# In Quest of a Sinoatrial Cell Model to Assess the Functional Effects of Mutations in the *HCN4* Funny Current Gene

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#### Abstract

Several loss-of-function mutations in the HCN4 gene have been associated with human sinus bradycardia. The HCN4 channel underlies the hyperpolarization-activated 'funny current' ( $I_f$ ), which plays an important role in sinoatrial node (SAN) pacemaker activity.

We attempted to assess the effects of HCN4 mutations on SAN pacemaker activity through computer simulations, using the most recent comprehensive mathematical models of single SAN cells. To this end, we incorporated the experimentally identified changes in expression and kinetics of  $I_f$  in the Severi-DiFrancesco and Maltsev-Lakatta models of a single rabbit SAN cell.

In the Severi-DiFrancesco model, 5 out of 11 mutations tested led to cessation of pacemaker activity rather than bradycardia, emphasizing the critical role of  $I_f$  in this model. In contrast, the decrease in pacing rate amounted to a maximum of only 5.2% for the most severe mutation in the Maltsev-Lakatta model. These results became even more distinct upon replacement of the  $I_f$  equations of either model by the ones that we recently derived from our experimental data on rabbit SAN cells.

We conclude that the most recent comprehensive mathematical models of single SAN cells do not allow adequate investigations of the functional effects of mutations in the HCN4 funny current gene.

### 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  $I_f$  channel is constituted by four hyperpolarization-activated, cyclic-nucleotide-gated (HCN) subunits, with the HCN4 protein, which is encoded by the *HCN4* gene, as 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, Milanesi et al. [9] reported on an Italian family with the S672R mutation. Average resting heart rate (mean  $\pm$  SEM) was 52.2  $\pm$  1.4 beats/min (range 43–60 beats/min) in the 15 mutation carriers vs. 73.2  $\pm$  1.6 beats/min (range 64–81 beats/min) in the 12 non-affected family members. Similarly, Nof et al. [10] reported on a family with the G480R mutation. The average heart rate of the 8 mutation carriers was 48  $\pm$  12 beats/min, whereas that of the 8 non-carriers was 73  $\pm$  11 beats/min. In three families of Moroccan Jewish decent, Laish-Farkash et al. [11] observed an average heart rate of 58  $\pm$  6 beats/min in 14 carriers of the A485V mutation and 77 $\pm$ 12 beats/min in 6 non-carriers.

Only recently, Baruscotti et al. [12] reported on a gainof-function mutation in *HCN4*. Five family members and carriers of this R524Q mutation exhibited inappropriate sinus tachycardia rather than bradycardia, with a daytime heart rate of 98.5  $\pm$  14.2 beats/min (mean  $\pm$  SD) in the proband.

The S672R, G480R, and A485V loss-of-function mutations in *HCN4* reduce resting heart rate by  $\approx$ 30%, whereas the R524Q gain-of-function mutation increases it substantially. Voltage-clamp experiments on HCN4 channels expressed in cell lines have revealed changes in the expression and kinetics of these and other mutant channels [13,14], but the functional effects of these changes on SAN pacemaker activity remain unresolved.

In the present study, we attempted to assess the effects of HCN4 mutations on SAN pacemaker activity by incorporating the experimentally identified changes in expression and kinetics of HCN4 channels, conducting  $I_{\rm f}$ , in the most recent comprehensive mathematical models of single SAN cells. These are the models by Maltsev and Lakatta [15] ('Maltsev-Lakatta model') and Severi et al. [16] ('Severi-DiFrancesco model'). Both models represent the electrophysiological behavior of a single isolated 32-pF rabbit SAN cell and are based on the large amount of experimental data that have become available over the years, largely obtained in patch-clamp experiments on isolated rabbit SAN cells.

Mutation	Scaling factor	Shift (mV)	Maltsev-Lakatta model		Severi-DiFrancesco model	
			CL (ms)	Δf (%)	CL (ms)	Δf (%)
Control	1	0	332.7	0	354.7	0
P257S	0.5	0	340.6	-2.3	409.6	-13
A414G	1	-23.9	347.7	-4.3	_	_
G480R	0.5	-15.0	347.2	-4.2	_	_
Y481H	1	-43.9	350.9	-5.2	_	_
G482R <sup>a</sup>	1	-38.7	350.6	-5.1	_	_
G482R <sup>b</sup>	0.35	0	343.4	-3.1	444.5	-20
A485V	0.33	-30	350.5	-5.1	—	_
R524Q	1	+4.2	328.4	+1.3	319.5	+11
K530N	1	-14	343.2	-3.1	577.5	-39
D553N	0.37	0	343.0	-3.0	438.8	-19
S672R	1	-4.9	336.9	-1.2	402.4	-12
G1097W	0.55	-7.6	343.9	-3.3	509.6	-30
Full block	0	0	351.0	-5.2	_	_

Table 1. Parameter settings and simulation results.

Scaling factor applied to fully-activated  $I_f$  conductance. Shift in voltage dependence applied to steady-state activation curve as well as time constant of (de)activation.  $\Delta f$ , percent change in pacing rate. <sup>a</sup>According to Milano et al. [19]. <sup>b</sup>According to Schweizer et al. [20].

