Effects of the Small Conductance Calcium-Activated Potassium Current (I_{SK}) in Human Sinoatrial Node

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Abstract

The description of the underlying phenomena that modulate the heart rate is crucial to better understand arrhythmias. Computational models are powerful tools to investigate the contribution of ion currents to the changes of membrane potential. Among them, the calcium-activated small conductance K^+ current (I_{SK}) is able to modulate the action potential (AP) duration and rate.

The aim of this work was to assess how the inclusion of I_{SK} affected the AP and calcium transient features of the human sinoatrial node model we recently developed.

The formulation of I_{SK} was adopted according to Kennedy et al. and a sensitivity analysis on g_{SK} (g_{SK} = 0, 4, 10, 41.70 μS/μF) was carried out.

The main effects of I_{SK} were an overall reduction of cycle length (CL) (from 814 ms in CTRL to 764, 668 and 439 ms for g_{SK} = 4, 10, 41.70 μS/μF, respectively) due to a decrease of the AP duration at 90% of repolarization (APD_{90}) (from 161 ms in CTRL to 155.0, 143.0 and 96.0 ms) and an increase of the diastolic depolarization rate in the first 100 ms (DDR_{100}) (from 48.1 mV/s to 52.9, 60.6 and 87.2 mV/s).

The reduction of CL due to the shortening of APD_{90} was predictable, since I_{SK} is an outward current. The increase of DDR_{100} led to the shortening of the DD phase. This was an unexpected effect of the inclusion of I_{SK}: the latter reduced the contribution of the rapid delayed rectifier K^+ current (I_{Kr}), which compensated and even overcame the outward contribution of I_{SK}.

1. Introduction

The study of the mechanism underlying the automaticity of the specialized cardiac tissue is crucial to understand how the heart rate is modulated; in particular, the sinoatrial node (SAN) plays a key role in physiological and pathological conditions.

The mathematical description of the cardiac action potential (AP) is a powerful tool to separately assess contributions of ion currents and to provide insights about cell behaviour.

We recently developed a single cell AP model of human SAN [1] able to mimic the experimental data in physiological conditions, to reproduce the effects on heart rate due to genetic mutations affecting the ion channels and to provide insights on heart modulation due to the block of the hyperpolarization-activated 'funny current' (I_f), the sodium-calcium exchange current (I_{NaCa}) and the activity of the autonomic nervous system through acetylcholine release and the administraton of isoprenaline, the synthetic analogue of adrenaline.

The small conductance K^+ current (I_{SK}) is an outward current which has the peculiarity to be activated by intracellular calcium ($[Ca^{2+}]_i$) concentration; its calcium-dependence couples $[Ca^{2+}]_i$ variations to the membrane potential (V_m). The presence of I_{SK} has been reported through the use of several techniques (qPCR, immunostaining, patch clamp) in murine [2], guinea pig [3], rabbit [4], and also in human [5] cardiac tissue. Gene expression essays showed that I_{SK} contributes to the V_m behaviour both in specialized SAN [2] and atrio-ventricular node [6] and working cardiac tissue (atrial and ventricular).

In particular, an enhanced activity of I_{SK} led to an AP shortening, whereas I_{SK} inhibition, e.g. with apamin, a highly selective I_{SK} blocker, or in an I_{SK} knock out model, showed an AP prolongation and a decrease of SAN rate [2]. The capability to modulate the AP and the beating rate makes I_{SK} a promising target to treat cardiac arrhythmias, especially the ones characterized by abnormal increases of $[Ca^{2+}]_i$.

The aim of this work is to assess the effects of the inclusion of I_{SK} on the biomarkers that describe the AP waveform and calcium transient of human SAN cells using increasing values of I_{SK} conductance.

