

An in Silico Investigation into the Role of SK Channels in Failing Ventricular Myocytes

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Abstract

SK channels are small conductance (~ 10 pS) calcium-activated potassium channels. Although they have been suggested not to play a relevant role in healthy ventricles, SK channel upregulation has been reported in failing ventricles, with potential implications for arrhythmogenesis. In this work, we aimed at uncovering the contribution of SK channels to ventricular repolarization in failing myocytes. To that end, we extended an in silico electrophysiological model of human ventricular failing myocytes by introducing the equations representing SK channel activity. To determine the value of the maximal SK current conductance, G_{SK} , we simulated action potentials (APs) at different pacing frequencies and we fitted the changes in AP duration induced by SK channel inhibitions to available experimental data recorded under pharmacological interventions. The SK block-induced effects in our simulations were consistent with experimental evidence. Early afterdepolarizations were observed at pacing frequencies below 0.7 Hz only when SK channels were blocked. In conclusion, our presented model of human ventricular failing myocytes integrating an SK channel formulation can allow dissecting the contribution of SK channels to ventricular repolarization and may help in understanding their role in arrhythmogenesis.

1. Introduction

The small conductance (~ 10 pS) calcium-activated potassium (SK) channels are an important group of potassium-selective ion channels. Some studies have shown that SK channels significantly contribute to the repolarization phase of the atrial action potential (AP), although their beneficial or adverse effects remain debated. SK channels were initially considered not to play a relevant role in ventricular repolarization. However, different studies in ventricular myocytes, tissues and whole hearts have shown that SK channel blockade has remarkable ventric-

ular effects in certain pathological states [1], highlighting these channels as a possible backup mechanism in diseased ventricles.

In heart failure (HF), a chronic, progressive condition in which the heart muscle cannot pump enough blood to meet the body's needs for blood and oxygen [1], blockade of SK channels has been experimentally reported to prolong AP duration (APD). It has been suggested that upregulation of SK channels under pathological conditions could be an adaptive physiological response to shorten the APD under conditions of reduced repolarization reserve [2]. Nevertheless, there is conflicting evidence on the pro- or antiarrhythmic role of these channels [3]. The development of mathematical models that incorporate descriptions of SK channel activity in failing ventricles could help to shed light on their role in cardiac pathophysiology.

The aim of this work was to uncover the contribution of SK channels to ventricular repolarization in human failing myocytes. We extended a mathematical model of cellular electrophysiology in failing myocytes by incorporating a formulation for the current generated by the SK channels, I_{SK} , based on experimental data available in the literature. We simulated APs with and without SK channel blockade and investigated the occurrence of proarrhythmic early afterdepolarizations (EADs).

2. Methods

2.1. Model of failing myocytes with SK channel formulation

The O'Hara *et al.* model (ORd) [4], which is the most widely used, validated human ventricular myocyte model in the literature, was used as a basis. We replaced the original formulation of the fast sodium current, I_{Na} , in the ORd model with the formulation in the ten Tusscher *et al.* model [5] to better represent the AP upstroke characteristics. HF-associated electrophysiological remodeling was simulated by scaling the maximal conductances and time constants

of various ionic currents and fluxes as in Gomez *et al.* [6].

A formulation for the I_{SK} current was integrated in the failing ventricular model by using the equations proposed in Landaw *et al.* [7]:

$$\begin{cases} I_{SK} &= G_{SK}x_{SK}(V - E_K) \\ \frac{dx_{SK}}{dt} &= \frac{x_{SK,\infty} - x_{SK}}{\tau_{SK}} \\ x_{SK,\infty} &= \frac{1}{1 + (K_d/Ca_{ss})^n} \\ \tau_{SK} &= \tau_0 + \frac{\tau_1}{1 + (Ca_{ss}/0.1)} \end{cases} \quad (1)$$

where Ca_{ss} is the calcium concentration in the subspace compartment (submembrane space near t-tubules), which is sensed by SK channels. In the equations, the following parameter values were set in concordance with experimental observations [7, 8]: $\tau_0 = 4 \text{ ms}$, $\tau_1 = 20 \text{ ms}$, $n = 3.14$, and $K_d = 0.000345 \text{ mM}$.

