyperpolarizing-activated current or the “funny” current that participates in spontaneous diastolic membrane depolarization of SA node cells.^{23–26} The HCN channels have a structure of six transmembrane segments (Fig. 1). cAMP binds to the cyclic nucleotide-binding domain (CNBD) located in the COOH terminus.²⁷ The modulation of these channels by cAMP is believed to be responsible for acceleration of the heart rate.²⁴ Muscarinic agonists shift the activation curve to more negative voltages; thus, less inward current is available at diastolic potentials, resulting in deceleration of the heart rate.²⁷ Four *HCN* gene family members have been cloned; all of them were found to be expressed in the heart at some level.^{9,28,29} *HCN4* is the most prominent HCN transcript in SA node, whereas *HCN2* is the dominant transcript in the ventricles of all the species investigated.^{9,28} In rabbits, *HCN1* is also expressed in the atria, representing approximately 20% of the total *HCN* mRNA.^{9,30} SA cells from knockout mice lacking *HCN4* have 75% less I_f ³¹ and SA cells from mice lacking *HCN2* have 25% less HCN current.³² Of note in humans, *HCN2* and *HCN4* were found to be the dominant mRNA transcripts.³³ Characteristics of I_f in atrial myocytes closely resemble those of *HCN4* + *HCN1*³⁰ or *HCN4* + *HCN2*³⁴ expressed in heterologous systems, supporting the thesis that atrial myocytes may carry heteromeric complexes composed not only of *HCN4* but also of *HCN1* or *HCN2*. Moreover, an ion pore mutation in *HCN2* has been shown not only to suppress the *HCN2* current in a dominant negative manner, but also to reduce *HCN4* current, further substantiating the fact that these channels may co-assemble.³⁵ However, the exact roles of *HCN2* and *HCN1* in the human sinus node are still in debate. A recent paper³⁶ demonstrated that *HCN2* is actually more abundant in the right atrium than in sinus node area while *HCN1* and *HCN4* are more abundant in the sinus node itself.

Debate continues over the precise ionic mechanisms responsible for diastolic depolarization in the human SAN. Ion channel currents thought to participate include the HCN current or “funny” current, I_f ; deactivation of the delayed rectifier current, I_K ^{11,12}; sustained inward current (I_{st})^{37,38}; and the L-type (I_{Ca-L}) and T-type (I_{Ca-T}) calcium channel currents,¹⁰ which participate in the final portion of phase 4¹⁰ (Fig. 2). In addition to these ion channel currents, an intracellular “calcium clock” has been proposed as contributing to spontaneous SA nodal activity.³⁹ Spontaneous rhythmic submembrane local calcium release from the sarcoplasmic reticulum is thought to activate the sodium–calcium exchanger. This inward current contributes to depolarization of the SAN cell. The frequency of this release is modulated by cAMP and can thus respond to sympathetic stimulation-mediated increase in heart rate.

In this review, we will focus on the contribution of *HCN4* to sinus node function in murine models and human cases.

The Role of *HCN4* in Animal Models

There is clear evidence from several studies that mouse embryos lacking functional *HCN4* fail to develop during the embryonic period.^{23,35,40,41} Homozygous embryo mice, lacking exon 4 encoding the *HCN4* ion channel pore and the transmembrane segment 6, died in utero.²³ Likewise, overexpression of a pore mutation of *HCN2* causing a dominant negative suppression or “knockout” of I_f , eliminated the spontaneous beating activity of neonatal rat

myocytes.³⁵ A recent study⁴⁰ demonstrated that even mice lacking the ability of cAMP to bind to the CNBD, died in utero. In contrast, the role of I_f in adult mice is controversial. Because *HCN4* knockout mice fail to develop, it has not been clear until recently whether I_f has the same prominent role in adult as in embryonic mice. Recently, Herrmann and colleagues⁴¹ succeeded in generating an adult mouse lacking *HCN4* channels. They used the cre/loxP system. Adding tamoxifen by week 8 to the floxed mice resulted in the deletion of exon 4 of *HCN4*. Surprisingly, in contrast to embryonic mice lacking *HCN4*, adult mice developed normally. I_f in SAN cells was 75% less intense as expected and the adult mice displayed sinus pauses only at rest, which diminished when the mice were active. Mice lacking *HCN4* did not show any differences in maximum heart rate achieved after isoproterenol infusion when compared to normal controls. When the heart rate of the knockout mice returned to basal rate, the sinus pauses reappeared. Even after the addition of an I_f blocker to abolish the remaining current, heart rate could still be accelerated. This study clearly demonstrated that in adult mice, *HCN4* did not appear to be the only mechanism required for adrenergic stimulation of sinus activity. In another adult mouse model, Harzheim and colleagues⁴⁰ disrupted the binding of cAMP to the CNBD with a single nucleotide missense mutation. As in previous reports, homozygous mice died in utero, but heterozygous mice developed normally. Similar to the previous report, the adult knockout mice had a basal heart rate comparable to the wild type, although sinus pauses were evident at rest. However, these mice exhibited more sinus pauses during exercise compared to rest.