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2. Methods

We added to the recently developed Fabbri-Severi human SAN AP model [1] the mathematical description of I_{SK} by Kennedy *et al.* [7]:

$$I_{SK} = g_{SK} x_{SK} (V_m - E_K), \quad (1)$$

where g_{SK} is the maximal conductance, x_{SK} is a time- and calcium-dependent gating variable, V_m is the membrane potential and E_K is the potassium reversal potential. The steady-state probability of the channel opening, $x_{SK,inf}$, is a function of the calcium concentration sensed by the channels in the subsarcolemmal space ($[Ca^{2+}]_{sub}$):

$$x_{SK,inf} = 0.81 \cdot \frac{[Ca^{2+}]_{sub}^n}{[Ca^{2+}]_{sub}^n + EC_{50}^n} \quad (2)$$

$$\tau_{SK} = \frac{1}{0.047 \cdot [Ca^{2+}]_{sub} + 1/76} \quad (3)$$

where EC_{50} = 0.7 µM is the half maximal effective calcium concentration, n = 2.2, τ_{SK} is in ms and $[Ca^{2+}]_{sub}$ in µM.

Several experimental studies have reported the maximal conductance of the SK channel in cardiac cells. Some reported g_{SK} values in ventricular myocytes are as high as 10 µS/µF [6,8–10], which would profoundly affect AP duration (APD). Kennedy *et al.* chose a more conservative range of g_{SK} (from 0.4 to 4 µS/µF), based on apamin (a widely recognized specific I_{SK} blocker) effects on APD (12% prolongation of APD of failing rabbit ventricular myocytes upon application of apamin [8]). More recently the amount of I_{SK} has been specifically assessed in (mouse) SAN cells by Torrente *et al.* [2]. We assessed the maximal conductance by linearly fitting the reported *I–V* relationship of the apamin-sensitive current elicited by voltage steps from a holding potential of −55 mV to a range of test potentials from −100 to +50 mV, as shown in Figure 1. Since such experiments were performed with EGTA into the pipette solution to buffer intracellular calcium, yielding an estimated free (unchelated) calcium concentration of 0.5 µM, it is possible using equations (1) and (2) to estimate g_{SK}. We obtained the extremely high value of 41.70 µS/µF. We therefore decided to explore the sensitivity of human pacemaking to I_{SK} by testing different g_{SK} values: 4, 10, 41.70 µS/µF.

The effects of I_{SK} on the AP were quantified by comparing cycle length (CL), maximum diastolic potential (MDP), AP duration at 20, 50 and 90% repolarization (APD_{20}, APD_{50} and APD_{90}), diastolic depolarization rate in the first 100 ms after MDP (DDR_{100}), diastolic ($Ca_{i,min}$) and systolic intracellular calcium concentrations ($Ca_{i,max}$) in presence and absence of I_{SK}, and the intracellular calcium transient duration at 20%, 50% and 90% of calcium decay (TD_{20}, TD_{50} and TD_{90}). Simulations were run for 100 s to drive the model to a steady state.

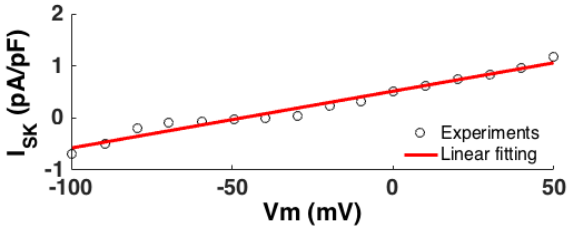

Figure 1. Linear fitting of the experimental I_{SK} data by Torrente *et al.* [2].

3. Results

The top panel of Figure 2 shows the original Fabbri-Severi AP and how it changes when I_{SK} is introduced in the model. In the bottom panel we show I_{SK}, which is an outward current, and the net membrane current (I_{net}) to compare the currents' magnitude.

In Table 1 we report the biomarkers computed on the four APs reported in Figure 2.

I_{SK} reached its maximum value during the AP (11, 30 and 116 pA corresponding to g_{SK} values of 4, 10 and 41.70 µS/µF, respectively). At all the tested g_{SK} values, its presence resulted in a CL shortening (up to -46% at g_{SK} = 41.70 µS/µF, see Table 1). Such CL shortening consistently affected the APD_{90}, which significantly shortened (up to -40% at g_{SK} = 41.70 µS/µF), MDP was virtually unchanged at 4 and 10 µS/µF, while g_{SK} = 41.70 µS/µF hyperpolarized MDP by 5%. DDR_{100} showed an appreciable increment at all the tested conductances, up to +81%. $Ca_{i,min}$ and $Ca_{i,max}$ were shifted towards higher values over +50% at g_{SK} = 41.70 µS/µF.

