The Contribution of I_f and I_{K1} to Focal Activity in Atrial Fibrillation

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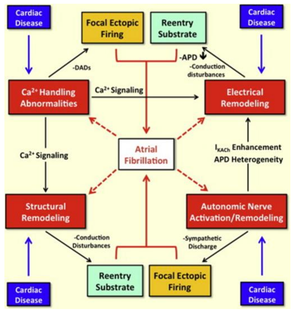
BACKGROUND

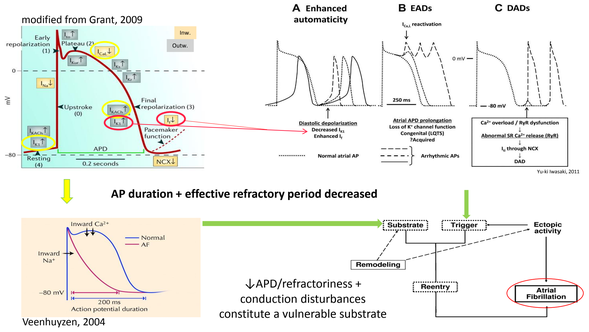


Nattel et al., 2014

Basic mechanisms in the pathophysiology of AF

There are four principal mechanisms (red boxes) contributing to AF. Each of these can result from cardiac disease (blue boxes). AF requires two preconditions, namely focal ectopic beats and a reentry substrate. A susceptible reentry substrate requires abbreviated refractoriness (which depends primarily on APD) and/or conduction abnormalities, which is a consequence of either structural or electrical remodeling. These mechanisms can be the result of AF-promoting cardiac diseases but can also result from the consequences of AF itself [2].

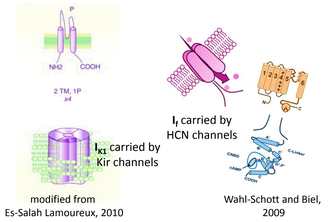
Atrial fibrillation (AF) ist the most common arrhythmia and is associated with increased morbidity and mortality. In this progressive disease electrical and structural remodeling are key components of arrhythmogenesis [1]. Electrical remodeling refers to alterations in ion channel expression and activity and can lead to ectopic firing, which can originate from the right (RAA), the left atrial appendages (LAA) or the pulmonary vein sleeve myocardium. Focal activity such as enhanced automaticity, early after depolarizations (EADs) or delayed after depolarizations (DADs) can trigger AF by entering a vulnerable substrate.



Basic mechanisms underlying enhanced automaticity

After repolarization, normal atrial cells remain at the resting potential. The resting potential is maintained by high resting K^+ conductance through the inward rectifier potassium current (I_{K1}). The human pacemaker current I_f (carried by HCN channels), which plays a significant role in the diastolic depolarization in pacemaker cells is also expressed in atrial cells but overwhelmed by a much larger I_{K1} guaranteeing a stable resting potential. Enhanced automaticity may occur through an imbalance resulting from decreased I_{K1} and/or enhanced I_f.

I_f and I_{K1} are potential contributors to focal activity



Studies indicate that several ionic currents, among them I_f [3] are altered with regard of the progression of AF, origin of the tissue (LAA or RAA) and the nature of the cardiac disease.

Modifications are observable at different levels
- mRNA level
- protein level
- posttranscriptional changes
- channel function

The majority of data is obtained from myocytes of the right atrial appendage.So far there are only few indications that the electrical remodeling process might be also chamber specific.

The human pacemaker current comprises two components

- I_f is the time- and voltage-dependent component, that reaches steady-state levels within a range of tens of milliseconds to several seconds when fully activated and can be blocked by cesium.
- I_{inst} is the voltage- and time-independent component, that activates fully within a few milliseconds and is insensitive to cesium.

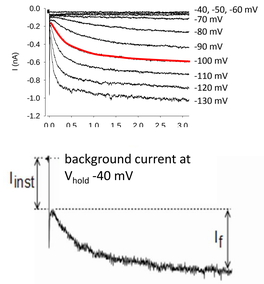
The molecular nature of I_{inst} and its role in the pacemaking process is still a matter of research. There is strong evidence for I_{inst} to flow through the main HCN channel pore. I_{inst} is discussed to contribute to a stable pacing rhythm in autorhythmic cardiac cells [4] and may form the sodium-sensitive background current ($I_{b,Na}$) [5]. Taking into account that at least for HCN2 channels I_{inst} amounts to approximately 10% of total pacemaker current [6] a physiological role in cardiac automaticity becomes reasonable. This contribution of I_{inst} to depolarizing pacemaker currents makes this component an interesting candidate for the generation and maintenance of pathological induced abnormal activities, in particular when considering that I_f is known to contribute to abnormal automaticity in cardiac non-pacemaker regions under pathological conditions [7,8].

Definitely, more studies are needed to elucidate the role of I_{inst} in pacemaking, in particular with regard to atrial fibrillation, since I_{inst} carries a quantitatively significant inward current within a few miliseconds.

