Development of a Sex-Specific Action Potential Model for Rabbit Atrial Cells

Camilla Pera[1], Katja E Odening[2], Ulrich Schotten[3], Simone Pezzuto[1]

[1] University of Trento, Trento, Italy
[2] University of Bern, Bern, Switzerland
[3] University of Maastricht, Maastricht, Netherlands

Abstract

This work presents an analysis of a mathematical model of action potentials (APs) in rabbit atrial cells, with a focus on gender differences. Gender medicine is increasingly recognized as essential, highlighting how sex and hormones influence the symptomatology and treatment of diseases. In particular, we focused on sex differences using a rabbit animal model. We adapted a previously proposed ionic model of rabbit atrial electrophysiology. A sensitivity analysis (SA) using Sobol' indices was performed to identify the ionic conductances that most influence the properties of the AP. We employed a Gaussian Process emulator, significantly decreasing the computational time required for the SA while maintaining high accuracy. Parameter optimization successfully reproduced experimental APs. Finally, we used Approximate Bayesian Computation with Sequential Monte Carlo methods (SMC-ABC) to estimate model parameters and quantify uncertainties, revealing varying degrees of parameter identifiability. Results showed that AP duration is primarily affected by L-type calcium conductance, while I_{K1} and the $Na-K$ pump influenced the resting membrane potential. We observed statistically significant sex differences in the I–V curve for I_{K1}, but differences in conductances were not significant, possibly due to the limited sample size.

1. Introduction

Gender medicine is increasingly recognized as crucial to understanding how biological sex influences the manifestation and treatment of diseases. In cardiac electrophysiology, sex differences have been observed in the prevalence, symptomatology and treatment of atrial fibrillation [1].

Rabbits are frequently used as animal models in the study of cardiac electrophysiology. Unlike rodents and other small animals, rabbits exhibit electrophysiological characteristics more similar to those of humans, making them particularly suitable for translational studies. Furthermore, compared to large animals, rabbits are easier to maintain, offering an ideal compromise between physiological relevance and experimental practicality [2]. The aim of the study is to develop and analyze a mathematical model for rabbit atrial action potential (AP), taking into account sex differences.

2. Methods

2.1. Mathematical Model

The Aslanidi model (2009) [3] was taken as the starting point for this study. The model consists of an equivalent circuit representing the cell membrane (sarcolemma) and a fluid compartmental model to describe ion fluxes between the cytoplasm and the extracellular and intracellular spaces.

The equivalent circuit represents the membrane as a capacitor C_m in parallel with various ionic currents, pumps and exchangers, each of which has been identified in the membrane of the rabbit atrial myocyte. These components include the fast sodium current I_{Na}, the transient potassium current I_{to}, the long-delayed $I_{\text{Ca,L}}$ and transient $I_{\text{Ca,T}}$ calcium currents, and the inward rectifier potassium current I_{K1}, the delayed potassium currents I_{Kr} and I_{Ks}, the background currents I_{b}, the sodium-potassium pump I_{NaK}, the calcium pump I_{CaP}, and the sodium-calcium exchanger I_{NaCa}. The membrane voltage $V(t)$ is described by the following equation

$$C_m \frac{\text{dV}}{\text{dt}} = I_{\text{stim}} - \sum_i I_i, \tag{1}$$

where I_i is the set of transmembrane currents and I_{stim} is the stimulation current applied. Eq. (1) is complemented by a set of ODEs for the concentration of intracellular potassium, sodium and calcium dynamics. The model has been implemented in Myokit [4], directly imported from the original CellML [3].

2.2. Global Sensitivity Analysis

In order to investigate the influence of the model parameters on the action potential properties we used global

Figure 1. Heat map of the sensitivity analysis.

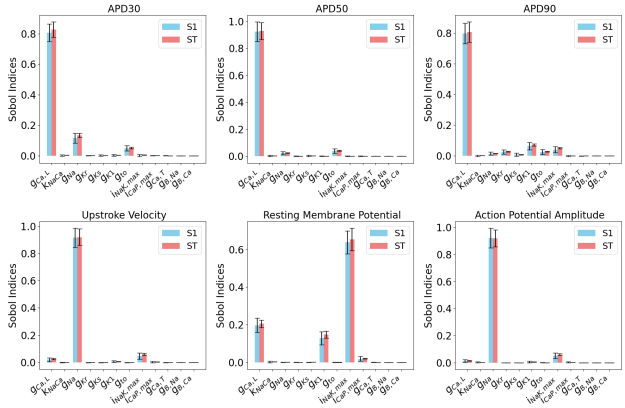

Figure 2. 95% confidence intervals for first-order and total effect Sobol' indices.

