Functional Role of the *HCN4* Encoded 'Funny Current' in Human Sinus Node Pacemaker Cells

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Abstract

We recently reported patch clamp data on the voltage dependence of HCN4 channels expressed in human cardiomyocyte progenitor cells. Their half-activation voltage was 15 mV less negative than previously observed for the HCN4 encoded hyperpolarization-activated 'funny current' (I_f) in isolated human sinus node cells. The time constant of (de)activation vs. voltage relationship showed a similar less negative voltage dependence as well as a 38% higher peak. We assessed the functional effects of these differences in I_f kinetics in the Fabbri–Severi model of a single human sinus node pacemaker cell.

The +15 mV shift in half-activation voltage per se resulted in a substantial increase in I_f, carrying 85 vs. 59% of the net diastolic depolarizing charge, and a 14% shortening of the cycle length from 813 to 699 ms. This effect was counteracted by the time constant vs. voltage relationship, which caused a slower activation of I_f in the diastolic membrane potential range. The resulting net effect was a 5.4% shortening of the cycle length from 813 to 770 ms, with I_f carrying 59% of the net diastolic charge, and limited effects on the autonomic modulation of pacing rate by isoprenaline and acetylcholine.

We conclude that the absolute value of the half-activation voltage of I_f may be less indicative of the functional role of I_f than commonly assumed.

1. Introduction

The hyperpolarization-activated 'funny current' (I_f), also known as 'pacemaker current', is a key player in sinus node pacemaker activity. This (mainly) inward current of mixed ionic nature is a determinant of the spontaneous depolarization that underlies the pacemaker activity of the sinus node and thus a modulator of its pacing rate [1,2]. The I_f ion channel in the cell membrane 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 the rabbit and human sinus node [3–6]. Not surprisingly, several loss-of-function mutations in the *HCN4* gene have been associated with human sinus bradycardia [7,8].

In 2007, Verkerk et al. [9] published patch clamp data on I_f in single pacemaker cells isolated from a human sinus node. These unique data were used by Fabbri et al. [10] in their comprehensive mathematical model of a single human sinus node pacemaker cell, which is known as the Fabbri–Severi model. Hoekstra et al. [11] recently reported comprehensive voltage clamp data on the kinetics of HCN4 channels expressed in human cardiac myocyte progenitor cells (CMPCs). As compared to the Verkerk et al. [9] data, the steady-state activation curve and the time constant of (de)activation vs. voltage relationship were both shifted by +15 mV. Furthermore, the time constant vs. voltage relationship showed a 38% higher peak.

In the present study, we replaced the I_f kinetics of the Fabbri–Severi model cell with kinetics based on the Hoekstra et al. [11] data and assessed the effects on the pacemaker activity of the model cell. These computer simulations were carried out with the standard model and at different levels of autonomic tone through the simulated administration of acetylcholine (ACh; vagal tone) or isoprenaline (Iso; β-adrenergic tone).

2. Methods

The HCN4 based I_f kinetics were implemented in the CellML code [12] of the Fabbri–Severi model [10] by replacing its I_f steady-state activation (y_{ss}) curve with a Boltzmann curve with a half-activation voltage of −81.7 mV and a slope factor of −8.5 mV (Fig. 1A) and replacing the time constant of (de)activation (τ_y, in s) vs. voltage relationship by

$$\tau_y = [\, A \times \exp(-B \times V_m) + C \times \exp(D \times V_m) \,]^{-1} + E \,,$$

with the membrane potential (V_m) in mV and parameters $A = 0.00191$ s^{-1}, $B = 0.0629$ mV^{-1}, $C = 24.41$ s^{-1}, $D = 0.0546$ mV^{-1}, and $E = 0.03$ s (Fig. 1B).