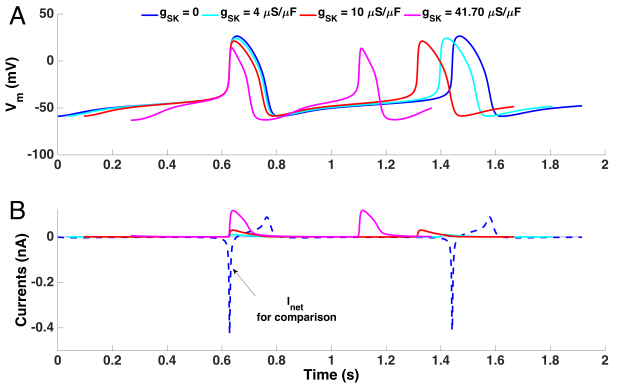

Figure 2. Comparison between the Fabbri-Severi SAN APs without (blue) and with I_{SK} computed with different g_{SK} values: 4 (cyan), 10 (red) and 41.70 (magenta) µS/µF (top panel). Simulated I_{SK} (blue, cyan, red and magenta solid lines) and I_{net} (blue dashed line) for magnitude comparison (bottom panel).

Table 1. Biomarkers computed on the Fabbri-Severi APs without and with I_{SK}. For each biomarker, we report its absolute value (in bold) corresponding to a specific g_{SK} and its percent variation with respect to the case $g_{SK} = 0$.

Biomarker	Units	g_{SK} = 0 µS/µF	g_{SK} = 4 µS/µF	g_{SK} = 10 µS/µF	g_{SK} = 41.70 µS/µF
APA	**mV**	**85.3**	**82.7**	**79.1**	**73.6**
ΔAPA			-3.0%	-7.3%	-13.7%
MDP	**mV**	**-58.9**	**-58.7**	**-58.5**	**-62.0**
ΔMDP			+0.4%	+0.7%	+5.4%
CL	**ms**	**813**	**764**	**668**	**439**
ΔCL			-6.0%	-17.8%	-46.1%
dV/dt max	**V/s**	**7.3**	**7.3**	**7.1**	**6.7**
ΔdV/dt max			-0.2%	-2.3%	-7.5%
APD_{20}	**ms**	**98.0**	**92.0**	**82.0**	**50.5**
ΔAPD_{20}			-6.1%	-16.3%	-48.5%
APD_{50}	**ms**	**135.5**	**129.0**	**119.0**	**76.0**
ΔAPD_{50}			-4.8%	-12.2%	-43.9%
APD_{90}	**ms**	**161.0**	**155.0**	**143.0**	**96.0**
ΔAPD_{90}			-3.7%	-11.2%	-40.4%
DDR_{100}	**mV/s**	**48.1**	**52.9**	**60.6**	**87.2**
ΔDDR_{100}			+10.0%	+25.9%	+81.1%
$Ca_{i,min}$	**nM**	**83.8**	**88.3**	**98.4**	**128.4**
$\Delta Ca_{i,min}$			+5.4%	+17.4%	+53.2%
$Ca_{i,max}$	**nM**	**189.2**	**198.3**	**218.0**	**291.8**
$\Delta Ca_{i,max}$			+4.8%	+15.3%	+54.3%
TD_{20}	**ms**	**136.7**	**130.0**	**117.0**	**69.0**
ΔTD_{20}			-4.9%	-14.4%	-49.5%
TD_{50}	**ms**	**206.3**	**194.0**	**170.0**	**97.3**
ΔTD_{50}			-6.0%	-17.6%	-52.8%
TD_{90}	**ms**	**552.3**	**518.0**	**451.5**	**276.0**
ΔTD_{90}			-6.2%	-18.3%	-50.0%

4. Discussion and conclusions

We have assessed the effect of I_{SK} in our computational model of human SAN AP. The main result is that depending on the amount of SK channels that are considered in the SAN cell, hence on the maximal conductance of the I_{SK} current, the effect on SAN electrical activity, in particular on pacemaking rate, can be substantial.

