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Single Cells Isolated from Human Sinoatrial Node: Action Potentials and Numerical Reconstruction of Pacemaker Current

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***Abstract*—Pacemaker activity of the sinoatrial node has extensively been studied in laboratory animals of various species, but is virtually unexplored in man. Most experimental data have been obtained from rabbit, where the hyperpolarization-activated 'funny' current (I_f), also known as the 'pacemaker current', plays an important role in diastolic depolarization and thus in setting pacing rate. Recently, we isolated pacemaker cells from excised human sinoatrial node tissue, and recorded action potentials and I_f using the whole-cell patch-clamp technique in current clamp and voltage clamp mode, respectively. Single sinoatrial node pacemaker cells showed a spontaneous beating rate of 73 ± 3 beats/min (mean ± SEM, *n* = 3) with a remarkably slow diastolic depolarization. I_f was identified in voltage clamp experiments as the 2 mmol/L Cs^+-sensitive inward current activating upon 2-s hyperpolarizing voltage clamp steps. The I_f reversal potential and (de)activation kinetics were similar to those in rabbit. However, the fully-activated I_f conductance was 3–4 times smaller than typically found in rabbit. Furthermore, the half-maximal activation voltage was ≈20 mV more negative than in rabbit. These differences would both act to reduce the functional role of I_f in human pacemaker cells. To assess this functional role, we carried out a numerical reconstruction of the I_f time course during an experimentally recorded human sinoatrial node action potential, based on the obtained data on I_f amplitude and kinetics. This reconstruction revealed that I_f provides a small but significant inward current in the voltage range of diastolic depolarization. We conclude that human sinoatrial node pacemaker cells functionally express I_f and that this I_f contributes to pacemaking in human sinoatrial node.**

I. Introduction

THE repetitive electrical impulse for excitation and contraction of the mammalian heart originates from a relatively small group of pacemaker cells located in the sinoatrial (SA) node region. In animal studies, mostly conducted on rabbit heart, the electrophysiological properties of SA nodal cells have been determined in detail and various ion currents underlying pacemaker activity have been identified. It is now clear that pacemaking in SA nodal cells of various species is due to diastolic depolarization driven by a net inward current, which results from a complex interaction of multiple inward and outward ion currents, including the hyperpolarization-activated 'funny' current (I_f), also known as the 'pacemaker current', which provides a substantial inward current during diastolic depolarization (see [1]–[4] for reviews).

Although the relative contribution of individual ion currents to diastolic depolarization remains a matter of debate, animal studies consistently show that I_f plays a key role. However, there is no direct evidence that I_f also plays a functional role in human SA nodal pacemaking. The electrophysiology of human adult SA nodal cells is virtually unexplored, because explanted hearts obtained during cardiac transplantation, which form the major source for isolating human myocytes, do not contain the SA nodal region as it is usually left in the chest of the recipient. In the present study, we assessed the role of I_f in pacemaker activity of isolated human SA nodal cells that were obtained from a patient undergoing sinus node excision as a last resort against recurrent supraventricular tachyarrhythmias [5].

II. Methods

A. Cellular Electrophysiology

Informed consent was obtained before sinus node excision, and all protocols complied with institutional guidelines. Single cells were enzymatically isolated from the excised tissue as described previously for rabbit [6].

Membrane potentials and currents were recorded at 36 ± 0.2°C in the whole-cell configuration of the patch-clamp technique. The pipette solution contained (in mmol/L): 125.0 K-gluconate, 20.0 KCl, 5.0 NaCl, 1.0 $MgCl_2$, 5.0 MgATP, 10.0 HEPES (pH 7.2 with KOH); the Tyrode's solution contained 140 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1.0 $MgCl_2$, 5.5 glucose, 5.0 HEPES (pH 7.4 with NaOH). Data were corrected for liquid junction potential, and cell capacitance and series resistance were compensated for >85%. I_f was examined as the 2 mmol/L Cs^+-sensitive, time-dependent current that activated upon 2-s hyperpolarizing voltage clamp steps from a holding potential of –40 mV [7], [8].

The I_f activation curve was obtained by plotting normalized I_f tail current amplitude against the test potential, and characterized by fitting it to the Boltzmann equation $I/I_{max} = A/\{1+\exp[(V_{1/2}–V_m)/k]\}$ to determine the membrane

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potential for half-maximal activation ($V_{1/2}$) and the slope factor (k). The time course of I_f (de)activation was fitted by the monoexponential equation $I/I_{max} = A\times[1–\exp(–t/\tau)]$, ignoring the variable initial delay in I_f (de)activation [9], [10].