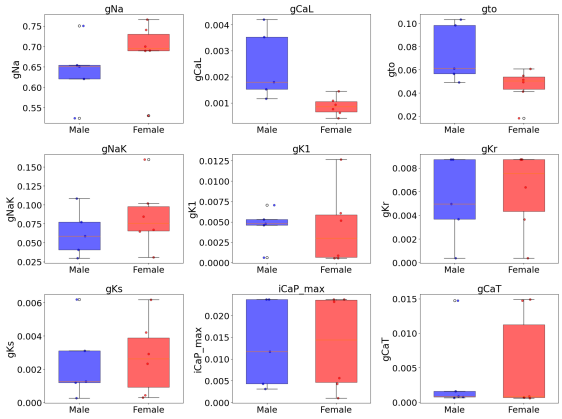

Figure 3. Boxplot of optimized conductance parameters obtained from the fitting of AP traces for female and male groups.

sensitivity analysis [5]. As inputs for GSA were chosen the maximum conductances of ion channels, pumps and exchangers (12 parameters in total), whereas as outputs we considered APD30, APD50, APD90, upstroke velocity, AP amplitude and resting membrane potential.

Model inputs were varied in a range $\pm 50\%$ of their reference value [3]. This range was selected to account for physiological variability without reaching biologically implausible values. The sensitivity analysis was performed utilizing Sobol' indices, in particular the first order indices and total-effect indices. The first order index measures the individual effect of a parameter, while the total effect index captures the combined effect of a parameter including its interaction with others.

Sensitivity analysis with Sobol' indices requires a large number of model evaluations. To overcome this limitation, we developed an emulator based on Gaussian Processes [6, 7]. We built separate emulators for each model output, employing Gaussian Process Regression (GPR) models using a Matérn kernel combined with a constant kernel for each emulator. The kernel hyperparameters were tuned through multiple random restarts (50 restarts).

We explored the parameter space through Latin hypercube sampling from uniform distributions centered on the baseline model values. The model was pre-paced for 50 beats to reach a steady state, and then outputs were calculated over a single cycle length. In order to built a robust emulator we considered two different sets: a training set (1000 samples) to train the GP and a test set (200 samples) to validate the model.

2.3. Fitting

With regard to the fitting of the experimental data, two datasets were available for consideration. The first set consists of current-voltage (I-V) curves of current I_{K1} measured on 81 cells from female rabbits and 68 cells from male rabbits. The second set consists of AP traces obtained by sharp electrode from atrial tissue, including 5 measurements from male atria and 5 from female atria.

I_{K1} curves were obtained by patch-clamp measurements. The current was measured across a range of membrane potential values, from -120 mV to -30 mV, in increments of 10 mV. To simulate the IV curves, only the I_{K1} current equation was considered, in order to simulate the patch-clamp measurements. The equation for the I_{K1} current

$$I_{\mathrm{K1}} = g_{\mathrm{K1}} \left(\frac{[K^+]_o}{[K^+]_o + K_{\mathrm{m,K1}}} \right)^3 \frac{V - E_K}{1 + e^{a(V - E_K + b)/RT}}. \tag{2}$$

We opted to fit the data using group (female and male) averages at each voltage rather than individual experimental curves, in order to mitigate the effects of random fluctuations. To perform the fitting of the average IV curves,

we used a non-linear optimization approach based on minimizing the square error of the differences between the experimental data and the proposed mathematical model. The optimization parameters chosen are the gating variables, a and b, and the conductance g_{K1}.

For the fitting of AP traces we treated all conductances as fitting parameters, setting their optimization ranges to $(0.1\times$reference value$, 2.5\times$reference value$)$. However, we made an exception for the sodium conductance, constraining g_{Na} to within $\pm 10\%$ of the reference value to ensure reliable propagation of the action potential. For parameter optimization we used a derivative-free minimization algorithm. The objective function was defined as the L^2 norm between the measured AP and the values obtained from the model simulations.

