Bradycardic Effects of Mutations in the *HCN4* Gene at Different Levels of Autonomic Tone in Humans

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Abstract

HCN4 channels are expressed in the human sinoatrial node (SAN) and conduct the hyperpolarization-activated 'funny current', I_f, also known as 'pacemaker current'. Several loss-of-function mutations in the HCN4 gene have been associated with human sinus bradycardia. Clinical observations suggest that bradycardic effects are present at all levels of autonomic tone.

We assessed the effects of three different mutations in HCN4 on human SAN pacemaker activity at different levels of autonomic tone by incorporating experimentally identified mutation-induced changes in I_f into the recently developed Fabbri et al. model of a single human SAN cell. Different levels of autonomic tone were obtained through simulated administration of specific levels of acetylcholine (ACh) or isoprenaline (Iso).

The G480R, A485V, and 695X mutations in HCN4 lowered the control beating rate from 74 to 62, 59, and 65 bpm, the ACh beating rate from 49 to 40, 37, and 45 bpm, and the Iso beating rate from 140 to 115, 100, and 109 bpm, respectively, all in accordance with the clinical observations.

We conclude that experimentally observed changes in the expression and kinetics of I_f channels can explain the clinically observed bradycardic effects of loss-of-function mutations in HCN4 at different levels of autonomic tone.

1. Introduction

The hyperpolarization-activated 'funny current' (I_f), also known as 'pacemaker current', is a key player in sinoatrial node (SAN) pacemaker activity. This (mainly) inward current of mixed ionic nature is a determinant of the spontaneous depolarization that underlies SAN pacemaker activity and thus a modulator of pacing rate [1,2]. The membrane ionic I_f channel is constituted by four hyperpolarization-activated, cyclic-nucleotide-gated (HCN) subunits. The HCN4 protein, which is encoded by the *HCN4* gene, is the dominant HCN isoform in rabbit and human SAN [3–6].

Since 2003, several loss-of-function mutations in the *HCN4* gene have been associated with human sinus bradycardia [7,8]. For example, Nof et al. [9] reported on an Israeli family with the G480R mutation. Average resting heart rate (mean ± SEM) was 49 ± 12 bpm (range 36–72 bpm) in the 7 mutation carriers vs. 73 ± 11 bpm (range 55–89 bpm) in the 8 non-affected family members. Similarly, Laish-Farkash et al. [10] reported on three families of Moroccan Jewish decent with the A485V mutation. The average heart rate of 14 mutation carriers was 58 ± 6 bpm (range 49–70 bpm), whereas that of 5 non-carriers was 77 ± 12 bpm (range 59–91 bpm). In a single German family with the 695X mutation, Schweizer et al. [11] observed an average heart rate of 56 ± 5 bpm (range 47–61 bpm) in 7 mutation carriers vs. 72 ± 10 bpm (range 58–86 bpm) in 6 non-carriers. The clinical data of Nof et al. [9], Laish-Farkash et al. [10], and Schweizer et al. [11] are not limited to the average heart rate. They also reported the minimum and maximum heart rate obtained with 24-hour Holter recording. These data are summarized in Table 1 and visualized in Figure 1.

The G480R, A485V, and 695X mutations in *HCN4* all reduce the minimum, average, and maximum heart rate by ≈20–30%, likely due to mutation-induced changes in the expression and kinetics of the *HCN4*-encoded I_f channels. Voltage clamp experiments on wild-type and heterozygous mutant HCN4 channels expressed in cell lines revealed changes in the expression and kinetics of these channels [12,13], but the functional effects of these changes on human SAN pacemaker activity remain unresolved.

In the present study, we attempted to assess the effects of the aforementioned *HCN4* mutations on human SAN pacemaker activity by incorporating the experimentally identified changes in expression and kinetics of HCN4

 ISSN: 2325-887X DOI:10.22489/CinC.2017.017-008

Table 1. Clinical observations on heart rate in carriers of mutations in *HCN4*.