B. Numerical Reconstruction of I_f

The time course of I_f during an experimentally recorded human SA nodal action potential was reconstructed using a first-order Hodgkin-Huxley type kinetic scheme, with parameters based on the measured I_f (de)activation kinetics. We calculated I_f according to $I_f = y \times g_f \times (V_m–E_f)$, where g_f, V_m, and E_f are the fully-activated I_f conductance, membrane potential, and I_f reversal potential, respectively, and y is the I_f gating variable, which ranges between 0 (no activation) and 1 (full activation). In our reconstruction we used g_f and E_f values of 75 pS/pF and –22 mV, respectively, based on the experimentally observed values. V_m values were obtained from the recorded action potentials. According to the Hodgkin-Huxley formalism, the time dependence of I_f is governed by $dy/dt = (y_\infty–y)/\tau_y$, where t is time and y_∞ and τ_y are the (voltage dependent) steady-state value and time constant of y, respectively.

The differential equation for y was solved using an Euler type integration scheme with a time step of 10 μs. The y_∞ values were defined by the above Boltzmann fit to the experimental data on steady-state activation. As in previous studies on I_f kinetics [9]–[13], the voltage dependence of τ_y was described by a bell-shaped function (see also Fig. 4 below). We used the Dokos *et al.* [11] type equations to fit our (de)activation data. Specifically, we used $\tau_y = (\alpha+\beta)^{-1} – 0.054$, where τ_y is in seconds and α and β are defined by

$$\alpha = 0.36 \times (V_m+148.8) / \{\exp[0.066\times(V_m+148.8)]–1\},$$

and

$$\beta = 0.1 \times (V_m+87.3) / \{1–\exp[–0.21\times(V_m+87.3)]\}.$$

III. Results

A. Action Potential of Human SA Nodal Pacemaker Cells

Figure 1 shows typical current clamp recordings from a single human SA nodal pacemaker cell. Both in this cell and the two other cells studied, action potential shape and duration were similar to those of rabbit SA nodal pacemaker cells. Maximal diastolic potential (–62 ± 4 mV, mean ± SEM, *n* = 3) and maximal upstroke velocity (4.6 ± 1.2 V/s) were also similar to those of rabbit. However, diastolic depolarization was considerably slower (49 ± 18 mV/s over the first 100 ms), resulting in an intrinsic beating rate of 73 ± 3 beats/min. Thus, the low beating rate of isolated human SA nodal cells compared to rabbit SA nodal cells is due to slow diastolic depolarization rather than long action potential duration.

B. Characteristics of I_f in Human SA Nodal Pacemaker Cells

Subsequent voltage clamp experiments revealed the presence of I_f, which was identified as the Cs^+-sensitive inward current that activated upon 2-s hyperpolarizing steps from –40 mV [7], [8]. I_f became larger and activated more rapidly at increasingly negative potentials (Fig. 2). Figure 3 shows the mean I_f step and tail current (Fig. 2, arrows) of three human SA nodal pacemaker cells. The fully-activated I_f conductance, as determined from the I_f step current, was 75.2 ± 3.8 pS/pF. The I_f reversal potential was –22.1 ± 2.4 mV, as determined from comparison of the fully-activated current (I_{step}) at –130 mV and the associated tail current (I_{tail}) at –40 mV. Thus, the reversal potential of human I_f is similar to values previously reported for rabbit, whereas its conductance is 3–4 times smaller [10], [14].

Voltage dependence of activation was characterized by plotting normalized I_{tail} amplitude against the preceding hyperpolarizing test potential. Figure 4A shows the thus

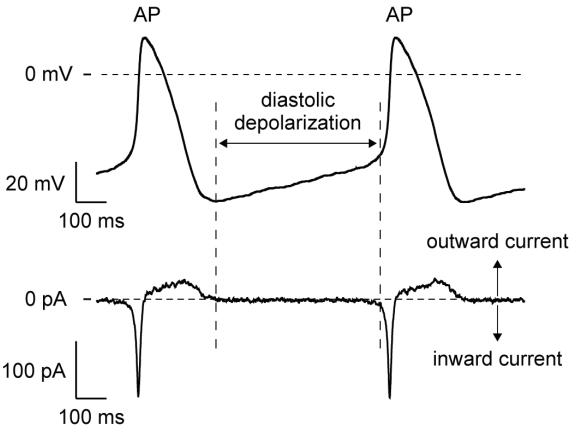

Fig. 1. Typical action potentials of an isolated human SA nodal pacemaker cell (top) and associated net membrane current (I_{net}), calculated from $I_{net} = – C_m \times dV_m/dt$, where C_m and V_m denote membrane capacitance and membrane potential, respectively (bottom). Note the small inward current underlying the slow diastolic depolarization.

