If in left human atrium: a potential contributor to atrial ectopy

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Abstract

Objective: The left human atrium plays an important role in initiation of atrial fibrillation (AF) and the hyperpolarization activated cation current (If) is a candidate for contributing to abnormal automaticity. However, electrophysiological data concerning If are not available in this cardiac region and we therefore investigated If in human left atrial tissue.

Methods: Human atrial myocytes were isolated from the left atrial appendage (LAA) and the left atrial wall (LAW) obtained from patients undergoing open heart surgery. If was measured with the whole-cell patch-clamp technique.

Results: If densities between −70 and −110 mV were found to be significantly higher in LAA than in LAW cells. Furthermore, in the group of LAA cells the half maximal activation potential (V1/2) was found to be less negative (V1/2 of −84.3 ± 1.9 mV, n=14/9) compared to LAW cells (V1/2 of −97.8 ± 2.1 mV, n=28/9). Beta-adrenergic receptor stimulation with isoproterenol (1 μM) caused an acceleration of current activation and a V1/2 shift to more positive potentials in cells of both regions (LAA: 8.8 ± 2.3 mV, n=6/4 and LAW: 8.9 ± 2.6 mV, n=6/4). Simulations using a mathematical model of the human atrial myocyte demonstrated that If was able to induce spontaneous activity in the model at a regular rhythm due to the interplay of If, Na+/Ca2+ exchange current and Ca2+ release of the sarcoplasmic reticulum (SR).

Conclusions: Our study revealed the presence of If in left atrial myocytes and showed that If parameters depend on atrial region. If current densities were sufficient to convert the mathematical model of a quiescent human atrial cell into a “pacemaker cell”. These data support the hypothesis of If as a contributor to abnormal automaticity in human atrial tissue.

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1. Introduction

The cardiac hyperpolarization activated cation current (If) is known to be present not only in regions with primary or secondary pacemaker activity, but also in non-pacemaking regions of atrium and ventricle. In pacemaking regions, If is believed to contribute to spontaneous diastolic depolarization [1,2]. The physiological relevance of If in the working myocardium is not clear, because the activation range is negative to the resting membrane potential and the current density is much smaller compared to sinus node [1,2]. However, it was speculated that If could elicit abnormal automaticity and thus play a role in atrial and ventricular arrhythmias [3–6]. This could be favoured by catecholamine release which is known to shift If activation to less hyperpolarized potentials and/or by a pathological upregulation of If with a concomitant downregulation of the inward rectifier current (IK1), which plays a major role in stabilizing the resting membrane potential [7]. Since atrial IK1 is of a several times smaller amplitude than ventricular IK1 [8,9], the arrhythmogenic potential of a depolarizing current, such as If, should be more pronounced in the atrium [10]. So far,
electrophysiological investigations on human atrial $I_f$ were restricted to the right atrium (right atrial appendage, RAA) [4,11], but the left atrium (LA) is known to play an important role for initiation and maintenance of atrial fibrillation (AF), the most common arrhythmia [12,13]. Furthermore, expression of cardiac ion channels depends on the cardiac region and can differ between right and left atrium as shown for $I_{Kr}$ in dogs [14,15]. Therefore, it was the aim of our study to investigate $I_f$ in two different regions of human LA using tissue from the left atrial appendage (LAA) and the left atrial wall (LAW).

