Populations of models: A new approach for understanding the mechanisms of atrial fibrillation

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Abstract

Inter-subject variability explains the diverging effects to pharmacological treatments of AF reported in the literature. A population of 173 mathematical models of remodeled human atrial tissue with realistic inter–subject variability was developed based on action potential recordings of 149 patients diagnosed with AF. The ranges of 6 biomarkers measured from the patch-clamp studies were appropriately covered. The resulting population shows that multiple combinations of variations in the ionic conductances result in AF models that cover the range of biomarkers measured experimentally.

Introducing restrictions associated with pacing rate limits the number of models, restricts calcium transient amplitudes, and shows significant differences in the distribution of variation of parameters associated with I_{Na}, I_{Kr} and I_{NaK} ionic currents. Populations of models will improve the development of new antiarrhythmic treatments preventing undesired effects associated with differences between individuals.

1. Motivations

Atrial Fibrillation (AF) is the most common cardiac arrhythmia, affecting more than 33 million people in the world and requiring 2% of the Spanish sanitary cost (1,545 million €/year), accordingly with other developed countries [1]. However, antiarrhythmic drugs have a modest efficacy at the time of terminating the arrhythmia and sustaining sinus rhythm [2]. Further research is thus needed to yield a deeper understanding of the ionic and structural mechanisms sustaining the arrhythmia.

Mathematical models are useful tools to evaluate hypotheses of AF perpetuation mechanisms and drug response, both in terms of action potential (AP) and rotors dynamics [3,4]. However, most of these in–silico studies use a single set of parameters fitted to the average of many experiments. Although this approach has allowed the reproduction and a better understanding of multiple AF mechanisms, it hides the variability between patients observed in the clinical practice [5,6]. It has been observed that AF remodeling and its interaction between ion channel currents depends on the underlying clinical scenario and genetics of each patient [6]. This variability may explain contradictory responses to pharmacological treatments reported in the literature.

The aim of this study is to develop a realistic population of mathematical models of atrial cardiomyocytes that reproduce the inter-subject variability observed in patients. It may help in the understanding of the ionic mechanisms of AF, and will prevent from undesired effects associated with the development of new pharmacological treatments.

2. Methods

2.1. Experimental Dataset and Biomarkers

Right atrial appendages were obtained from 149 patients diagnosed with chronic AF and undergoing surgery for myocardial or mitral/aortic valve replacement revascularization. All experimental recordings were performed conforming the declaration of Helsinki, and the study was approved by the Ethics Committee of the Dresden University of Technology (Nº EK790799). All patients gave written, informed consent. Antiarrhythmic medication was interrupted before the study [6].

Standard intracellular microelectrodes were used to record APs in atrial trabeculae of the samples described (n=215 of 149 patients) [2]. The preparations were stimulated at 1 Hz for 1 h before data acquisition. A subset of this database (n=9) was also recorded at different pacing frequencies of 1, 2, 3 and 4 Hz.

Variability in APs was quantified by the following biomarkers: APD (AP duration) at 20, 50, and 90% of repolarization (APD20, APD50, APD90 respectively), AP amplitude (APA), resting membrane potential (RMP) and AP plateau potential at 20% of APD90 (V20). Graphical representation of these parameters is given in Figure 1.

In addition to the 1Hz static biomarkers, a rate dependent biomarker (APDratio) was defined as the ratio between the APD at each pacing rate (i.e. 2, 3 and 4Hz) divided by the APD at 1 Hz. This biomarker was measured for each pacing rate both for APD50 and APD90, as they were the most sensitive biomarkers to the pacing.

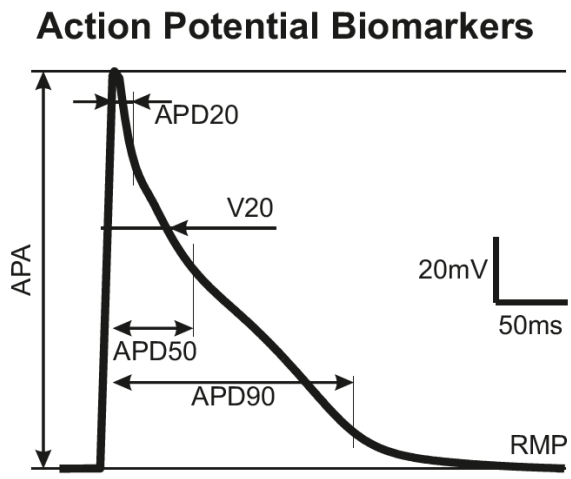

Figure 1.*Representation of the biomarkers measured in this work: APD (AP duration) at 20, 50, and 90% of repolarization (APD20, APD50, APD90 respectively), AP amplitude (APA), resting membrane potential (RMP), and V20 the potential at 20% of APD90 (V20).*