2.4. Approximate Bayesian Computation

We utilized `pyABC` [8] to perform parameter estimation using the Approximate Bayesian Computation with Sequential Monte Carlo (ABC-SMC) method. For the distance function, we selected the Euclidean distance between the experimental action potential traces and those obtained from the simulations. The prior distributions for the parameters (conductances) were defined as uniform distributions within the ranges specified in the previous section. The ABC-SMC algorithm was executed for a maximum of 15 populations or until a minimum tolerance level was achieved.

3. Results

The sensitivity analysis, performed using Sobol' indices, revealed that g_{CaL} exerts the strongest influence on action potential duration (APD30, APD50, APD90). In contrast, g_{Na} primarily determines the upstroke velocity and AP amplitude. The resting membrane potential is most sensitive to variations in g_{K1} and $I_{\mathrm{NaK,max}}$. These results are visualized in the heat map of first-order Sobol' indices (Figure 1).

Figure 2 presents the 95% confidence intervals for both first-order and total effect Sobol' indices. The total effect indices are comparable to the first-order indices. This indicates that interaction effects among parameters are minimal, consequently the model is characterized by a more straightforward and direct dependence of parameters on outputs, with fewer significant interaction effects.

The fitting results, displayed in Figure 4, demonstrate a good agreement between the model and the experimental data for both groups. Notably, significant differences were observed in the optimized parameters between females and males, suggesting intrinsic variations in the electrophysiological properties of I_{K1} between the two groups. Indeed, the Mann-Whitney U-test showed significant differences

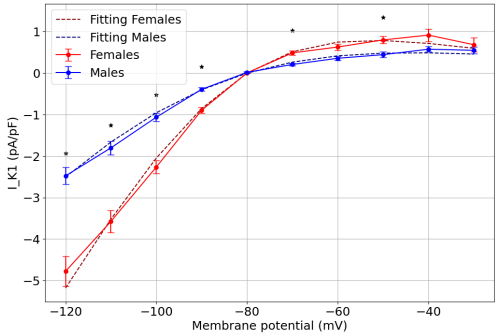

Figure 4. I-V curves of I_{K1} for female and male groups from experimental data. Stars indicate voltages where significant differences are found ($p < 0.05$).

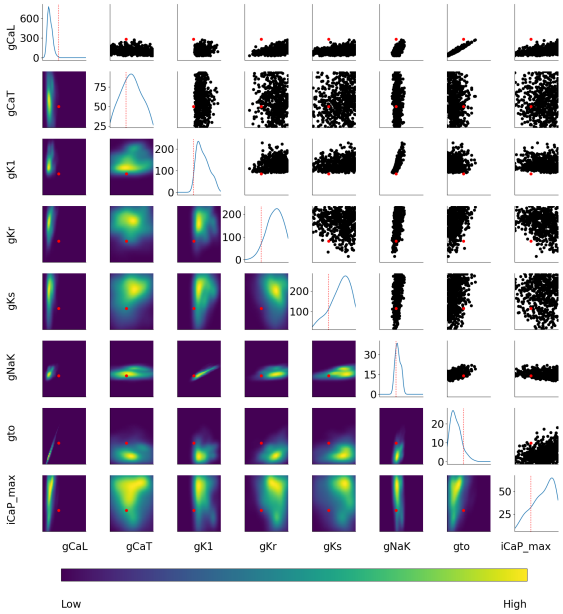

Figure 5. KDE matrix of parameters estimated via ABC-SMC. The marginal distributions are shown along the diagonal, while the correlation between parameter pairs are shown in the off-diagonal sections. Dotted lines and red points represent the reference value for each conductance. Axes span 10%–250% of reference values.

for 6 out of 10 voltages in the experimental data, indicated by stars in Figure 4.

The results of the fit, shown in Table 1, reveal distinct differences in current parameters I_{K1} between the female and male groups. Females exhibit a higher conductance (g_{K1}) than males, indicating a higher potassium current density. Both b and a differ between groups, potentially reflecting differences in channel gating kinetics.

The fitting approach of the AP traces demonstrated favorable outcomes in both groups; consequently, an analysis of the optimized parameters (ionic conductances) was

Table 1. Optimized parameters of I_{K1}.