2. Methods

2.1. Patients

Small pieces (0.1–0.3 g; in case of LAA excision during ablation: ≥0.8 g) of left human atrium (LAA/LAW) were obtained from 18 (9/9) patients undergoing cardiac surgery due to mitral valve disease. They were transported to the laboratory (10–15 min) in saline [16] and immediately used for cell isolation. Most of the patients (16) showed a normal ejection fraction (EF>55%), pulmonary artery pressure (PAP<35 mm Hg) and left atrial diameter (LAD<4 cm). The two remaining patients (chronic atrial fibrillation; LAW tissue) had end stage mitral valve disease (EF≤30, PAP≥46, LAD≥4.3). Patient’s mean age was 67.1±2.7 years (68.3±4.1/65.8±3.9); 10 patients were female (5/5), 8 patients were male (4/4). Nine patients had chronic atrial fibrillation (4/5), two patients had paroxysmal atrial fibrillation (4/5), two patients had paroxysmal atrial fibrillation (0/2) and all other patients were in sinus rhythm. The patients were treated with digoxin (4/3), beta-1-blockers (3/1), Ca²⁺ antagonists (1/0), a class III antiarrhythmic drug (0/1), ACE inhibitors (3/4) and diuretics (4/1). The investigation conforms to the principles outlined in the Declaration of Helsinki [17]. All patients gave informed consent and the use of human tissue was approved by the ethics committee of the Karl-Franzens-University.

2.2. Cell isolation and electrophysiological measurements

Atrial cells were isolated by enzymatic dissociation and mechanical disaggregation using trypsin (Sigma, MO, USA) and collagenase (Type 2, Worthington, NJ, USA) as described in detail previously [11]. The isolated myocytes were stored in cell culture medium (M-199, Sigma), supplemented with 50 μg/ml penicillin and 50 IU/ml streptomycin and were kept in an incubator at 37 °C. Experiments were performed 4–8 h after cell isolation. Electrophysiological measurements were carried out at 36–37 °C. The external solution contained (in mM): NaCl 137, KCl 25, CaCl₂ 1.8, MgCl₂ 1.2, BaCl₂ 1, MnCl₂ 2, CdCl₂ 0.2, 4-aminopyridine 3, glucose 5, HEPES 5, adjusted to pH 7.35 with NaOH. The pipette solution contained (in mM): K⁺-aspartate 100, KCl 30, Na⁺-ATP 5, CaCl₂ 4, EGTA 11, HEPES 10, adjusted to pH 7.2 with KOH. Mn²⁺, Ba²⁺, Cd²⁺ and 4-aminopyridine were added to reduce the interference with potassium and calcium currents. Currents were measured in the whole-cell mode of the ruptured patch-clamp technique using a List L/M EPC-7 amplifier. Electrodes had resistances from 2 to 4 MΩ when filled with internal solution.

The amplitude of $I_f$ was measured as the difference between instantaneous current at the beginning of the hyperpolarizing step and steady-state current at the end of the voltage clamp step and was normalized to cell membrane capacitance. Ionic conductance of $I_f$ was determined for each cell according to the equation $g=I_f(V_m-V_{rev})$, where $g$ is the conductance calculated at the membrane potential $V_m$. $I_f$ is the current amplitude, and $V_{rev}$ the reversal potential (−13 mV as described for $I_f$ in human right atrial myocytes for an external potassium concentration of 25 mM [11]). For calculation of steady-state activation curves, conductances were normalized to the maximal conductance. A Boltzmann equation was fitted to these normalized values.

The voltage-dependency of $I_f$ activation/deactivation time constants were fitted according to the equation $\tau_y=1/(\alpha_0 \exp(-V/V_0) + \beta_0 \exp(V/V_0))$, whereby the parameters $\alpha_0$, $\beta_0$ and $V_0$ had to be determined ($\alpha_0$, $\beta_0$ are opening and closing rates at zero voltage $V_0$). Activation and deactivation time constants were calculated by fitting $I_f$ recordings to a single exponential equation. Results are presented as means±standard error of the mean (S.E.M.). Statistical significance was tested by Student’s t-test and a value of $P<0.05$ was considered significant.

2.3. Human atrial single cell model

A modeling approach was used to estimate the role of $I_f$ for eliciting spontaneous activity in the human atrium. Therefore, we used a modified version of a mathematical cell model based on human right atrial myocyte data developed by Nygren et al. [18]. We replaced the Na⁺ influx by sodium entry due to the stimulus current for the calculation of intracellular ionic concentrations [19]. Since Na⁺ current assignment produced the same results as the original model (after 20 s and 10 min of pacing) Na⁺ ions were used as stimulus carrier in all simulations. $I_f$ was included in the model according to the equation

$$ I_f = g_f(y)[f_{Na}(V - E_{Na}) + f_K(V - E_K)] $$

where $f_{Na}=0.2677$ ($f_K=1-f_{Na}$) and $g_f$ is the maximal conductance. The gating variable ($y$) was described by a differential equation of the form

$$ dy/dt = (y_{\infty} - y)/\tau_y $$

where $\tau_y$ is the time constant and $y_{\infty}$ is the steady-state value of $y$. 