These studies suggest that although I_f is crucial in early development, it may not be the sole mechanism responsible for determining heart rate at rest or during sympathetic drive in adult mice. Other ionic mechanisms contributing to the spontaneous diastolic depolarization, such as described above, may take over when *HCN4* is knocked out or may even be the main mechanism responsible for the increase in heart rate in the murine adult heart.

The Role of *HCN4* in Humans

To date, only five *HCN4* mutations have been described in humans. The first report²⁰ described a single patient with malignant syncope, sinus bradycardia, and bouts of atrial fibrillation. During exercise, the patient did not reach the predicted maximal heart rate for her age and gender. There was no family history of bradycardia. Genetic analysis revealed a heterozygous stop codon at position 573, creating a protein lacking the CNBD unit (Fig. 1, No. 4). A functional expression revealed that the mutant channels were insensitive to cAMP and had slower activation and faster deactivation kinetics. These findings correlate with the patient's inability to accelerate her heart rate during exercise. The altered kinetics of the mutant channel explains the sinus bradycardia at rest.

Another report²¹ described a patient with syncope, prolonged QTc (670 ms), and *torsade de pointes* (TdP). This patient was found to have a heterozygous missense mutation (Fig. 1, No. 3) affecting trafficking of the mutant channel to the membrane. A functional expression showed decreased current amplitudes for both homozygous and heterozygous states. This mutation segregated with long QTc among family members. No other mutations were found in *KCNQ1*, *KCNH2*, *SCN5A*, *KCNE1*, *KCNE2*, and *RYR2*. The reason for a prolonged QTc association with a decrease in *HCN4* current amplitude is unclear, since a loss of I_f function is not expected to prolong the QT interval, other than through a reduction in heart rate. Studies in both humans^{42,43} and mice⁴⁴ have not observed prolongation of the QTc in response to I_f blockers. It is also noteworthy that *HCN2* rather than *HCN4*⁹ is the predominant HCN in the ventricle. Of note, bradycardia by itself has been shown to contribute to QT and prolongation and the subsequent development of TdP.⁴⁵

Two large families with mutations in *HCN4* causing asymptomatic bradycardia have been reported by us and others.^{19,22} A missense mutation (S672R; Fig. 1, No. 5) found by Milanese et al.¹⁹ was associated with an asymptomatic bradycardia. Despite its location in the CNBD, this mutation did not affect the binding properties of cAMP, but changed the biophysical properties of the channel. Mutant channels deactivated more slowly and showed a voltage-dependent shift of activation toward hyperpolarization, leading to a decrease in I_f , which was in turn responsible for slowing down the heart rate.¹⁹ We described²² a family with asymptomatic sinus bradycardia with no extracardiac abnormalities, managed conservatively during long-term follow-up (14 ± 11 years). Eight family members were classified as affected. All the affected family members were asymptomatic with a normal exercise capacity during long-term follow-up. A genetic analysis revealed a missense mutation (G480R) in the *HCN4* channel pore (Fig. 1, No. 1). The reduction in I_f in this family was due to both functional changes in channels that reach the plasma membrane, as well as reduced membrane expression of the *HCN4* channels.²²