Group	g_{K1}	b	a
Female	0.0117	4.1984	1.3315
Male	0.0076	-12.0258	0.8886
Model	0.005088	-3.6	1.393

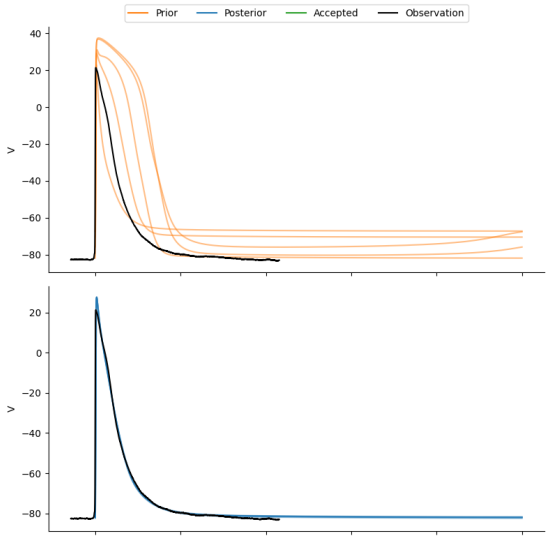

Figure 6. Comparison of simulated AP and experimental observations. Specifically, the top panel shows samples generated from the a prior distribution (orange), the middle panel shows samples from the posterior distribution (blue) compared to the experimental observation (black).

conducted. However, no statistically significant differences were observed between the two groups. The boxplots of the optimized conductances are displayed in Figure 3.

Parameter estimation revealed varying degrees of identifiability, displayed in Figure 5, with some parameters estimated with high confidence while others remained uncertain. Figure 6 shows the comparison between the simulations of the cardiac action potential generated from the samples distributed according to the prior, posterior, and accepted, compared to the experimental data. The differences between the accepted samples and the observed potential are minimized. We can therefore conclude that the ABC-SMC algorithm was effective in reducing the parameter space to a set that provides a better approximation.

4. Conclusions

This study proposes a framework for analyzing sex differences in cardiac electrophysiology through mathematical modelling. Sensitivity analysis enabled the identification of the most influential ionic currents in modulating action potential properties, providing useful insights for future targeted studies. The absence of significant differences in conductances between males and females could be due to the limited sample size. However, the observed differences in current I_{K1} suggest the presence of relevant physiological variations to be further investigated. Looking forward, the integration of larger experimental data and the inclusion of hormonal influence represent essential next steps for a more complete understanding of sex-specific dynamics in cardiac physiology.

Acknowledgements

This work has been supported by the CSCS production grant s1275 and by the MICROCARD-2 project (project ID 101172576). SP also acknowledges the PRIN-PNRR [no. P2022N5ZNP] and the FWF-SNSF [no. 214817 (CardioTwin)].

References

[1] Westerman S WN. Gender differences in atrial fibrillation: A review of epidemiology, management, and outcomes. Current Cardiology Reviews 2019;15(2):136–144. ISSN 1573-403X/1875-6557.

[2] Milani-Nejad N, Janssen PM. Small and large animal models in cardiac contraction research: Advantages and disadvantages. Pharmacology Therapeutics 2014;141(3):235–249. ISSN 0163-7258.

[3] Aslanidi OV, Boyett MR, Dobrzynski H, Li J, Zhang H. Mechanisms of transition from normal to reentrant electrical activity in a model of rabbit atrial tissue: Interaction of tissue heterogeneity and anisotropy. Biophysical Journal 2009;96.

[4] Clerx M, Collins P, De Lange E, Volders PG. Myokit: a simple interface to cardiac cellular electrophysiology. Progress in Biophysics and Molecular Biology 2016;120(1-3):100–114.

[5] Saltelli A, Annoni P, Azzini I, Campolongo F, Ratto M, Tarantola S. Variance based sensitivity analysis of model output. design and estimator for the total sensitivity index. Computer Physics Communications 2010;181(2):259–270.

[6] Williams CK, Rasmussen CE. Gaussian processes for machine learning. MIT Press Cambridge, MA, 2006.

[7] Gander L, Pezzuto S, Gharaviri A, Krause R, Perdikaris P, Sahli Costabal F. Fast characterization of inducible regions of atrial fibrillation models with multi-fidelity gaussian process classification. Frontiers in Physiology 2022;13:757159.

[8] Klinger E, Rickert D, Hasenauer J. pyABC: Distributed, likelihood-free inference. Bioinformatics 2018; 34(20):3591–3593.

Address for correspondence:

Camilla Pera
Via Sommarive 13, Trento (Italy)
camillapera7@gmail.com