Parameter values of the LAA cell that showed the most positive half-maximal activation voltage ($V_{1/2}=I_{f(LAA)}$) are:

\[
y_{\infty} = \frac{1}{1 + \exp[(V_m + 74)/6.9]}
\]

\[
\tau_y = \frac{1}{2.1056 \times 10^{-4} \exp(-V_m/9.1787) + 115.4489 \exp(V_m/9.1787)}
\]

$g_f = 4.75 nS$

and mean parameter values of LAA cells:

\[
y_{\infty} = \frac{1}{1 + \exp[(V_m + 84.94)/10.1]}
\]

\[
\tau_y = \frac{1}{1.2783 \times 10^{-4} \exp(-V_m/9.2424) + 121.6092 \exp(V_m/9.2424)}
\]

$g_f = 2.5 nS$.

The atrial cell model was paced for 10 min (1 Hz) to achieve steady state for each scaling of $g_f$ and was then simulated for 10 min without stimulation in order to test for the occurrence of spontaneous action potentials (APs) in steady state. The differential equations system of the model was implemented on a personal computer in Simulink 5.0 and a variable step solver (ode 15 s) was used for numerical integration.

3. Results

The atrial myocytes used for this study were rod-shaped and showed clear cross striation. Cell membrane capacitance did not differ between the cells isolated from the two left atrial regions (101.9±9.1 for LAA, n=29/9 vs. 116.2±11.5 pF for LAW, n=29/9, P=0.33).

3.1. $I_f$ occurrence

The presence of a hyperpolarization activated inward current ($I_f$) was determined in each cell by application of hyperpolarizing voltage steps from $-40$ to $-140$ mV in 10 mV steps of 3-s duration (holding potential=$-40$ mV). Fig. 1 shows current traces obtained in representative cells from LAA (A) and LAW (B). In both atrial regions, the voltage clamp protocol elicited a time-dependent inward current that increased in amplitude and activated more rapidly with progressively more negative test potentials. However, the current in the LAA cell activated at a less negative membrane potential.
potential (−70 mV) compared to the LAW cell (−80 mV) and showed a greater density at −140 mV (−3.9 pA/pF in LAA vs. −3.1 pA/pF in LAW). Addition of CsCl (2 mM) reversibly eliminated the time-dependent part of the current in both, the LAA (C) and LAW (D) myocyte. These electrophysiological characteristics and the modulation by Cs⁺ revealed If to underlie the current in both cells. If could be found in 18 out of 22 LAA myocytes (81%) and in 27 out of 40 LAW myocytes (68%), whereby the pacemaker current was considered to be present if its density was larger than 0.5 pA/pF at −120 mV.

3.2. Density, kinetics and steady-state activation of If

Fig. 2A illustrates the mean If current–voltage relationships for LAA (closed circles, n=14/9) and LAW cells (open circles, n=28/9). If current densities were significantly higher in LAA than in LAW cells at potentials between −70 and −110 mV (e.g. at −70 mV: −0.47±0.14 pA/pF vs. −0.13±0.04 pA/pF and at −80 mV: −1.22±0.35 pA/pF vs. −0.36±0.01 pA/pF) and the activation threshold in LAA cells was about 10 mV more positive (between −60 and −70 mV) compared to LAW cells (between −70 and −80 mV). At test potentials more negative than −110 mV, If current densities were not significantly different between both groups (e.g. at −120 mV: −3.2±0.7 pA/pF, LAA vs. −1.9±0.2 pA/pF, LAW; P=0.083) which was mainly due to the large current variability in LAA cells. The inserts in Fig. 2A show current density values of individual myocytes at −80 and −120 mV for both groups. We did not observe a significant influence of the presence or the absence of AF on If densities by either comparing data from the same region (LAA or LAW) or pooling LAA and LAW data into an AF and sinus rhythm (SR) group.