Up to now the presence of I_{SK} and its contribution to SAN pacemaker activity has been investigated only by Torrente *et al.* [2] , who concluded that SK channels have demonstrable effects in the mouse. However, the translation of mouse data to humans is not straightforward for SAN pacemaker cells. In mice, the heart rate is dramatically higher, due to higher diastolic depolarization slope, which in turn is due to higher inward net current during that phase. In line with this observation, it has been observed that several ionic currents (both inward and outward) are smaller in human than in rabbit SAN. It is therefore likely that also I_{SK} maximal conductance is smaller in humans with respect to the experimental data reported in mouse.

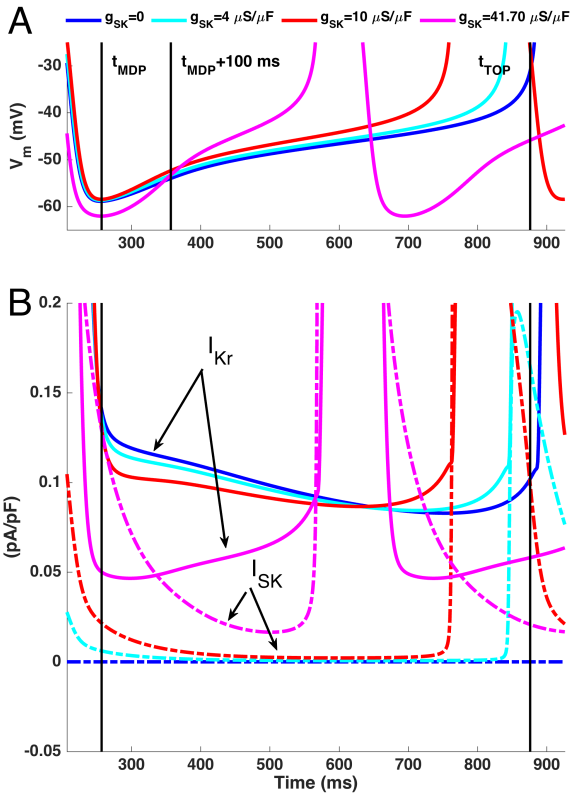

Figure 3. Effects of the different expressions of I_{SK} on the diastolic depolarization. Increasing levels of I_{SK} remarkably increased DDR_{100} (top panel). The increasing contribution of I_{SK} during the diastolic depolarization is partially compensated by a reduction of I_{Kr} contribution (bottom panel).

Whilst the actual size (= maximal conductance value) of the I_{SK} current in human SAN is still an open issue, it has been possible, through simulations, to make a sort of sensitivity analysis of the pacemaking to different levels of the current. Results confirm that it is critical to determine the real amount of I_{SK} maximal conductance in human SAN in order to quantitatively evaluate its eventual contribution to pacemaking.

In any case, simulation results suggest that even a quite small amount of I_{SK} leads to small but not negligible changes in the main biomarkers. As a relevant example, CL was reduced by 6%, down to 764 ms, which is out of the experimental range (828 ± 21 ms, mean ± SD) reported by Verkerk *et al.* [9] in human SAN cells. We observed that the model itself tends to compensate for the introduction of I_{SK}. In Figure 3B we showed how the I_{Kr}

outward contribution decreases together with the increasing expression of I_{SK}. During the AP, V_m reaches lower peak values due to the outward contribution of I_{SK} that leads to a reduced contribution of I_{Kr}, not predictable *a priori*. The reduced outward current compensates and even overcomes the effect of the inclusion of I_{SK}. Therefore, the inclusion of I_{SK} into the model should be compensated by changes in other ionic currents in order to recover proper overall behaviour of the model.

Further developments will include other calcium-dependent potassium currents such as BK channels and will analyze the impact of these currents in conditions of enhanced intracellular calcium concentrations, like beta-adrenergic stimulation, high rates, or pathological conditions.

Acknowledgements

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