	HR in mutation carriers (bpm)				HR in non-carriers (bpm)				
Mutation	Min	Avg	Max	n	Min	Avg	Max	n	Study
G480R	32 ± 8*	49 ± 12*	101 ± 21*	7	55 ± 9	73 ± 11	126 ± 17	8	Nof et al. [9]
A485V	37 ± 3*	58 ± 6*	117 ± 27	14	49 ± 11	77 ± 12	140 ± 33	5	Laish-Farkash et al. [10]
695X	36 ± 6*	56 ± 5*	131 ± 17	7	47 ± 6	72 ± 10	157 ± 26	6	Schweizer et al. [11]

Minimum (Min), average (Avg), and maximum (Max) heart rate (HR) obtained with 24-hour Holter recording. Data are mean ± SEM. *P<0.05 vs. non-affected family members.

channels in the comprehensive mathematical model of a single human SAN pacemaker cell that was recently developed by Fabbri et al. [14]. This model allows different levels of autonomic tone through the simulated administration of acetylcholine (ACh; vagal tone) or isoprenaline (Iso; β-adrenergic tone).

2. Methods

Mutations in *HCN4* were implemented in the CellML code [15] of the Fabbri et al. model [14] by scaling the fully-activated conductance of I_f and/or shifting its voltage dependence, based on the data from literature obtained in voltage-clamp experiments on HCN4 channels expressed in cell lines [12,13]. The G480R mutation was simulated through a 50% reduction in fully-activated conductance (i.e., a scaling factor of 0.5) and a −15 mV shift in voltage dependence, whereas a scaling factor of 0.33 and a −30 mV shift were used to simulate the A485V mutation. The loss of cAMP sensitivity in case of the 695X mutation was simulated by a fixed −10.1 mV shift in voltage dependence, in line with the maximum effect of acetylcholine.

The default Fabbri et al. model [14] has a beating rate of 74 bpm. This rate was lowered to 49 bpm (vagal tone) through the simulated administration of 20 nM ACh. The major effects of the administration of ACh are the activation of the ACh-activated potassium current $I_{K,ACh}$, which is zero in the default model, and the inhibition of I_f through a negative shift in its voltage dependence [14]. A beating rate of 140 bpm (β-adrenergic tone) was obtained through the simulated administration of Iso, tuning the parameters affected by Iso to arrive at this beating rate. The simulated effects of Iso included a +10 mV shift in the voltage dependence of I_f.

The CellML code was edited and run in the Cellular Open Resource (COR) environment [16], version 0.9.31.1409. All simulations were run for a sufficiently long time to reach steady-state behaviour.

3. Results

3.1. Autonomic modulation

Fig. 2A shows the effects of autonomic modulation through ACh and Iso on the electrical activity of the human SAN pacemaker cell model of Fabbri et al. [14]. Under control conditions, the model cell shows pacemaker activity with a cycle length of 813 ms (Fig. 2A, top panel, grey trace). The associated time course of I_f is shown in the bottom panel of Fig. 2A. The amplitude of I_f is ≈1 pA, whereas the amplitude of the net inward current is ≈2 pA (not shown). Thus, I_f is an important inward current during the diastolic depolarization phase.

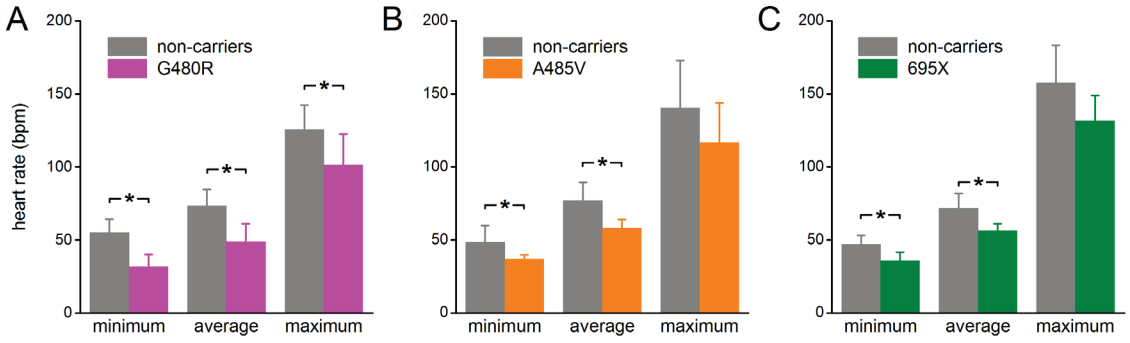

Figure 1. Minimum, average, and maximum heart rate in carriers of the (A) G480R, (B) A485V, and (C) 695X mutations in the *HCN4* gene. Data are from 24-hour Holter recording and presented as mean ± SEM. *P<0.05 vs. non-affected family members ('non-carriers').