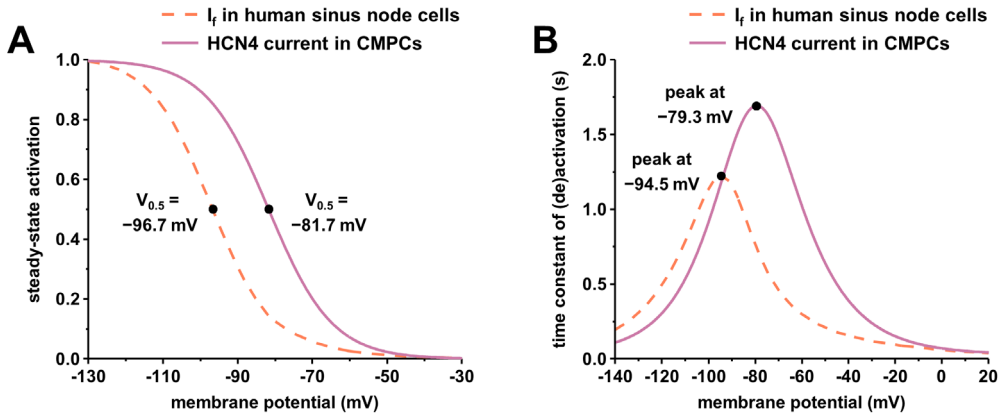

Figure 1. (A) Steady-state activation curve of I_f and (B) its time constant of (de)activation as used in the Fabbri–Severi model of a single human sinus node pacemaker cell [10] (dashed lines) and curves obtained in voltage clamp experiments on human cardiomyocyte progenitor cells (CMPCs) expressing HCN4 channels [11] (solid lines). The half-activation voltage ($V_{0.5}$) of the steady-state activation curve amounts to −96.7 and −81.7 mV, respectively. The slope factor is −8.2 and −8.5 mV, respectively.

The CellML code was edited and run in the Cellular Open Resource (COR) environment [13], version 0.9.31.1409. All simulations were run for a sufficiently long time to reach steady-state behaviour.

3. Results

The cycle length of the model cell is reduced by 44 ms upon introduction of the HCN4 based I_f kinetics, without any major changes in the time course of I_f and the net membrane current (I_{net}), as illustrated in Fig. 2, A–C. The pacing rate is increased by 5.7% (Fig. 2D), whereas the diastolic charge carried by I_f (Q_f) remains 59% of the net diastolic charge (Q_{net}) (Fig. 2E). With an increase in cycle length of 272 instead of 229 ms and a decrease in pacing rate of 26 instead of 22% the effect of full block of I_f is slightly increased (Fig. 2, A and D).