We recently⁴⁶ identified three additional families with symptomatic bradycardia. The affected members presented with a history of presyncope, except for one subject who had a poorly documented event of loss of consciousness with apparent cardiopulmonary arrest, which resolved following basic cardiopulmonary resuscitation; he recovered without defibrillation. There were no documented events of syncope and all had a normal exercise test. Sequencing of the *HCN4* gene in the probands of these families revealed a new heterozygous C to T transition in exon 4. This change predicted a substitution of alanine to valine at position 485 within the pore-forming region of the membrane (Fig. 1, No. 2). A485 is a conserved residue and the mutation was not found in unaffected relatives as well as in 50 controls. The reason why the affected family members in our first family reported and some of the affected family members in our previous report carry such a benign prognosis without any chronotropic incompetence despite a mutation in such a conserved pore region is not clear. Among possible explanations are the following: (1) the WT *HCN4* protein is up-regulated resulting in only mild reduction of I_f ; (2) expression of other currents may increase to compensate for the loss of *HCN4* current; or (3) I_f may not be the only current responsible for spontaneous diastolic depolarization of the sinus node or responsible for sympathetic stimulation in humans.

Clinical Correlation between Animal Models and Human Studies

The families described^{19,22,46} and the knockout mice lacking *HCN4*^{40,41} both display abnormalities of the sinus node function. Moreover, as in the adult *HCN4* knockout mouse model reported by Herrmann et al.,⁴¹ the affected family members and the knockout mice had no impairment in a heart rate response during exercise. It was not clear whether or which channel/current compensated for the loss of *HCN4* in this model. Herrmann et al.⁴¹ did not observe upregulation of *HCN2*, L-type and T-type calcium channels, potassium channels, or Na/Ca exchanger. In another adult mouse model,⁴⁰ neither chronotropic incompetence nor bradycardia was observed at rest, but sinus pauses were evident mainly during exercise. The main difference between the two mouse models and the families with bradycardia described above was the resting heart rate. While normal basal heart rates were observed in the knockout mice, basal heart rate was reduced in the case of the humans,^{19,22} a finding that may be explained by the difference in basal heart rate between humans and mice. Mouse heart rate frequency is 10 times faster than that of the human heart, and I_f may play a more significant role at slower heart rates.^{40,41} Another explanation may be that a current, not expressed in the human heart, but expressed in the knockout mouse model, provides a compensatory force. Taken together, the available animal and human studies suggest that I_f is a major contributor to spontaneous diastolic depolarization at rest, evident by the significant bradycardia that all humans carrying mutations in *HCN4* display, some of

them symptomatic. The role in response to sympathetic stimulation is less clear. It seems that I_f makes a critical contribution during embryonic development. In adult life, there seems to be some discrepancy between animal models and humans. In most cases, humans with heterozygous mutations in *HCN4* did not have chronotropic incompetence with the exception of one case with a stop codon mutation creating an *HCN4* channel lacking the CNBD. In adult mice, one study⁴⁰ in which adult mice had a heterozygous mutation reported that sinus pauses were accentuated during exercise, whereas in another report⁴⁰ in which the adult mice lacked *HCN4*, all had a normal increase in heart rate during sympathetic drive. The reason for this different observation between the two models is not clear. One possibility is that mice completely lacking *HCN4* have some kind of compensatory mechanism that mice only deficient in *HCN4* do not have. This hypothesis remains to be tested.

The Role of *HCN4* in Other Cardiac Diseases

HCN4 may also contribute to other cardiac pathological conditions. A decrease in *HCN4* in the SAN of tachy-paced dogs that develop congestive heart failure (CHF) was found to be associated with prolonged sinus node recovery time.⁴⁷ This may contribute, at least in part, to sinus node dysfunction in CHF. I_f was also found to be increased in ventricular myocytes of rats suffering from ventricular hypertrophy due to hypertension.⁴⁸ *HCN4* is present in other regions of the rat's right atrium in addition to the SAN,⁴⁹ which under certain conditions may lead to atrial ectopy and tachyarrhythmias.