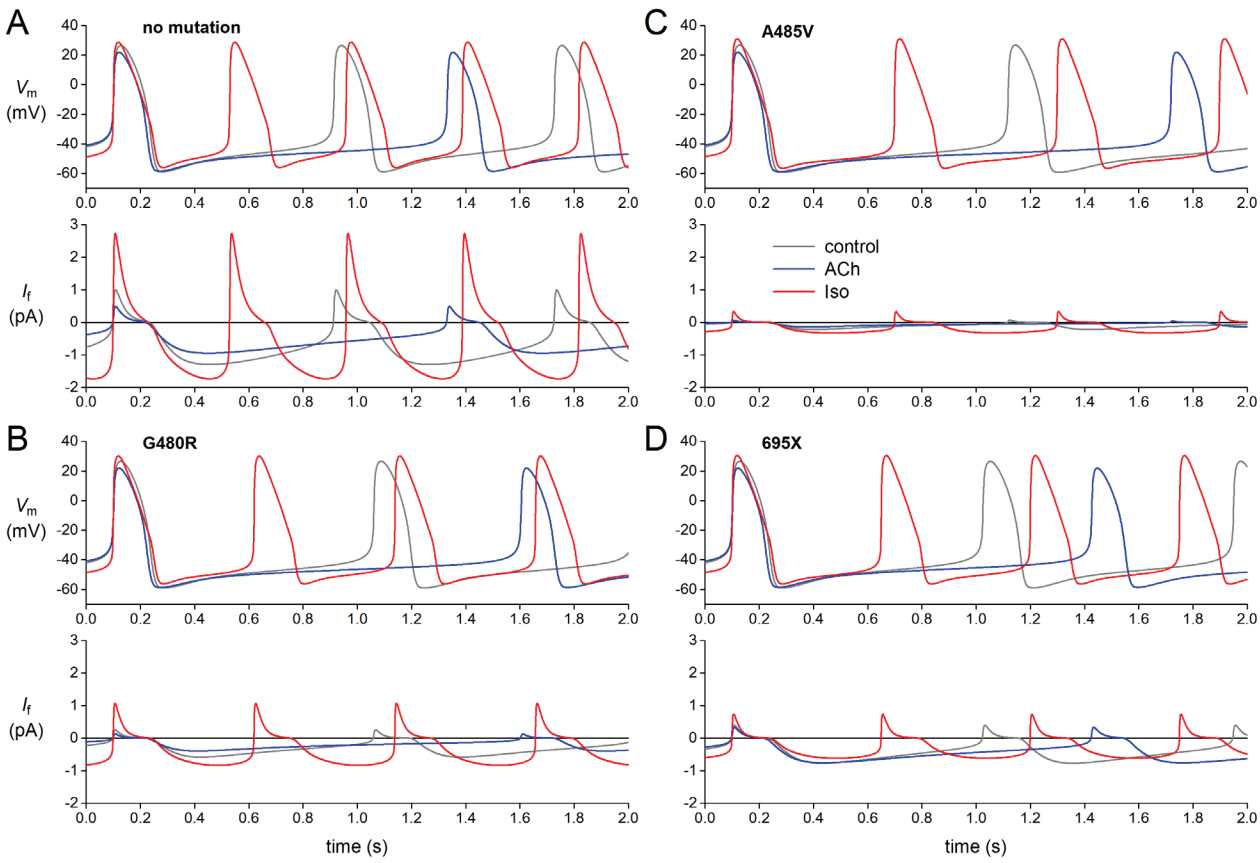

Figure 2. Effects of mutations in *HCN4* on the pacemaker activity of the Fabbri et al. [14] model of a single human sinoatrial node cell at different levels of autonomic tone. Membrane potential (V_m) and associated hyperpolarization-activated 'funny current' (I_f). (A) Default model (no mutation). (B) G480R, (C) A485V, and (D) 695X mutations.