2.2. Electrophysiological Cellular Model

As a basis of the study, the human atrial myocyte model by Koivumaki et al [4] was used, with modifications to account for electrophysiological remodelling in chronic AF: I_{CaL} (–59%), I_{to} (–62%), I_{Kur} (–38%), I_{K1}(+ 62%), I_{NCX} (+50%), expression of SERCA (–16%) and PLB to SERCA (+ 18%) and SLN to SERCA (–40%). We will refer to this model as 'baseline AF model'; the model with no modifications associated with the remodeling will be referred as 'Koivumaki original sinus rhythm model'.

2.3. Population of Models

In order to model variability between subjects, a population of human atrial models was generated from the 'baseline AF model' described above, by varying from –100% to +200% of the original value the following parameters: g_{Na}, maximum I_{NaK}, g_{K1}, g_{CaL}, k_{NCX}, g_{to}, g_{Kur}, g_{Kr}, g_{Ks} as well as the expression of cpumps in SERCA and the availability of Ca^{2+} release channels from sarcoplasmic reticulum $J_{rel,RyR}$.

Latin Hypercube Sampling Method [7] was used to generate 16.384 combinations of the currents above. The method allows the generation of combinations of the N=11 parameters by means of sampling the N–dimensional space with a high resolution, efficiently and without bias. It is implemented by establishing the inferior and superior and bounds and sampling the range in each parameter in M=16.384 intervals, which results in M^N locations. Then, M of these locations are randomly chosen meeting that, for each dimension N. In no case each interval is chosen more than once.

The resulting 16,384 unicellular models were simulated at different pacing frequencies: 1, 2, 3 and 4 Hz with square stimulus with duration 2 ms and amplitude 1250 pA, followed by negative amplitude during the inactive time interval to maintain current conservation in the model, and simulate the effect of neighboring cells in the tissue. Mathematical simulations were performed on the cardiac simulation GP–GPU platform [8]. APs from period 91 to 100 were stored at sampling frequency of 1kHz and used to measure the AP biomarkers described above.

A subset of the initial population of 16,384 mathematical models was selected by identifying those that satisfied physiological ranges of the biomarkers identified by using the experimental recordings. The identification of that subset of models was carried out in two different steps.

First, only those models for which biomarkers APD90, APD50, APD20, APA, RMP and V20 at 1 Hz remained restricted to the maximum and minimum values measured experimentally in the 215 APs were selected. To ensure biological depolarization transient, two extra constraints in biomarkers were introduced, the time between the stimuli and AP peak must be lower than 20 ms, whilst dV/dtmax higher than 20 V/s.

Next, with the aim to ensure that the resulting population reproduces the rate dependence of the experimental data, only those models that reproduced physiological rate dependence were selected. Specifically, linear regressions of APDratios vs APD at 1 Hz were computed for each frequency (2, 3 and 4 Hz) and biomarker (i.e. APD50 and APD90) in all samples recorded at different frequencies. The models that presented APDratios for APD90 and APD50 at 2, 3 and 4Hz within a standard deviation to the estimated regression were finally accepted (see Figure 3).

We will refer to the set of models which only satisfied the 1Hz biomarkers as the '1Hz population', whereas the set of models that satisfied both the 1Hz and rate dependent biomarkers will be referred as 'AF population'.

2.4. Statistical Analysis

Mann–Whitney U–test, which is robust against non-Gaussian distributions, was used to evaluate statistical significance between variables ($p<0.01$).

3. Results

3.1. Experimental Dataset and Biomarkers

Minimum and maximum biomarker values, as measured at a pacing frequency of 1Hz, are presented in Table 1.

Minimum and maximum values of APD90 and APD50 in the subset recorded at different frequencies were: 167-236, 148-220, 134-199 and 121-176 ms in case of APD90 for 1, 2, 3 and 4 Hz, respectively; and 59-121, 65-118, 62-108, 56-96 ms in case of APD50. The rest of biomarkers did not exhibit significant changes due to the increase in pacing rate.