To estimate τy of the If model, we performed monoexponential fits of current activation and tail current deactivation. Tail currents were elicited in LAA cells (n=6/3) by 10 mV steps from −50 to +20 mV, preceded by a hyperpolarizing step to −120 mV. Current activation was well fitted by a single exponential decay equation at potentials positive to −100 mV. At more negative test potentials in most cases a biexponential fit was more accurate in describing the If time course. However, we decided to describe current activation at all membrane potentials by a monoexponential fit since for the subsequent purpose of modeling only membrane potentials positive to −100 mV were of interest. Fig. 2B shows mean time constants of current activation in LAA (closed circles, n=11/7) and in LAW cells (open circles, n=17/7). If activated significantly faster in cells from LAA than in LAW cells at all membrane potentials (between −80 and −140 mV; e.g. at −80 mV: 1135±87 ms vs. 1661±139 ms and at −140 mV: 60±7 ms vs. 96±13 ms). The insert in Fig. 2B illustrates the mean activation/deactivation values of If and the corresponding τy curve (solid line, a0=1.2783×10⁻⁴, b0=121.609 and V0=9.2404) for LAA cells, which were used to gain the τy equation as described in Methods.

Fig. 3 illustrates the voltage dependence of If activation. A Boltzmann function fitted to the normalized conductances of LAA cells yielded a \( V_{1/2} \) of −84.3±1.9 mV and a slope factor of 10.1±0.6 mV (n=14/9, closed

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**Fig. 2.** (A) Mean current density to voltage relation in LAA (n=14/9, closed circles) and in LAW (n=28/9, open circles) myocytes. The inserts show current density values at −80 and −120 mV for each individual myocyte of both regions. (B) Mean time constants of If activation in LAA (n=11/7) and LAW (n=17/7) myocytes. The insert illustrates time constants (τ) of activation and deactivation of LAA cells (n=6/3), which were used to gain τy (solid line) for If modeling.

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**Fig. 3.** Voltage dependence of activation in LAA (n=14/9) and LAW cells (n=28/9) calculated by fitting a Boltzmann function to normalized conductances. The insert illustrates individual \( V_{1/2} \) values of both regions.
circles). The activation curve of LAW cells (open circles, \( n = 28/9 \)) showed a significant more negative \( V_{1/2} \) value \((-97.8 \pm 2.1 \text{ mV})\) and a slope factor of \(9.6 \pm 0.4 \text{ mV} \). The insert in Fig. 3 shows \( V_{1/2} \) values of individual LAA and LAW myocytes.

### 3.3. Beta-adrenergic modulation of \( I_f \)

Since \( I_f \) is a presumptive target for autonomic nervous system regulation (beta-adrenergic stimulation increases \( I_f \) activity), we also investigated the effect of beta-adrenergic stimulation by isoproterenol (1 \( \mu \text{M} \)). In both groups of cells, we found an acceleration of \( I_f \) activation (LAA: Fig. 4A, LAW: Fig. 4B) by beta-adrenergic stimulation and a significant shift of \( V_{1/2} \) to more depolarized potentials. This shift amounted for \(8.8 \pm 2.3 \text{ mV} \) in LAA (\( V_{1/2} \) was \(-87.0 \pm 2.3 \text{ under control conditions, closed circles and -78.3 \pm 2.2 \text{ with isoproterenol, closed squares, } n=6/4, \text{ Fig. 4C}\)) and \(8.9 \pm 2.6 \text{ mV} \) in LAW (\( V_{1/2} \) was \(-99.2 \pm 2.5 \text{ under control conditions, open circles and -90.3 \pm 2.4 \text{ with isoproterenol, open squares, } n=6/4, \text{ Fig. 4D}\)).