AIMS AND METHODS

To compare human myocytes of the LAA and the RAA in SR and AF regarding their

- **Electrophysiology**
  - relative size of I_f, I_{CaL} and I_{K1} to assess their contribution to abnormal automaticity
  - β-adrenergic stimulation of I_f
  - contribution of I_{inst} to the pacemaker current and to clarify its ionic nature
- **Molecular biology**
  - expression of isoforms underlying I_f, I_{K1}, I_{CaL} on mRNA and protein level
  - expression of relevant microRNAs that regulate the expression of channel protein
- **Biochemical analyses**
  - energy metabolism via determination of high energy phosphates
  - oxidative stress via determination of reduced and oxidized glutathione
- **Computer modeling**
  - single cell excitability: alterations of ionic currents integrated in atrial cell models
  - these results in turn will be implemented in a 3D model of the atria



Determination of the instantaneous current component:
Cells were clamped to a holding potential of -40 mV. Pacemaker current was elicited by hyperpolarizing voltage steps (3 s duration) from -40 mV to -130 mV. I_{inst} was determined as the amplitude of the instantaneous current immediately after the decline of the capacitive transient, and the background current was subtracted. I_f represents the difference between I_{inst} and the current at the end of the hyperpolarizing voltage steps.

PRELIMINARY RESULTS

Sinus Rhythm

Comparing cells of the LAA and RAA shows that
- I_f is larger in cells of the RAA
- I_f activates at more positive V_m in cells of the RAA

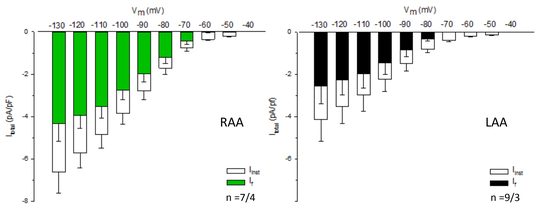


Figure 1. Comparison between the left and the right atrium regarding the contribution of I_f and I_{inst} to the total pacemaker current. Myocytes of the RAA show higher I_f current densities than cells from the LAA. In the LAA there is no I_f at V_m more positive than -80 mV. I_{inst} is already present at V_m -50 mV where I_f is not activated yet.

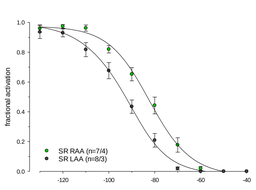


Figure 2. In sinus rhythm I_f activates at more positive membrane voltages in the RAA cells than in the LAA cells. $V_{0.5}$ was tested significantly different with -82.12 ± 2 mV for the RAA and -92.94 ± 1.8 mV for the LAA. The slope factor was similar with 8.54 ± 2 mV and 9 ± 0.68 mV for the RAA and the LAA, respectively.

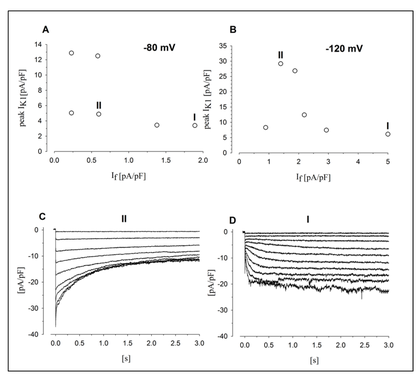


Figure 3. Data plots of I_f vs. I_{K1} current density measured in 6 left atrial cells from patients at -80 mV **(A)** and -120 mV **(B)**. Note that the two cells with large I_f showed only small I_{K1} (I_{K1} was measured as the instantaneous inward current of the Ba^{2+} sensitive current). Panel **C** and **D** show corresponding original current recordings of two LAA cells (I, II) obtained in I_f Tyrode (25 mM K^+) in the absence of external Ba^{2+}. Cell I showed large I_{K1} but small I_f, whereas cell II showed small I_{K1} but large I_f.

Atrial Fibrillation

Comparing cells of patients in AF to SR shows that
- I_f is smaller in AF
- I_{inst} and background current are increased in AF

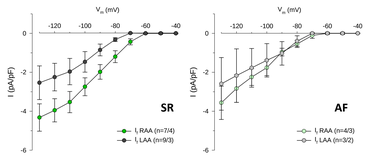


Figure 4. In SR, there is a significant difference in the mean I_f current densities between the RAA and the LAA. This difference equalizes in AF since I_f in the RAA becomes substantially smaller.

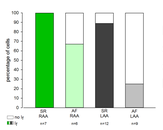


Figure 5. The number of cells with detectable I_f decreases in AF. This effect is more pronounced in the LAA.

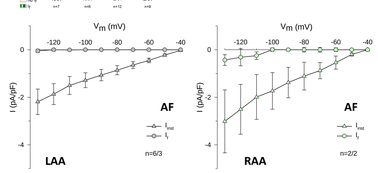


Figure 6. In AF, even if I_f is close to zero, the mean I_{inst} current densities in both atria are increased compared to SR.

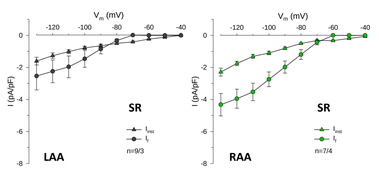


Figure 7. These graphs illustrate that in AF, in both atria, the instantaneous current component increases whereas I_f current densities tend to get smaller.

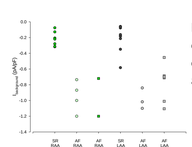


Figure 8. In AF, the background current at V_{hold} -40 mV increases compared to SR. This effect can also be seen in cells without I_f.

CONCLUSIONS AND OUTLOOK

In AF
- The mean I_f current densities of the RAA and the LAA are smaller compared to SR.
- The instantaneous current component as well as the background current are increased in both atria compared to SR.

Therefore, the instantaneous current and the background current seem to be study objects of high importance regarding the electrical remodeling in AF.

Furthermore, when investigating cells of patients in SR, we observed that cells which show a large I_f tend to show a relatively small I_{K1}. Hence, these two currents will also be examined in the same cells of AF patients to detect a possible imbalance in these two counterbalancing currents.

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