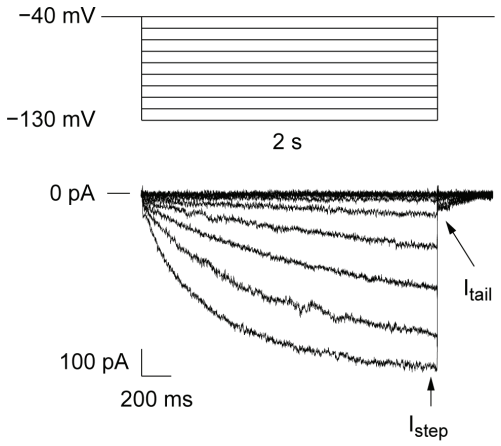

Fig. 2. Time and voltage dependence of the hyperpolarization-activated inward current (I_f) in a single human SA nodal pacemaker cell. Voltage clamp protocol (top) and associated I_f current traces (bottom). Arrows indicate I_f step and tail current (I_{step} and I_{tail}, respectively). I_f current traces obtained by digital subtraction of current traces recorded in the absence and presence of 2 mmol/L external Cs^+.

obtained steady-state activation curve, together with data from previous experimental or model studies on rabbit [9]–[13]. Activation threshold was between –50 and –60 mV, and the half-maximal activation voltage and slope factor of the Boltzmann fit to the data were –96.9 ± 2.7 and –8.8 ± 0.5 mV, respectively. This half-maximal activation voltage is ≈20 mV more negative than the values of –76.1 mV [10] and –69.6 ± 2.0 mV [8] previously reported for rabbit.

Figure 4B shows the activation and deactivation time constants as obtained from monoexponential fits of the I_f step and tail currents, respectively. Reliable fits of the step current could only be obtained at –100 mV and more negative test potentials. The time course of I_f (de)activation was comparable to that of rabbit (Fig. 4B).

C. Role of I_f in Pacemaker Activity of Human SA Nodal Cells

In human, the fully-activated I_f conductance was 3–4 times smaller than typically found in rabbit. Furthermore, the half-maximal activation voltage was ≈20 mV more negative than in rabbit (Fig. 4A). These differences would both act to reduce the functional role of I_f in human pacemaker cells. To assess this functional role, we carried out a numerical reconstruction of the I_f time course during a recorded human SA nodal action potential, based on the obtained data on I_f amplitude and kinetics. In this reconstruction, we used a first-order Hodgkin-Huxley type kinetic scheme, which provided satisfactory fits to our experimental data (cf. Fig. 4). Figure 5 shows a human SA nodal action potential and the net membrane current, I_{net}, underlying diastolic depolarization. The numerically reconstructed I_f is shown as a thick dashed line. Note that I_{net} during diastolic depolarization (Fig. 5, bottom) is much smaller than I_{net} during the action potential upstroke (Fig. 1).

Figure 5 demonstrates that I_f provides a significant inward current in the voltage range of diastolic depolarization, suggesting a prominent role of I_f in human cardiac pacemaker activity. In line with this reconstruction, we observed that blocking I_f by application of Cs^+ (2 mmol/L) reduced beating rate by slowing diastolic depolarization [5].

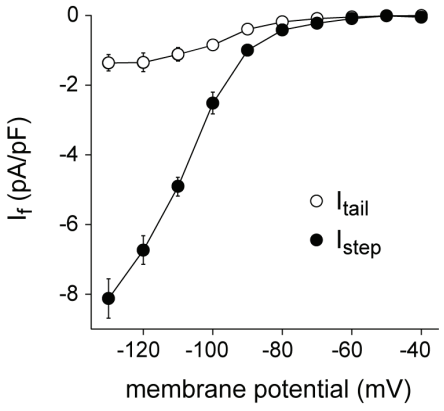

Fig. 3. Average current-voltage relationship of I_f step and tail current (I_{step} and I_{tail}, respectively), as determined from voltage clamp experiments on three isolated human SA nodal pacemaker cells.