### 3.4. Simulation of \( I_f \)

Fig. 5A shows original \( I_f \) recordings from the LAA cell with largest current densities at test potentials between \(-50 \text{ and } -80 \text{ mV} \) and the simulated current (dashed line= \( I_f(\text{LAA}_{\text{single}}) \)). Fig. 5B illustrates time constants from this LAA cell (closed circles) for modeling \( \tau_y \) curve (solid line). The external and internal Na\(^+\) and K\(^+\) concentrations as well as the clamp protocol were identical with the experimental conditions.

### 3.5. Pacemaking mechanism in the human atrial cell model

\( I_f(\text{LAA}_{\text{single}}) \) and \( I_f(\text{LAA}_{\text{mean}}) \) were implemented in the atrial cell model and simulations were performed using different scaling factors for \( I_f \) conductance in order to test for the occurrence of spontaneous action potentials (APs).

Fig. 6 shows selected ionic processes during the first two spontaneous APs (AP1 and AP2) and two spontaneous APs in steady state (AP3 and AP4). The right panel of Fig. 6 (framed box) shows an overlay comparison between a paced steady state AP and corresponding ionic processes of the original Nygren model (1 Hz, dotted line) and AP3 (solid line). First spontaneous APs in steady state (AP3 and AP4) occurred at 35.4% of \( I_f(\text{LAA}_{\text{single}}) \) conductance (scaling factor 0.354; Fig. 6A). Spontaneous activity was initiated by \( I_f \) inducing additional depolarizing inward current (Figs. 6B and 7A) leading to depolarization of the membrane potential (\( V_m \)). The depolarized \( V_m \) reduced (via decreased driving force) inward current through the Na\(^+\)/Ca\(^{2+}\)
exchanger (\(I_{\text{NCX}}\), by \(-1.4\) pA at a time of 0 s in right panel of Fig. 6C), which diminished NCX Ca\(^{2+}\) efflux leading to a rise of internal Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\); of \(-110\) nM at a time of 0 s in right panel of Fig. 6D). This small rise in [Ca\(^{2+}\)]\(_i\) was sensed by the release mechanism of the sarcoplasmic reticulum (SR) which responded with a small Ca\(^{2+}\) release further elevating [Ca\(^{2+}\)]. The positive feedback process culminated in an SR Ca\(^{2+}\) release (\(I_{\text{re}}\)) of 3.69 nA elevating [Ca\(^{2+}\)] to a maximal value of 0.68 \(\mu\)M. This release was not induced by Ca\(^{2+}\) influx via L-type Ca\(^{2+}\) current (\(I_{\text{Ca,L}}\)). The rise in [Ca\(^{2+}\)] increased inward \(I_{\text{NCX}}\) which reached a maximum of \(-71.61\) pA 1.6 ms after \(I_{\text{re}}\) peak. This in turn depolarized \(V_m\) to the threshold for activation of sodium current (\(I_{\text{Na}}\)), which peaked to a value of \(-1.59\) nA (Fig. 6E) and brought \(V_m\) to an overshoot potential of 13.03 mV. Depolarization of \(V_m\) also resulted in activation of \(I_{\text{Ca,L}}\) which induced \(I_{\text{re}}\) in the unmodified cell. However, in the \(I_f\) pacemaker cell this \(I_{\text{Ca,L}}\) induced \(I_{\text{re}}\) coincided with the trailing edge of the previous SR Ca\(^{2+}\) release and therefore elicited a second, very small second \(I_{\text{re}}\) peak (arrow in insert of Fig. 7A). The potassium currents (Fig. 6G, H) repolarized \(V_m\) to maximal diastolic potential of \(-72.57\) mV but at this value they could not counterbalance the total inward current due to \(I_\alpha\) inward \(I_{\text{NCX}}\) and inward background currents. This imbalance caused slow phase-4 depolarization that lead to generation of a subsequent AP (via the mechanism described above) and thus continuous pacemaking.