2.2. Fitting of SK current conductance

The maximal conductance G_{SK} of the SK current was adjusted so that the resulting model reproduced the experimental AP prolongation induced by I_{SK} block in human ventricular failing myocytes [1]. In the experiments, APD at 50% and 90% repolarization, APD_{50} and APD_{90} , were measured in midmyocardial ventricular cells from seven HF patients, at baseline and after apamine-induced I_{SK} block while pacing at 0.5, 1 and 2 Hz (Figure 1).

From the experimental APD values, we calculated the relative change (R) in APD_{50} and APD_{90} induced by SK channel block, for 1 Hz and 2 Hz pacing:

$$R = \frac{APD_{\text{apamin}} - APD_{\text{baseline}}}{APD_{\text{baseline}}} 100 \quad (2)$$

where APD represents APD_{50} or APD_{90} . Experimental data at 0.5 Hz pacing were not used for G_{SK} fitting since

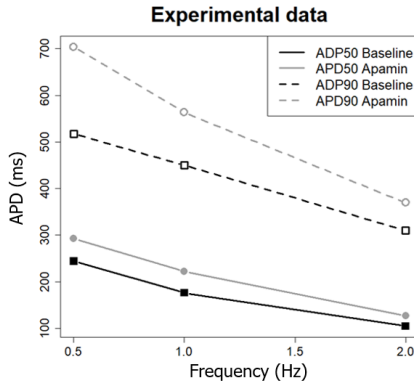


Figure 1. Experimental APD_{50} and APD_{90} with and without I_{SK} block. Figure replicated from [1].

various AP traces presenting EADs had been excluded from the data in [1]. The four experimental R values are presented in Table 1.

The optimal value of G_{SK} was found by minimizing the difference between the experimental and simulated relative changes in APD_{50} and APD_{90} using the method proposed in [9]:

$$f(G_{SK}) = \sum_{i=1}^4 (R_i - S_i(G_{SK}))^2, \quad (3)$$

where R_i represents each of the four experimental relative changes and $S_i(G_{SK})$ represents the corresponding simulated relative change for a given conductance value G_{SK} . Simulations were ran for twenty different values of G_{SK} and the resulting S values were interpolated by quadratic polynomials, from which the search for the minimum was performed using Brent's method. A search range for optimal G_{SK} was established from 0 to 0.01 based on experimental reports [7]. The value of G_{SK} associated with the lowest error f was selected.

Simulations were carried out at baseline (with the optimal G_{SK} value) and under full SK channel block ($G_{SK} = 0$) at pacing frequencies varying from 0.5 to 2 Hz in 0.1-Hz steps. For comparison purposes, additional simulations were performed using the non-failing ORd model, both with and without SK channels.

All simulations were ran using the cardiac electrophysiology simulator DENIS [10]. Forward Euler was used for numerical integration with a time step of 0.002 ms . Monophasic current pulses with an amplitude of $-80 \mu\text{A}/\mu\text{F}$ were used for stimulation. The model was paced for 20 cycles after steady-state was reached. The effect of apamin was simulated as full SK channel block ($G_{SK} = 0$), as the concentration used in the experiments (i.e. 100 nM) was an order of magnitude higher than the IC50 value reported in the literature [11].

2.3. Sensitivity analysis

A sensitivity analysis was conducted to determine the influence of variations in ionic conductances on the generation of EADs at a low pacing rate of 0.5 Hz. Each conductance in the model was varied by $\pm 5\%$ and $\pm 10\%$ and the absence or presence of EADs was assessed both with and without I_{SK} block.