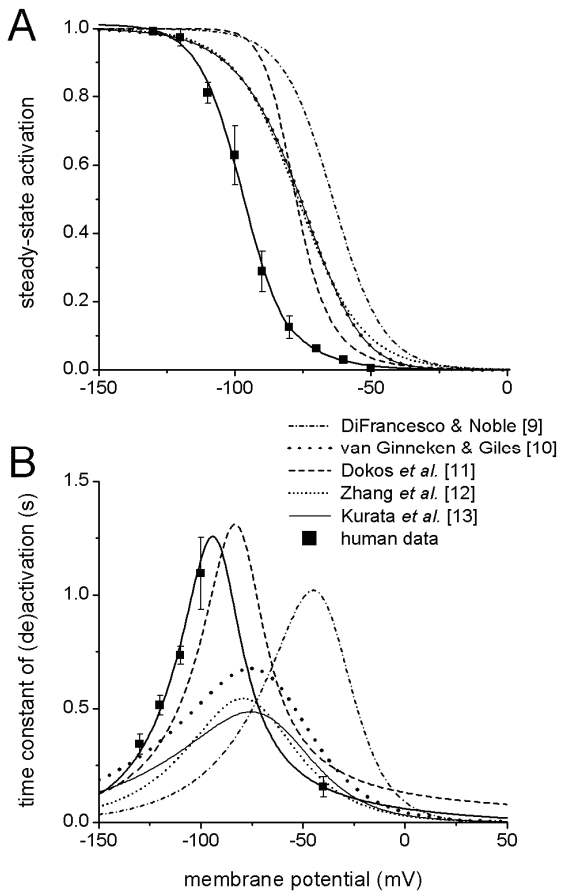

Fig. 4. Kinetics of I_f in various experimental or model studies on rabbit [9]–[13]. Data from isolated human SA nodal myocytes are shown as filled squares and the associated fits as solid lines. (A) Steady-state activation curve. (B) Time constant of (de)activation.

IV. Discussion

In the present study, we have shown that single pacemaker cells isolated from the human SA node show spontaneous beating at 70–75 beats/min with a remarkably slow diastolic depolarization. Like SA nodal pacemaker

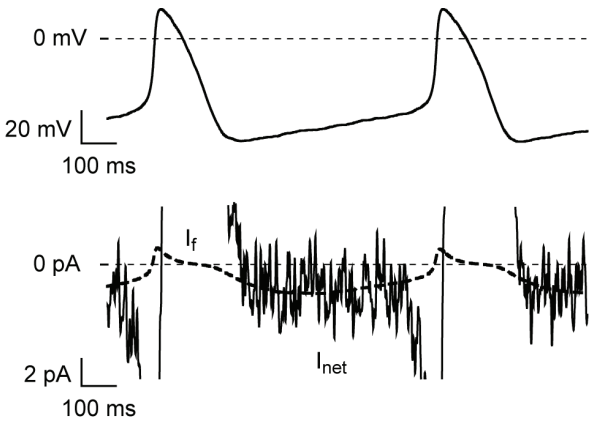

Fig. 5. Numerical reconstruction of I_f during human SA nodal pacemaker activity. Experimentally recorded action potentials of a single human SA nodal pacemaker cell (top), associated net membrane current (I_{net}, bottom), and computed I_f (thick dashed line, bottom). Same cell as Fig. 1 (note difference in current scale).

cells from animal species, these cells have a hyperpolarization-activated 'pacemaker current' I_f, but its fully-activated conductance, as identified in voltage clamp experiments, is 3–4 times smaller than typically found in rabbit and its half-maximal activation voltage is ≈20 mV more negative. Despite these differences, which would both act to reduce the functional role of I_f in human pacemaker cells, numerical reconstruction of the I_f time course during an experimentally recorded human SA nodal action potential revealed that I_f provides a small but significant inward current in the voltage range of diastolic depolarization. This finding was substantiated by our experimental observation that blockade of I_f reduces beating rate by slowing diastolic depolarization.

V. Limitations

Using the whole-cell patch-clamp technique and hyperpolarizing 2-s voltage clamp steps, we found an I_f half-activation voltage of –96.9 ± 2.7 mV. We cannot exclude that this value was shifted to hyperpolarized potentials due to cell dialysis or the absence of cyclic AMP from our pipette solution. Also, the activation curve may have been shifted to negative values because I_f was not fully activated during all of the 2-s voltage clamp steps (Fig. 2). Thus, we may have actually underestimated I_f in our numerical reconstruction.

A second and major limitation is that we studied a limited number of SA nodal cells that were isolated from just one patient with inappropriate supraventricular tachy-arrhythmias. We cannot exclude that these tachycardias were associated with I_f remodeling in the SA nodal pacemaker cells.

VI. Conclusion

The present study shows that adult human SA nodal pacemaker cells functionally express a significant I_f. Both numerical reconstruction of the I_f time course during a human SA nodal action potential and effects of Cs^+ on pacemaker activity demonstrate that this I_f contributes to pacemaking in human sinus node.

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