To confirm that the reduction of Ca\(^{2+}\) efflux by NCX is the main trigger for the SR Ca\(^{2+}\) release, replacement of the NCX Ca\(^{2+}\) efflux in addition to \(I_f\) implementation (see below) should prevent the model cell from pacemaking. Therefore, a stimulated AP without \(I_f\) (Fig. 7A, solid line, NCX Ca\(^{2+}\) efflux=Efflux\(_{\text{spon}}\)), a spontaneous AP after implementation of \(I_{\text{fLAA single}}\) (arrow in Fig. 7A, dashed line, NCX Ca\(^{2+}\) efflux=Efflux\(_{\text{spon}}\)) and finally the effect of replacing Efflux\(_{\text{spon}}\) by Efflux\(_{\text{stim}}\) (dotted line in Fig. 7A) were simulated. Replacement of Efflux\(_{\text{spon}}\) by Efflux\(_{\text{stim}}\) synchronous to \(I_f\) implementation resulted in a stable membrane potential without generation of a spontaneous AP. The inserts in Fig. 7A illustrate \(I_{\text{re}}\) from the stimulated AP (solid line) and the spontaneous (dashed line) and suppressed AP (dotted line). The arrow labels the \(I_{\text{re}}\) peak due to \(I_{\text{Ca,L}}\).

Furthermore, a spontaneous AP could be suppressed by reduction of \(I_{\text{NCX}}\) immediately before the peak of \(I_{\text{re}}\), indicating that \(I_{\text{NCX}}\) is necessary to bring the membrane potential to activation threshold of \(I_{\text{Na}}\) (data not shown).

3.6. Influence of \(I_f\) parameters on pacemaking in the model

The effects of different \(I_{\text{fLAA single}}\) and \(I_{\text{fLAA mean}}\) conductances and shifting \(V_{1/2}\) to less negative potentials on eliciting spontaneous activity in the single cell model are shown in Fig. 7B. First spontaneous activity occurred at 35.4% of \(g_f\) of \(I_{\text{fLAA single}}\) (closed circles) at a frequency of 15 beats per minute (bpm). A stepwise increase of the scaling factor up to 1.0 resulted in higher beat frequencies (at a scaling factor of 1.0, which corresponds to the experimentally observed maximal \(I_{\text{fLAA single}}\) conductance at [K\(^{+}\)]\(_o\)=25 mM, spontaneous activity was observed at a frequency of 47 bpm). Implementation of \(I_{\text{fLAA mean}}\) did not result in spontaneous activity within the parameter values yielded from the experimental data (closed diamonds in Fig. 7B). The \(I_{\text{fLAA mean}}\) conductance had to be scaled by a factor of 1.7 to elicit spontaneous activity (at 15 bpm). Further enlargement of \(I_{\text{fLAA mean}}\) again initiated beating at higher frequencies. Finally, shifting \(V_{1/2}\) of \(I_{\text{fLAA mean}}\) to more positive membrane potentials (by 8 mV as observed under beta-adrenergic stimulation) decreased threshold for evoking spontaneous activity to a scaling factor of 0.9 (14 bpm; \(I_{\text{fLAA mean iso}}\), open diamonds in Fig. 7B).

4. Discussion

The present study provides first evidence of the functional presence of the hyperpolarization activated cation current, \(I_\alpha\), in the human LA. \(I_f\) was found in both left atrial
regions (LAA/82% and LAW/68%) and showed typical properties, i.e. activation at hyperpolarized membrane potentials (with faster activation at more negative potentials) and block by Cs⁺ [1]. Our data on \( I_f \) occurrence agree with the values reported for human myocardium where \( I_f \) was also shown to be abundant (82% [11] and 95% [4] in the RAA and 75% in the ventricle [6]).

Myocytes with \( I_f \) showing low threshold activation (i.e. activation between \(-60\) and \(-70\) mV) were mainly found in LAA where we also observed significantly higher \( I_f \) current.