3. Results

3.1. Model development and validation

The optimal value for the SK current conductance was $G_{SK} = 0.0038 \text{ mS}/\mu\text{S}$. Relative changes in APD_{50} and APD_{90} for simulations calculated with that G_{SK} value differed from the experimental relative changes, R , by less

Table 1. Relative changes in APD₅₀ and APD₉₀ induced by I_{SK} block in simulations and experiments.

| | Simulated | Reference (R) | Error (%) |
|-------------------------|-----------|---------------|-----------|
| APD ₅₀ (1Hz) | 27.27 | 26.43 | 0.84 |
| APD ₅₀ (2Hz) | 19.85 | 21.22 | 1.37 |
| APD ₉₀ (1Hz) | 21.77 | 25.26 | 3.49 |
| APD ₉₀ (2Hz) | 20.51 | 19.21 | 1.3 |

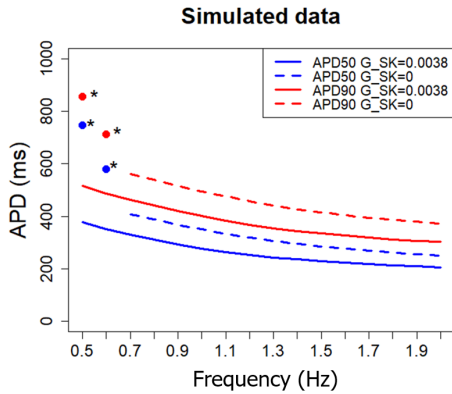


Figure 2. Simulated APD₅₀ and APD₉₀ with and without I_{SK} block. EADS appear below 0.7 Hz (*).

than 3.5% (Table 1). Both APD₅₀ and APD₉₀ were prolonged under I_{SK} block, in line with experimental observations for pacing frequencies above 0.7 Hz (Figure 2). For frequencies below 0.7 Hz, simulated I_{SK} block led to EAD generation. Simulated AP traces at 0.5, 1 and 2 Hz are depicted in Figure 3, both with and without I_{SK} block.

Simulated APD₅₀ and APD₉₀ values for the non-failing ventricular myocyte model are shown in Figure 4. In this case, I_{SK} block prolonged both AP durations, but did not lead to EADs at any pacing frequency.

3.2. Ionic variability

When variability in ionic currents other than I_{SK} was simulated in our extended failing myocyte model, EADs could not be observed for any pacing frequency. Following simulation of I_{SK} block, EADs developed for most of the ionic current variations (Figure 5).

4. Discussion and conclusions

In this work, we extended an existing cardiac electrophysiology model of failing human ventricular myocytes by adding the equations for the I_{SK} current. To do so, a formulation without rectification was chosen for I_{SK} . Alternative formulations with rectification, as in [12], would require the estimation of a larger number of parameters, which, given the lack of sufficient experimental data, could

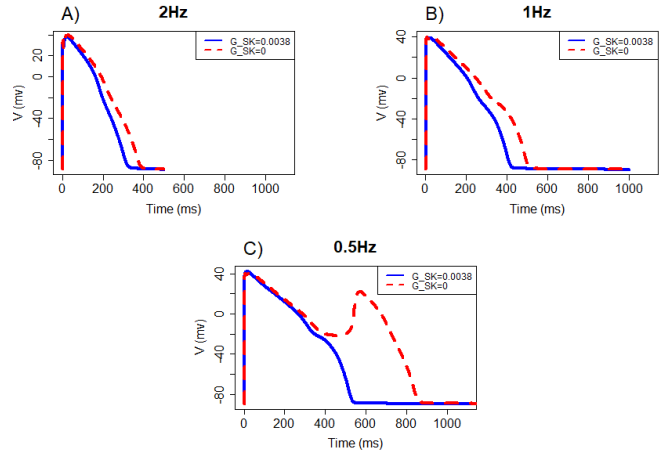


Figure 3. APs computed with and without I_{SK} block at pacing frequencies of (A) 2 Hz, (B) 1 Hz and (C) 0.5 Hz.