The Role of *HCN4* in Treatment of Brady- and Tachycardia

Blockade of I_f has the potential to reduce heart rate without reducing contractility. This is particularly important in patients with heart failure, ischemia, or both. Although several I_f blockers exist, ivabradine is the prototype and the most studied. Of note, these drugs, particularly at higher concentrations, have the ability to block other channels, such as I_{Ca-L} and I_K ,^{50,51} and therefore, some of their effects may not be attributed solely to the blockade of the "funny" current. Two large clinical studies evaluated the antianginal and antiischemic effects of ivabradine.⁵²⁻⁴³ The main conclusion was that ivabradine improves the time required to develop exercise-induced ischemia. Patients in the ivabradine group had lower resting and lower maximal heart rates during exercise. These effects were dose dependent. The highest dose used reduced maximal heart rate by an average of <20 beats/min. In studies on patients with CHF, ivabradine reduced heart rates without reducing left ventricular ejection fraction.⁵³ The most serious and common adverse reaction in this study and others⁵⁴ was visual symptoms thought to be related to blockade of ion channels in the retina. Currently, the effect of this drug is under investigation by researchers in two clinical trials, BEAUTIFUL⁵⁵ and SHIFT.⁵⁶ BEAUTIFUL is a multicenter, double-blind, placebo-controlled trial to evaluate the superiority of ivabradine over placebo as a lowering heart rate agent in reducing cardiovascular events in patients with stable coronary artery disease and left ventricular ejection fraction <40%. The primary end point is the composite of cardiovascular mortality and hospital admission for acute myocardial infarction or new onset or worsening of heart failure. The main results from this trial were recently published.^{57,58} Ivabradine significantly reduced heart rate over time. The difference in the mean heart rate (placebo minus ivabradine) at 12 months was 6.4 beats/min and 5.6 beats/min at 24 months. In patients with a baseline heart rate over 70 beats/min, the difference in heart rate was even greater. In this patient group (a baseline heart rate ≥ 70 beats/min), ivabradine reduced the incidence of hospitalization for myocardial infarction and subsequent coronary revascularization procedures. Interestingly, in this group ivabradine did not affect cardiovascular death or hospitalization for worsening heart failure. There were no major side

effects reported. SHIFT will investigate patients with reduced left ventricular function because of various causes in addition to myocardial ischemia.⁵⁶

I_f blockers may also serve as a tool to study the effect of “funny” current inhibition on the sinus node. Mice treated with ivabradine exhibited a decrease in heart rate and at higher doses displayed fluctuations in the R–R interval.⁴⁴ Treated mice did not achieve the same maximal heart rate during exercise as before the drug. These results are somewhat at odds with the studies discussed above in which mice lacking *HCN4* showed normal chronotropic competence even after adding Cilobradine (another I_f blocker).⁴¹ One explanation may be that the mice genetically lacking *HCN4* may have another channel/current compensating for the loss of the “funny” current. Theoretically, *HCN* blockade may also play a role in the treatment of inappropriate sinus tachycardia although, with the exception of one case, clinical data are lacking.⁵⁹

Recently another drug with the capability to block I_f was discovered.⁶⁰ Clonidine decreases heart rate without lowering blood pressure when administered in low doses to mice. In isolated atrial cells, clonidine inhibits I_f in the low micromolar range (approximately 3 $\mu\text{mol/L}$).

At present there is no effective alternative to pacing for patients with SN dysfunction. THEOPACE, a trial designed to assess the effect of theophylline,⁶¹ showed that although oral theophylline increases resting and exercise heart rate, it did not reduce the occurrence of syncope. Thus, there is a need for alternatives that more completely replicate the normal function of SN, other than electrical pacing. Overexpression of I_f may also serve as a therapeutic modality. Recent studies^{62,63} have implicated *HCN* channels in gene-based therapy. Modification of *HCN1* or *HCN2* DNA resulted in overexpression of I_f and an increase in heart rate, thus offering a potential alternative to artificial pacing in SN and conduction system disease. Cai and colleagues⁶⁴ transfected an adenoviral vector containing *HCN4* into the free left ventricular wall of pigs that underwent AV-nodal ablation. These pigs maintained a rapid idioventricular rhythm compared to pigs that were not transfected with *HCN4*. Whole cell recordings from the transduced myocytes demonstrated significant differences in I_f between transfected and nontransfected cells.

Summary and Conclusion

The role of I_f in maintaining slow diastolic depolarization in SAN cells and its contribution to adrenergic stimulation are still not completely understood. From recent animal and human studies, it appears that the role of *HCN* channels in autonomic regulation of heart rate in adults is important especially in maintaining normal heart rate at rest. Loss of their function has been shown to cause varying degrees of sinus bradycardia. The role during sympathetic drive is still under debate. Although most of the reported *HCN4* mutation carriers had asymptomatic bradycardia, at present, there is no reason why clinical evaluation of these patients should differ from any other patient presenting with bradycardia. Understanding the pathophysiology of these channels provides an opportunity for new treatment options for a wide range of patients. Blocking these channels may help in reducing heart rate without causing undesirable systemic cardiovascular reactions in patients with CHF and coronary artery disease and in patients suffering from conditions such as inappropriate sinus tachycardia. Overexpression of these channels may provide exciting opportunities in the development of biological pacemakers.