	Minimum Value.	Maximum Value
APD90(ms)	140	330
APD50(ms)	30	180
APD20(ms)	1	75
APA(mV)	80	130
RMP(mV)	-85	-65
V20(mV)	-30	20

Table 1. *Minimum and maximum values of biomarkers at 1Hz.*

3.2. Physiological AF Population of Mathematical Models

From the total of 16,384 mathematical models with different combinations of ion channels expressions that were analyzed, 945 models (5.76%) satisfied all the measured ranges of biomarkers at 1Hz (Table 1), and constituted our '1 Hz population'. From this, only 173 (1.06%) additionally met the rate dependence constraints and constituted our 'AF population'. Therefore, although a large number of ion channel combinations reproduced 1Hz patch–clamp experiments for a pacing rate of 1 Hz, only few of them were physiologically realistic in terms of pacing rate dependence.

In Figure 2, the variability of APs, calcium transients and biomarkers for the different groups is presented. In Figure 2A biomarkers for the simulated models, together with the experimental data are shown. Notice that the observed variability on experimental biomarkers is appropriately covered by the obtained populations of mathematical models. Figure 2B shows the coverage of APD50 and APD90 ratios for the experimental data, and the models in our '1 Hz' and 'AF' populations. It can be observed, both in experimental and simulated values, that the larger the APD at 1 Hz, the lower the APDratio. This reflects a pronounced shortening of the APD as a function of the pacing rate and baseline APD. Notice that many of the models in the '1 Hz population' do not reproduce the rate dependence as measured in the experimental recordings, which highlights the importance of calibrating the population at multiple pacing frequencies.

Figure 2C shows APs and calcium transients of all models in the populations. It is of note that imposition of only APD biomarkers at 1 Hz allows for the selection of a class of models with exacerbated calcium transients. However, imposition of rate-dependence biomarkers allowed discarding those models with possibly non physiological calcium transients.

Figure 3 shows the distribution of the variation in parameters for the 173 models that reproduced both 1Hz and rate dependent biomarkers (i.e. AF population) and those 945 models that reproduced only in the 1Hz biomarkers (i.e. 1Hz population). As a result of accounting for physiological variability, it is of note that most currents inside the 1 Hz and AF populations were not circumscribed to the midpoint of the simulated range (-100% to +200%) and showed median values that often diverged from the average baseline model. While the median values of g_{K1} and g_{Kur} matched with their baseline values, g_{Na} was clustered around lower values. On the other hand, g_{CaL} and k_{NCX} presented higher median values than the baseline model and closer to that of the control Koivumaki original sinus rhythm model.

The parameters that differed from 1 Hz and AF populations to a greater extent were g_{Na}, $I_{NaK,max}$ and g_{Kr} ($p<0.01$). In particular, g_{Na} tended to be lower while $I_{NaK,max}$ and g_{Kr} tended to be larger to fulfill the imposed rate-dependence criteria. The largest variations were observed for I_{NaK}, which highlights its importance for AP rate adaptation, consistent with previous works [9].