Fig. 6. Selected processes during spontaneous AP initiation (AP1 and AP2) and steady-state oscillations in the modified Nygren human atrial cell model (AP3 and AP4) with \( I_{f,LAA} \) single by 0.354 scaled implemented. A paced AP of the unmodified Nygren model (dotted line) and AP3 (solid line) are shown for comparison (framed right box). (A) Membrane voltage, \( V_m \). (B) Hyperpolarization activated cation current, \( I_f \). (C) \( \text{Na}^+/\text{Ca}^{2+} \) exchange current, \( I_{\text{NCX}} \). (D) Intracellular calcium concentration, \([\text{Ca}^{2+}]_i\). (E) Sodium current, \( I_{\text{Na}} \). (F) L-type calcium current, \( I_{\text{Ca,L}} \). (G) Inward rectifier potassium current, \( I_{\text{K1}} \) and delayed rectifier potassium current, \( I_{\text{K}} \). (H) Sum of transient outward potassium current, \( I_t \), and sustained outward potassium current, \( I_{\text{sus}} \).
spontaneous AP could be suppressed by replacing Effluxspon by Effluxstim simulation time 1 s (arrow) resulted in a spontaneous AP (dashed line). This shows a simulated AP of the Nygren model without the arrow labels the spontaneous and suppressed AP (dashed and dotted lines, respectively, subtypes HCN 1–4 are known, encode LAA (~ more positive densities (between −70 and −110 mV) and significantly more positive $V_{1/2}$ values than in LAW cells. The mean $V_{1/2}$ of LAA (−84 mV) is similar to that reported for $I_f$ in the human RAA (−87 mV [11], −89 mV [4]), whereas the mean $V_{1/2}$ of LAW was found to be more negative (−98 mV). The $I_f$ current densities at hyperpolarized membrane potentials found in LAA myocytes agree to the published data from RAA myocytes (−3.8 pA/pF at −120 mV [11] and at −130 mV [4]).

The nature of the difference in $I_f$ activation threshold and $V_{1/2}$ values between LAA and LAW is unclear at present. Molecular cloning revealed that hyperpolarization-activated cyclic nucleotide gated cation channels, from which four subtypes HCN 1–4 are known, encode $I_f$-like currents [20]. Data from various studies suggest that HCN4 expression is correlated with spontaneously active cells of the cardiac conduction system, whereas HCN2 expression is found in “non-spontaneously” active myocytes [2]. Both channel types (HCN2 and HCN4) have been detected in the right human atrium [21] and the amount of HCN2 transcripts did not differ between human left (LAA and LAW) and right atrium (RAA and RAW) [5]. Whether differences in the HCN2/HCN4 expression pattern are underlying the observed differences in $I_f$ activation curves (between LAA and LAW) needs further investigation.

An $I_f$ with properties as measured in cells of LAA may be of particular interest in contributing to abnormal automaticity in atrium. It is well known that the resting membrane potential stabilizing current $I_{K_1}$ is much smaller in atrium (6–10 fold) than in ventricle and therefore any inward cation current would have a higher arrhythmogenic potential in atrial cells [9,10]. It has also been shown that localized HCN2 overexpression in canine left atrium was able to transform the region near injection in an $I_f$-based pacemaker which was sufficient to drive the heart [22] further supporting the arrhythmogenic potential of atrial $I_f$.

$I_f$ was also affected by beta-adrenergic modulation resulting in a shift of $V_{1/2}$ to more positive potentials (about 9 mV in LAA and LAW), which is similar to right atrial myocytes (6 mV [11] and 7 mV [4]). Although there is no evidence of an elevated sympathetic tone in chronic AF, the sensitivity to beta-adrenergic stimulation may be of interest for the adrenergic type of paroxysmal AF which occurs during states of increased adrenergic activity [23] and in which spontaneous termination is accompanied by a reduction of the elevated sympathetic tone [24].