Next, we tested the effects of the change in the steady-

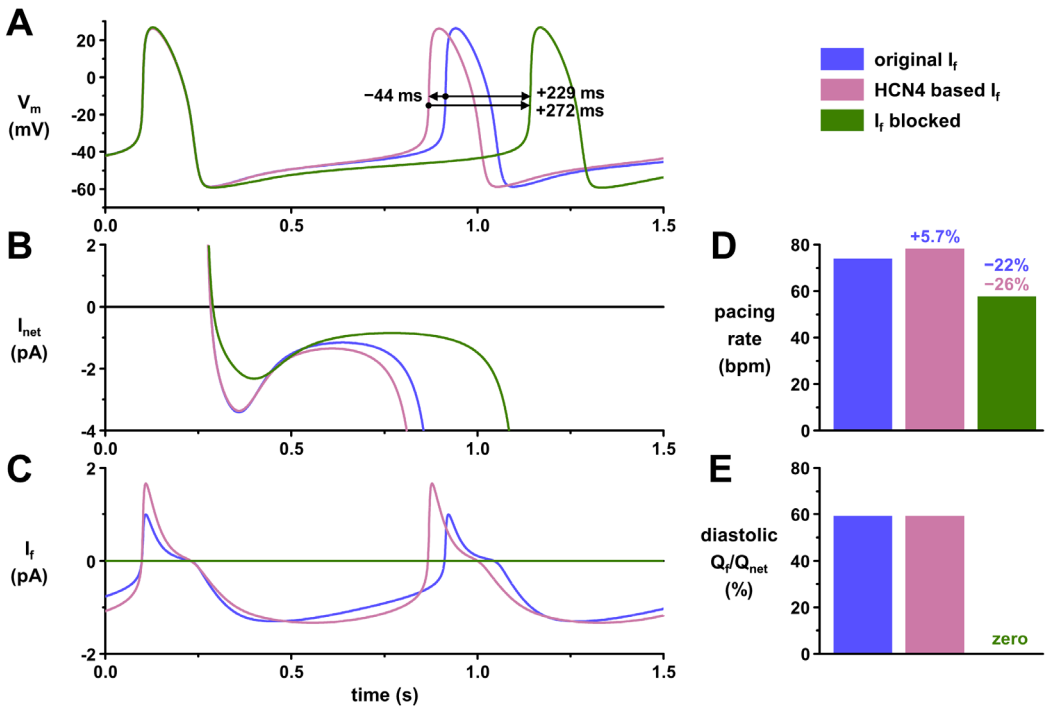

Figure 2. Pacemaker activity in the Fabbri–Severi model of a single human sinus node pacemaker cell [10] with its original I_f, with the HCN4 based I_f, and upon full block of I_f. (A) Membrane potential (V_m). (B) Net membrane current (I_{net}). (C) I_f. (D) Pacing rate. (E) Diastolic charge carried by I_f (Q_f) as percentage of the net diastolic charge (Q_{net}).

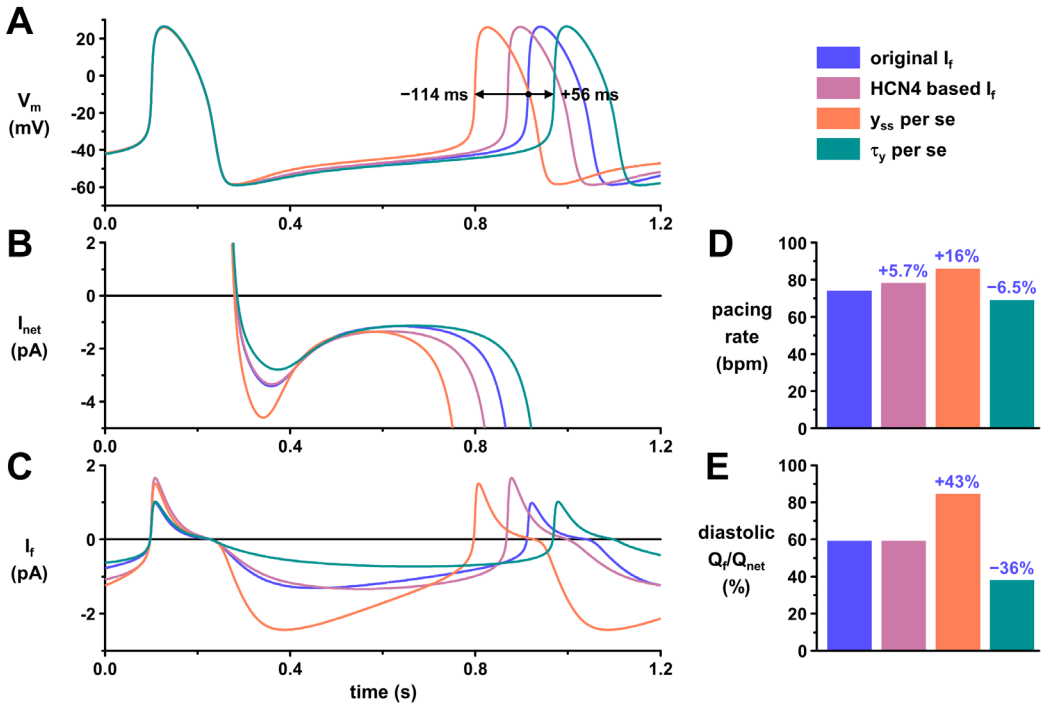

Figure 3. Pacemaker activity in the Fabbri–Severi model of a single human sinus node pacemaker cell [10] with its original I_f, with the HCN4 based steady-state activation (y_{ss}) and time constant of (de)activation (τ_y) of I_f ('HCN4 based I_f'), with the HCN4 based y_{ss} *per se*, and with the HCN4 based τ_y *per se*. (A) Membrane potential (V_m). (B) Net membrane current (I_{net}). (C) I_f. (D) Pacing rate. (E) Diastolic charge carried by I_f (Q_f) as percentage of the net diastolic charge (Q_{net}).

state activation curve and the change in the time constant of (de)activation vs. voltage relationship *per se*. As shown in Fig. 3, these changes have opposite effects. The change in the steady-state activation curve *per se* results in a substantially larger diastolic I_f and a 16% increase in pacing rate, whereas the change in the time constant vs. voltage relationship *per se* results in a substantially smaller diastolic I_f and a 6.5% decrease in pacing rate. The net effect is a considerably smaller increase in pacing rate than might be anticipated from the +15 mV shift in the steady-state activation curve.