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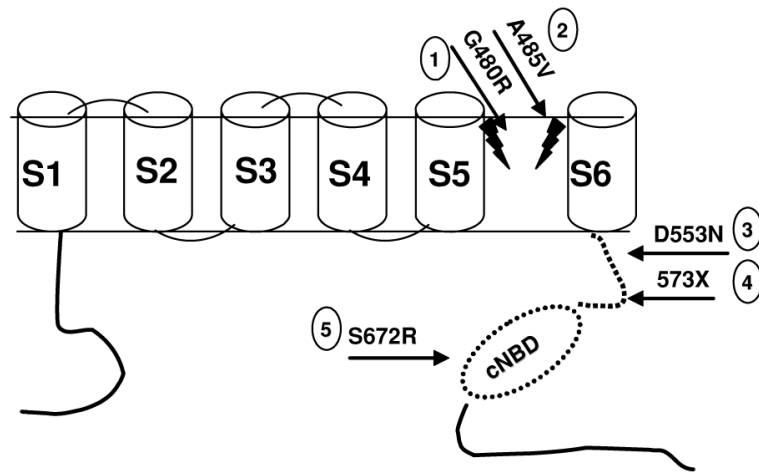


Figure 1. Schematic topology of HCN4 and related mutations in humans. Mutations 122 and 246 are in the ion channel pore. Mutations 321 and 420 are in the region between the core domain and cyclic nucleotide binding domain (CNBD; dashed line). Mutation 519 is in the CNBD itself. Reproduced from Nof et al.²² with permission.

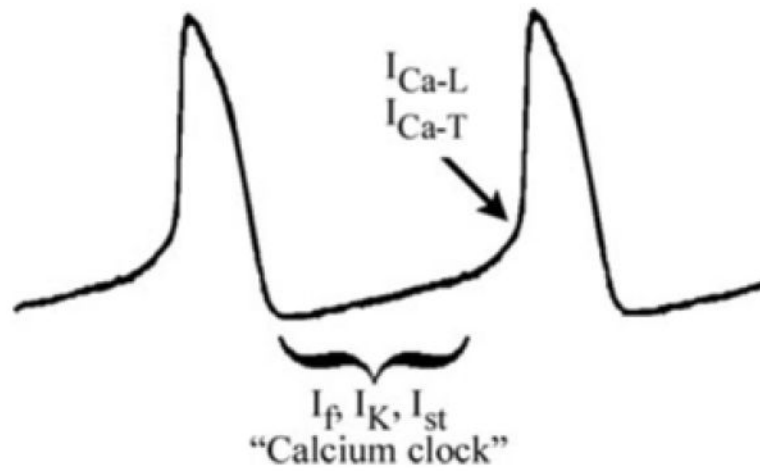


Figure 2. Schematic of the sinus node action potential and key players thought to contribute to spontaneous diastolic depolarization: I_f = hyperpolarization-activated non-selective cation current or “funny” current; I_K = deactivation of delayed rectifier current; I_{st} = sustained inward current; I_{Ca-L} = L-type calcium channel current and I_{Ca-T} = T-type calcium channel current; “Calcium Clock” (see text for further details).

Table 1
Basic Clinical and Expressional Study Characteristics of Described Human Mutant HCN4 Channels

Mutation	Patients (pt)	Bradycardia at Rest	Symptoms	Chronotropic Incompetence	Need for Permanent Pacemaker	Loss of Channel Function Due to	
						Trafficking Defect	Change in Biophysical Properties
573× 20	Single pt	Yes	Syncope	Yes	Yes	No	Yes
D553N21	Single pt	Yes	Syncope	No	Yes	Yes	No
S672R19	One family	Yes	No	No	No	No	Yes
G480R22	One family	Yes	No	No	No	Yes	Yes
A585V46	Three families	Yes	Presyncope	No	No	Yes	Yes