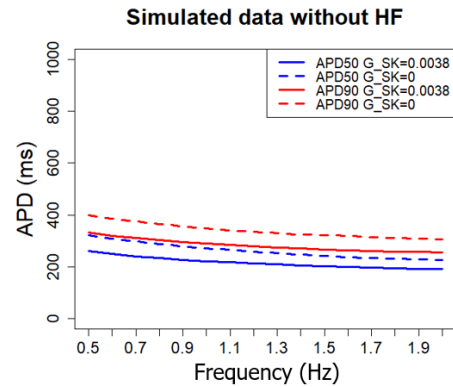


Figure 4. Simulated APD₅₀ and APD₉₀ with and without I_{SK} block.

introduce a high degree of uncertainty in the model. In our selected formulation, the values for the time constants and the dissociation constant were taken from the literature. The value for the conductance G_{SK} was determined by solving an optimization problem so that the model best replicated the experimental prolongation of APD₅₀ and APD₉₀ induced by SK channel block at pacing frequencies of 1 and 2 Hz (Table 1). Although lower pacing frequencies, like 0.5 Hz, were simulated, the results were not used to fit G_{SK} because AP traces exhibited EADs when SK channels were blocked (see Figures 2 and 3), in agreement with experiments in [1].

By defining a set of failing myocyte models built by varying ionic current conductances up to $\pm 10\%$, the results of our sensitivity analysis suggested that I_{SK} plays a major protective role from EADs formation at low pacing frequencies. No EADs were found when I_{SK} was active, while EADs developed for most ionic variations when I_{SK} was

| | Channels blocked ($G_{SK}=0$) | | | |
|-------------|---------------------------------|-------------|-------------|-------------|
| | -10% | -5% | +5% | +10% |
| <i>Ito</i> | Dark Red | Dark Red | Dark Red | Dark Red |
| <i>IKs</i> | Dark Red | Dark Red | Dark Red | Dark Red |
| <i>IpCa</i> | Dark Red | Dark Red | Dark Red | Dark Red |
| <i>IKr</i> | Dark Red | Dark Red | Light Green | Light Green |
| <i>IK1</i> | Dark Red | Dark Red | Dark Red | Dark Red |
| <i>INa</i> | Dark Red | Dark Red | Dark Red | Dark Red |
| <i>INaL</i> | Light Green | Light Green | Dark Red | Dark Red |
| <i>IKb</i> | Dark Red | Dark Red | Dark Red | Dark Red |
| <i>Incx</i> | Light Green | Light Green | Dark Red | Dark Red |
| <i>ICaL</i> | Light Green | Light Green | Dark Red | Dark Red |
| <i>INaK</i> | Dark Red | Dark Red | Dark Red | Dark Red |
| <i>INab</i> | Dark Red | Dark Red | Dark Red | Dark Red |
| <i>ICab</i> | Dark Red | Dark Red | Dark Red | Dark Red |

Figure 5. EAD development (dark red) under ionic current variations and I_{SK} block.

blocked (Figure 5). These findings are consistent with the experimental observations in [1], where EADs were frequent after apamin infusion but did not occur at baseline, and suggest that SK channels may exert an antiarrhythmic effect by compensating the low repolarization reserve in failing ventricles, as hypothesized in the literature [8].

As limitations of this work, it should be noted that the adjusted value for the conductance G_{SK} was estimated for midmyocardial cells and may not apply for epi- or endocardial cells, as transmural differences in I_{SK} have been reported experimentally [8]. Also, while the proposed parameter values in the I_{SK} formulation reproduce the APD prolongation in failing myocytes, it is not applicable to non-failing myocytes. Indeed, results in Figure 4 show that including exactly the same I_{SK} formulation in the non-failing model would lead to APD prolongation under SK channel block, which would disagree with the understanding that SK channels do not contribute to AP repolarization in ventricular cells under physiological conditions [13].