#### 2. Methods

The source code of the Maltsev-Lakatta and Severi-DiFrancesco models [15,16] was taken from the model repository on the CellML website [17]. For consistency and to prevent slow drifts, we set the intracellular Na<sup>+</sup> concentration of the Severi-DiFrancesco model [16] to a constant value, as in the Maltsev-Lakatta model [15].

Mutations in *HCN4* were implemented by scaling the fully-activated conductance of  $I_{\rm f}$  and/or shifting its voltage dependence, based on the data from literature obtained in voltage-clamp experiments on HCN4 channels expressed in cell lines [13,14]. The scaling factors and shifts are listed in Table 1 (left columns).

The CellML code was edited and run in the Cellular Open Resource (COR) environment [18], version 0.9.31.1409. All simulations were run for a sufficiently long time to reach steady-state behavior.

#### 3. **Results**

## 3.1. Maltsev-Lakatta model

Fig. 1 shows results obtained with the Maltsev-Lakatta model. All loss-of-function mutations result in a decrease in  $I_{\rm f}$  and an accompanying increase in cycle length.

However, the decrease in pacing rate ( $\Delta f$ ) is limited to only a few percent (Table 1), much less than what might be expected from the aforementioned clinically observed decrease in resting heart rate of  $\approx 30\%$ . Similarly, the sole gain-of-function mutation, R524Q, results in an increase in pacing rate of only 1.3% (Table 1).

The small changes in pacing rate point to a typical feature of the Maltsev-Lakatta model: with a slowing of 5.2%, full block of  $I_f$  has a marginal effect on pacing rate (Fig. 1, dashed grey lines). This reflects the viewpoint that the contribution of  $I_f$  to heart rate regulation is only modest [21], which is subject of an ongoing debate that will not be repeated here.

# 3.2. Severi-DiFrancesco model

Next, we carried out simulations with the Severi-DiFrancesco model. The observed effects of mutations in *HCN4* are much larger than with the Maltsev-Lakatta model (Fig. 2), up to a decrease in pacing rate of 39% for the K530N mutation (Table 1). However, pacemaker activity ceases for 5 out of the 11 loss-of-function mutations tested (Fig. 2). Pacemaker activity also ceases upon full block of  $I_{\rm f}$ . This illustrates a typical feature of the Severi-DiFrancesco model: pacemaker activity is critically dependent on  $I_{\rm f}$ . Thus,  $I_{\rm f}$  surely has "a major pacemaking role" [22].

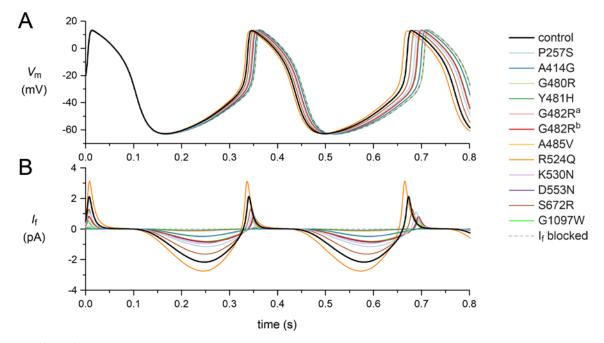


Figure 1. Effect of heterozygous mutations in *HCN4* on pacemaker activity in the Maltsev-Lakatta rabbit SAN cell model. (A) Membrane potential ( $V_m$ ). (B) Associated hyperpolarization-activated 'funny current' ( $I_f$ ).

## **3.3.** Unification of *I*<sup>f</sup> equations

If we replace the  $I_{\rm f}$  equations of either model by the ones that we recently derived from our experimental data on rabbit SAN cells [23], the above results become even more distinct: the Maltsev-Lakatta model shows even smaller changes in pacing rate, whereas pacemaker activity of the Severi-DiFrancesco model now even ceases under control conditions (data not shown).

## 4. Conclusion

The two most recent comprehensive mathematical models of single SAN cells show widely different results upon implementation of mutations in the HCN4 funny current gene. We conclude that these models do not allow adequate investigations of the functional effects of mutations in HCN4.

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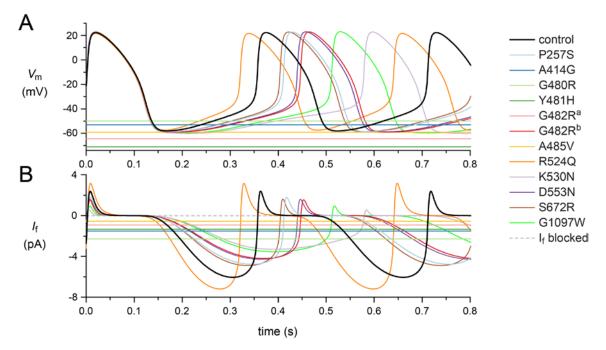


Figure 2. Effect of heterozygous mutations in *HCN4* on pacemaker activity in the Severi-DiFrancesco rabbit SAN cell model. (A) Membrane potential ( $V_m$ ). (B) Associated hyperpolarization-activated 'funny current' ( $I_f$ ). Pacemaker activity ceases in 5 out of 11 loss-of-function mutations tested.

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