The inhibition of I_f by ACh contributes to the increase in cycle length to 1,231 ms (Fig. 2A, blue traces). The application of Iso stimulates I_f and decreases cycle length to 429 ms (Fig. 2A, red traces). A loss-of-function mutation in *HCN4* will lead to a smaller I_f, and thus a decrease in inward current during diastolic depolarization, at all levels of autonomic tone.

3.2. Mutations in *HCN4*

Fig. 2, B–D, shows the effects of mutations in *HCN4* on the pacemaker activity of the model cell at different levels of autonomic tone. The G480R mutation (Fig. 2B) reduces I_f by ≈50%. As a result, the cycle length increases from 813 to 961 ms (+18%) under control conditions. With ACh and Iso, the cycle length increases from 1,231 to 1,503 ms (+22%) and from 429 to 519 ms (+21%), respectively.

With a −30 mV shift in voltage dependence and a scaling factor of 0.33, the A485V mutation is more 'severe' than the G480R mutation, which shows a −15 mV shift and a scaling factor of 0.5. This is reflected in a smaller amplitude of I_f and slower pacemaking (Fig. 2C). The cycle length now amounts to 1,018 ms under control conditions (+25%) and to 1,618 ms (+31%) and 599 ms (+40%) with ACh and Iso, respectively.

The functional effects are somewhat different for the 695X mutation, which leads to a loss of cAMP sensitivity of I_f rather than a shift in its voltage dependence and/or a reduction in its fully-activated conductance. With an increase in cycle length to 1,326 ms (+8%), the effects of the 695X mutation are relatively mild at vagal tone (Fig. 2D). Under control conditions and with Iso, cycle length increases to 924 ms (+14%) and to 550 ms (+28%), respectively.

The functional effects of the three different mutations are summarized in Fig. 3, which shows the beating rate of the model cell at different levels of autonomic tone for each of the mutations. The grey bars show the beating rate in the absence of a mutation.

4. Discussion

We assessed the effects of three different *HCN4* mutations (G480R, A485V, and 695X) on human SAN pacemaker activity at different levels of autonomic tone,

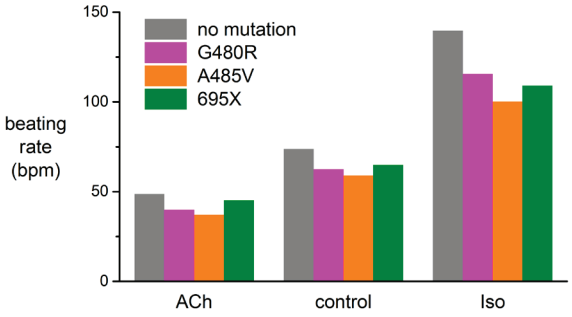

Figure 3. Effects of the G480R, A485V, and 695X mutations in *HCN4* on the beating rate of the Fabbri et al. [14] human sinoatrial node model cell at different levels of autonomic tone. Vagal tone (ACh, left), control (middle), and β-adrenergic tone (Iso, right).

using the model of a single human SAN pacemaker cell that was recently developed by Fabbri et al. [14]. These particular mutations were selected for their different electrophysiological effects and availability of clinical data on minimum, average, and maximum heart rates in multiple mutation carriers as well as non-carriers from the same family. The effects of the mutations on the beating rate of the model cell match the clinically observed effects on heart rate, at least qualitatively.

There are several factors, both physiological and non-physiological, that obscure a direct quantitative comparison between our simulation results and the available clinical data. One is the hyperpolarizing effect of the surrounding atrium in the intact heart, which will result in a more prominent role of I_f in vivo. Another is that experimental data from voltage clamp experiments on mutant HCN4 channels are often incomplete and potentially strongly dependent on the expression system and experimental protocol that were employed.

5. Conclusion

We conclude that experimentally observed changes in the expression and kinetics of I_f channels can explain the clinically observed bradycardic effects of loss-of-function mutations in *HCN4* at different levels of autonomic tone.

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