Finally, we tested to which extent the autonomic modulation of the pacemaker activity of the model cell was affected by the introduction of HCN4 based I_f kinetics. To this end, we repeated the simulations of Fig. 2 at different levels of autonomic tone through the simulated administration of 1 μM isoprenaline (Iso; β-adrenergic tone) and 10 nM acetylcholine (ACh; vagal tone). The results are shown in Fig. 4. In the original model, the cycle length decreases by 175 ms with Iso and increases by 214 ms with ACh (Fig. 4A), which corresponds to a 27% increase and 21% decrease in pacing rate, respectively (Fig. 4E). With the HCN4 based I_f kinetics the decrease in cycle length with Iso and the increase with ACh amount to 173 and 194 ms, respectively (Fig. 4C), corresponding to a 29% increase and 20% decrease in pacing rate, respectively (Fig. 4F). Thus, the introduction of HCN4 based I_f kinetics has only small effects on the autonomic modulation of the pacing rate.

4. Conclusions

We conclude that the Fabbri–Severi model of a human sinus node cell does not show major changes in its pacemaker activity if its I_f kinetics are based on recent experimental data on HCN4 current in human CMPCs, which emphasizes that the absolute value of the half-activation voltage of I_f may be less indicative of the functional role of I_f than commonly assumed. Accordingly, voltage clamp studies on the effects of mutations in *HCN4* should not be limited to expression level and half-activation voltage.

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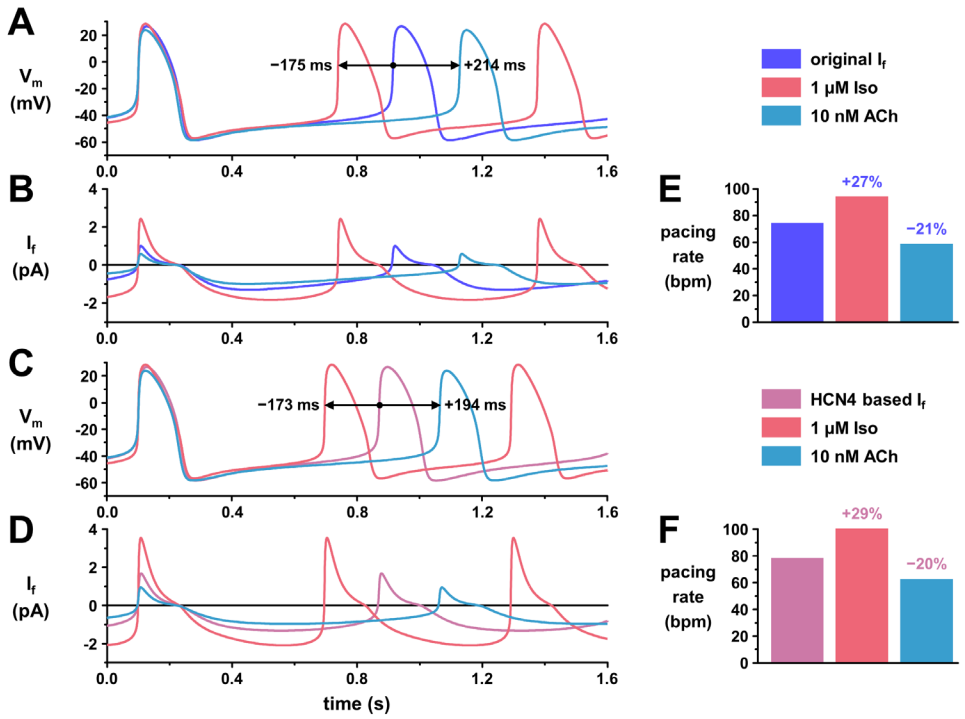

Figure 4. Autonomic control of the pacemaker activity by 1 µM isoprenaline (Iso) and by 10 nM acetylcholine (ACh) in the Fabbri–Severi model of a single human sinus node pacemaker cell [10]. (A) Membrane potential (V_m) and (B) I_f in the Fabbri–Severi model with its original I_f. (C) V_m and (D) I_f in the Fabbri–Severi model with the HCN4 based I_f. (E, F) Associated pacing rates.

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