We used a modeling approach to estimate the arrhythmogenic role of $I_f$ in the human atrium. This was achieved by implementing two formulations of human atrial $I_f$ ($I_{f(LAA)single}$ and $I_{f(LAA)mean}$) into the established human atrial cell model of Nygren et al. [18]. The $I_{f(LAA)single}$ described the LAA cell showing the most positive $V_{1/2}$ value, the $I_{f(LAA)mean}$ was based on the mean $I_f$ data from LAA. Our simulations demonstrated that $I_f$ (both formulations) was able to induce spontaneous activity at a regular rhythm. Spontaneous activity was not generated by $I_f$ solely but due to the interplay of $I_f$ induced depolarization, subsequent alteration of $I_{NCX}$ and SR Ca$^{2+}$ release. The frequency of activity could be modulated by scaling of $g_f$ or shifting $V_{1/2}$ to more positive potentials as seen under beta-adrenergic stimulation. The $I_{f(LAA)single}$ model was able to elicit spontaneous activity at only 35.4% of $g_f$ determined experimentally in a $[K_o]$ of 25 mM. An elevation of $[K_o]$ is known to strongly increase $g_f$ (which is utilized in the experiment to amplify $I_f$) but also to shift the reversal potential (and also the activation threshold) of the current to more positive potentials [1]. For human left ventricular myocytes, a $[K_o]$ of 25 mM was reported to amplify $I_f$ by 64% compared to $[K_o]$ 5.4 mM [3]. Therefore, the reduction of $g_f$ by 65.6% in the simulation compensates well for the enlargement of $g_f$ in the voltage clamp experiments due to the use of 25 mM $[K_o]$. It remains open how the $I_f$-induced transformation of quiescent atrial myocytes into spontaneously beating cells as
shown in our single cell simulations can take place in vivo. Our simulation results suggest the importance of SR Ca\(^{2+}\) release and \(I_{\text{NCX}}\) and confirm experimental work in atrial and sinoatrial pacemaker cells in which diastolic SR Ca\(^{2+}\) release and stimulation of inward NCX was shown to play an essential role in pacemaker activity [25,26]. Furthermore, \(I_{K1}\) downregulation (81\%) in a ventricular myocyte model demonstrated a central role of \(I_{\text{NCX}}\) for pacemaking [27].

It is well known that initiation of AF arises from focal activity of locally restricted regions within the atrium (mainly from the pulmonary veins) and therefore AF initiation has to be seen as a local phenomenon [12,13]. This dependence of the focal activity on atrial location is also emphasized by the large regional variability of \(I_f\) density. Therefore, spatial information seems to be a crucial element even in cells of a given patient (independent of rhythm state) and considering different stages of AF (paroxysmal/persistent/permanent) much larger sample sizes are warranted for a thorough statistical analysis of a correlation between \(I_f\) and AF. Furthermore, we are not entirely able to exclude an influence of clinical parameters and medication on the lack of difference in \(I_f\) properties between SR and AF cells due the limited number of patients (e.g. see Ref. [29] for the influence of digitalis on \(I_{K1}\) in AF patients). However, in the view of focal activity and considering the observed local heterogeneity of \(I_f\) the individual extreme current densities, which are usually lost during averaging and thereby resulting in an underestimation of the arrhythmogenic potential of \(I_f\), are of particular interest.

Our results implicate that cells from LAA may have a higher arrhythmogenic potential due to larger \(I_f\) than LAW cells. In this context, it is noteworthy that drivers of AF may be of particular interest. \(I_f\) as a contributor to atrial arrhythmia control. However, to estimate the full arrhythmogenic potential simultaneous information on \(I_{K1}\) (from the same cell where \(I_f\) was measured) is of great importance and a massive \(I_{K1}\) increase as reported for chronic AF [28,29] would abolish spontaneous pacemaker activity. Interestingly, RAA cells with prominent \(I_f\) show a relatively small \(I_{K1}\) [4] which would further emphasize a high arrhythmogenic activity of cells with large \(I_f\). Ongoing experiments in our laboratory are directed at elucidating this issue for LAA.

In summary, this study is the first electrophysiological characterization of \(I_f\) in left human atrium. We found \(I_f\) densities, activation kinetics and activation range to be independent on the left atrial region. These results offer a framework for further studies elucidating the correlation between HNC expression and functional \(I_f\) properties. Furthermore, our simulations suggest that human atrial \(I_f\) is able to induce membrane depolarization which is sufficient for the initiation of ectopic activity in the human atrium.

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References

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