Acknowledgments

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References

[1] Bonilla IM, Long VP, Vargas-Pinto P, Wright P, Belevych A, Lou Q, Mowrey K, Yoo J, Binkley PF, Fedorov VV, Györke S, Janssen PM, Kilic A, Mohler PJ, Carnes CA. Calcium-activated potassium current modulates ventricular repolarization in chronic heart failure. *PLoS ONE* 2014; 9(10):1–11.

[2] Gu M, Zhu Y, Yin X, Zhang DM. Small-conductance Ca^{2+} -activated K^{+} channels: insights into their roles in cardio-

vascular disease. *Experimental molecular medicine* 2018; 50(4):1–7.

[3] Darkow E, Nguyen TT, Stolina M, Kari FA, Schmidt C, Wiedmann F, Baczkó I, Kohl P, Rajamani S, Ravens U, Peyronnet R. Small Conductance Ca^{2+} -Activated K^{+} (SK) Channel mRNA Expression in Human Atrial and Ventricular Tissue: Comparison Between Donor, Atrial Fibrillation and Heart Failure Tissue. *Frontiers in Physiology* 2021; 12(April):1–16.

[4] O’Hara T, Virág L, Varró A, Rudy Y. Simulation of the undiseased human cardiac ventricular action potential: Model formulation and experimental validation. *PLoS Computational Biology* 2011;7(5). ISSN 1553734X.

[5] ten Tusscher KHWJ, Noble D, Noble PJ, Panfilov aV. A model for human ventricular tissue. *American journal of physiology Heart and circulatory physiology* 2004; 286(4):H1573–H1589.

[6] Gomez JF, Cardona K, Romero L, Ferrero JM, Trenor B. Electrophysiological and structural remodeling in heart failure modulate arrhythmogenesis. 1D simulation study. *PLoS ONE* 2014;9(9).

[7] Landaw J, Zhang Z, Song Z, Liu MB, Olcese R, Chen PS, Weiss JN, Qu Z. Small-conductance Ca^{2+} -activated K^{+} channels promote J-wave syndrome and phase 2 reentry. *Heart Rhythm* 2020;17(9):1582–1590.

[8] Chang PC, Turker I, Lopshire JC, Masroor S, Nguyen BL, Tao W, Rubart M, Chen PS, Chen Z, Ai T. Heterogeneous upregulation of apamin-sensitive potassium currents in failing human ventricles. *Journal of the American Heart Association* 2013;2(1):1–9.

[9] Carro J, Pueyo E, Rodríguez Matas JF. A response surface optimization approach to adjust ionic current conductances of cardiac electrophysiological models. application to the study of potassium level changes. *PloS one* 2018; 13(10):e0204411.

[10] Monasterio V, Castro-Mur J, Carro J. DeniS: Solving cardiac electrophysiological simulations with volunteer computing. *PLoS ONE* 2018;13(10).

[11] Benton DCH, Garbarg M, Moss GWJ. The Relationship between Functional Inhibition and Binding for KCa_2 Channel Blockers. *PLoS ONE* 2013;8(9).

[12] Bronk P, Kim TY, Polina I, Hamilton S, Terentyeva R, Roder K, Koren G, Terentyev D, Choi BR. Impact of isk voltage and Ca^{2+}/Mg^{2+} -dependent rectification on cardiac repolarization. *Biophysical journal* 2020;119(3):690–704.

[13] Nagy N, Szűts V, Horváth Z, Seprényi G, Farkas AS, Acsai K, Prorok J, Bitay M, Kun A, Pataricza J, et al. Does small-conductance calcium-activated potassium channel contribute to cardiac repolarization? *Journal of molecular and cellular cardiology* 2009;47(5):656–663.

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