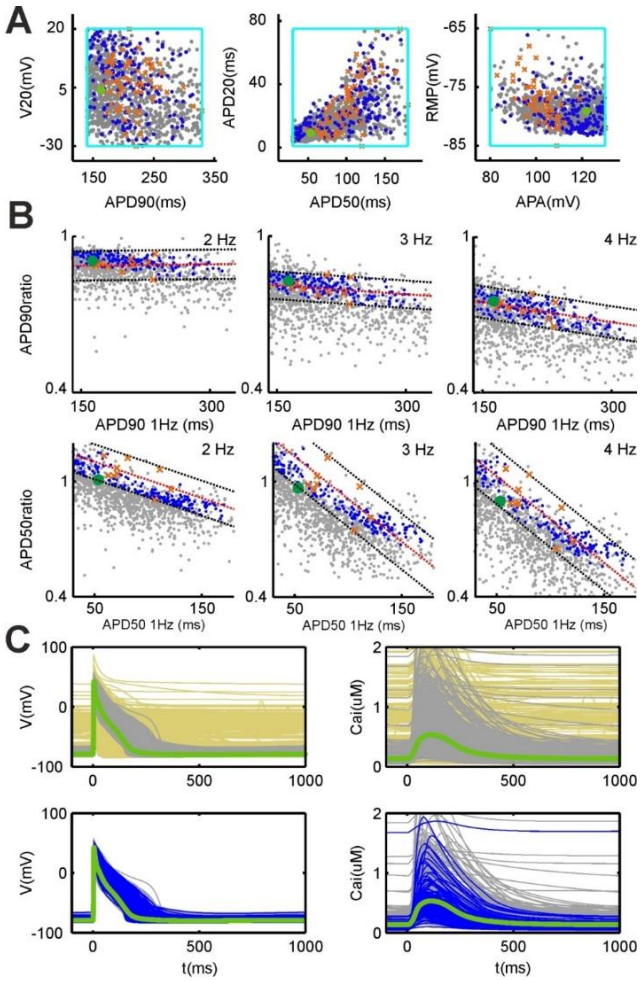

Figure 2. *Population of models calibration and biomarkers. Green, yellow, blue and gray respectively represent: baseline AF model, all simulated models (N=16384), the 'AF population' obtained including rate dependence constraints (N=173), and those that only reproduced experimental data at 1Hz (N=945). A: Biomarkers values (V20, APD20, APD50, APD90, RMP and APA) in the populations at 1Hz; orange marks correspond with a random sort of values measured in experimental preparations; upper and lower bounds for 1 Hz biomarkers are marked in light blue. B: Calibration process at different pacing frequencies: only models within a standard deviation from regression lines (red lines) as observed in experimental measurements were accepted. APD ratios are plotted versus APDs at pacing frequencies 2, 3 and 4 Hz. C: APs and intracellular calcium transients in the resulting populations.*

4. Discussion

A population of mathematical models of atrial tissues which mimics the inter–subject variability of AF patients including rate dependent response has been implemented.

Latin hypercube sampling allowed for an efficient sampling. After having established a wide population of models, AP biomarkers allowed us for a selection of ionic current combinations that result in APs covering the observed experimental range. However, reproduction of AP biomarkers at a single pacing rate does not account for the observed rate dependence of ionic currents. Cells with long basal APDs shorten their APDs under fast rates to a greater extent than those with shorter basal APDs.

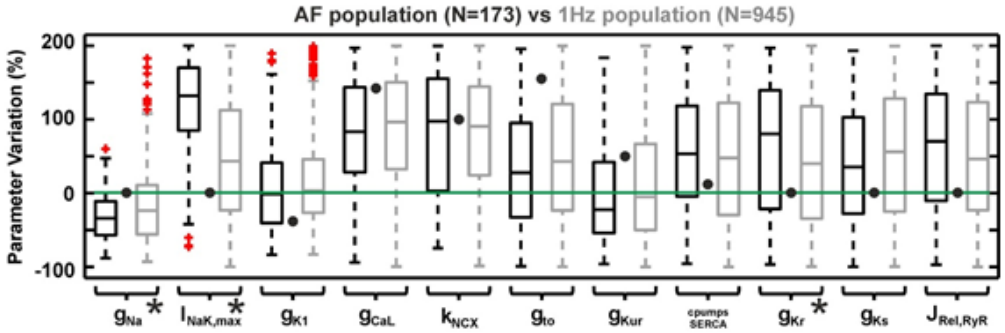

Figure 3. *Boxplot of the ionic current parameter variations in the population of models. The boxplots in black correspond to the AF population (models that satisfied the constraints of biomarkers at 1Hz and the conditions of rate dependence). Gray boxes indicate the models only satisfying the constraints of biomarkers at 1Hz. The green line depicts the baseline values (i.e. the baseline AF model), while black dots represent the original Koivumaki model in sinus rhythm. A high variability in conductances covering the whole range of values (from -100% to 200%) can be observed. It is also observed how the most constrained current is INa, with bounds in overexpression and blockade of +65% and -90% respectively in the resulting population.*

In order to account for this rate-dependence we have included restrictions in our database of models that reproduce this observed trend. It is of note that the introduction of these rate-dependence bounds, obtained from AP measurements, resulted in the rejection of models with exacerbated calcium transients. According to our results, the single most relevant parameter to account for this rate dependence was I_{NaK}, which is in accordance with previous reports [9].

Note how the 'baseline AF model' was close to the limits of the biomarkers ranges estimated from microelectrode recordings (APD90, APD50, APD20, APA, APD50ratio), which evidences that a single model may not be representative of the whole variability observed in AF. The population of models showed how a wide variability in ionic parameters reproduced physiological biomarkers. This variability has been useful to evaluate the effect of ionic currents in each biomarker and to better understand the effect of different currents in rotor dynamics [10].

Populations of models will improve viability studies in the development of new drug treatments, avoiding unexpected side effects associated to inter-subject variability.

Acknowledgements

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