Calcium-Activated Potassium Channels Inhibition in Autonomically Stimulated Human Atrial Myocytes

Chiara Celotto[1,3], Carlos Sánchez[1,2], Pablo Laguna[1,3], Esther Pueyo[1,3]

[1] Aragon Institute of Engineering Research, Univ. Zaragoza, Spain
[2] Defence University Centre, General Military Academy, Zaragoza, Spain
[3] CIBER in Bioengineering, Biomaterials and Nanomedicine, Zaragoza, Spain

Abstract

The autonomic nervous system has been reported to play a major role in the generation and maintenance of atrial fibrillation. Various investigations have suggested small-conductance calcium-activated potassium (SK) channels as potential targets for more effective pharmacological therapies. In this study, we used in silico modeling and simulation to investigate the effects of SK channel inhibition on the action potential (AP) of autonomically stimulated human atrial cardiomyocytes. The Grandi AP model, with a new formulation for the I_{SK} current, was used to represent human atrial electrophysiology. Cholinergic stimulation by different concentrations of acetylcholine (ACh) hyperpolarized the AP and shortened the AP duration (APD) in a dose-dependent manner, with up to 7 mV resting membrane potential elevation and >200 ms APD shortening for 1 μM ACh at 1 Hz pacing frequency. Additional β-adrenergic stimulation by 1 μM Isoproterenol (Iso) partially attenuated the effects of cholinergic stimulation by prolonging the APD by 41.6%. I_{SK} inhibition was able to reverse the effects of cholinergic activation, but only for moderate ACh doses and when combined with 1 μM Iso, leading to 58.3% prolongation of the AP stimulated with 0.01 μM ACh. In conclusion, I_{SK} inhibition combined with β-adrenergic stimulation can be effective in antagonizing cholinergic effects on human atrial myocytes.

1. Introduction

Atrial fibrillation (AF) is the most common sustained arrhythmia worldwide, but the efficacy of current anti-AF therapies is far from optimal. The autonomic nervous system (ANS) has been reported to play a major role in the generation and maintenance of atrial fibrillation [1]. Clinical and experimental studies have reported fluctuations in both sympathetic and parasympathetic nervous systems inducing atrial electrophysiological alterations that can potentially result in atrial tachyarrythmias and AF [2].

Acetylcholine (ACh), the parasympathetic neurotransmitter, has been shown to shorten the action potential (AP) duration (APD) of atrial cells by activating the ACh-activated potassium repolarizing current (I_{KACh}). Furthermore, ACh is rapidly broken down at its release site by acetylcholinesterase, which facilitates large regional variation in ACh concentration [3]. The shortened APD combined with the spatially heterogeneous ACh distribution increases susceptibility to AF [1].

Recently, various *in vivo* and *ex vivo* investigations have suggested inhibition of small-conductance calcium-activated potassium (SK) channels as a potential therapeutic option for AF treatment [3]. Inhibition of SK channels has anti-arrhythmic effects by prolonging atrial APD and increasing atrial effective refractory period [4]. An advantage of pharmacologically targetting SK channels is that they are preferentially expressed in the atria as compared to the ventricles [1, 5], thus they might constitute a relatively atrial-selective target. Further studies are needed to confirm the anti-arrhythmic benefits of SK channel inhibition in both short- and long-lasting AF, as AF-induced remodeling has been suggested to be associated with I_{SK} downregulation [1, 6].

In this work in silico modeling and simulation was used to investigate the effects of SK channel inhibition in adrenergically and cholinergically stimulated human atrial cardiomyocytes. We hypothesize that SK inhibition, individually or in combination with adrenergic stimulation, might act to counteract the very prominent APD shortening induced by cholinergic stimulation in the human atria.

2. Methods

2.1. Human atrial cell model with cholinergic and adrenergic effects

The Grandi AP computational model, built based on a wide set of experimental data from human atrial cardiomyocytes, was used to represent human atrial electrophysiol-

 ISSN: 2325-887X DOI: 10.22489/CinC.2019.334

ogy [7].

Parasympathetic effects were described by including I_{KACh}, which was defined as in Voigt et al., but corrected with the experimental data from Koumi et al. [7].Sympathetic stimulation of the heart activates the β-adrenergic signaling cascade by triggering PKA-dependent phosphorylation of different cellular substrates, namely ICa, IKs, IKur, PLN-SERCA2a, RyR2, troponin calcium affinity, and Na/K-ATPase. In the Grandi AP model, β-adrenergic effects were modeled similarly to the model developed by Shannon et al. [7].

2.2. Human atrial SK current

A new formulation for the I_{SK} current, based on experimental evidence [8], was introduced into the Grandi AP model. The Grandi model with the added current is considered, in this study, as the reference AP model. I_{SK} was defined as in Engel et al [8]:

$$I_{SK} = g_{SK} f^2 \left(\left[Ca^{2+}\right]_i\right)(V - E_K) \quad (1)$$

where g_{SK} is the conductance of I_{SK}, E_K is the Nernst potential for potassium and $f([Ca^{2+}]_i)$ is a gating variable that satisfies the equation:

$$\frac{df\left(\left[Ca^{2+}\right]_i\right)}{dt} = \frac{f_\infty\left(\left[Ca^{2+}\right]_i\right) - f\left(\left[Ca^{2+}\right]_i\right)}{\tau_f} \quad (2)$$

where the steady-state value of gate f is:

$$f_\infty\left(\left[Ca^{2+}\right]_i\right) = \frac{\left(\left[Ca^{2+}\right]_i / 0.0025\right)^2}{\left(1 + \left(\left[Ca^{2+}\right]_i / 0.0025\right)^2\right)} \quad (3)$$

and τ_f = 3 ms is the time constant associated with the activation gate f. Since the model by Engel et al. was built for neurons and not for cardiac cells, the conductance g_{SK} was adapted so that the contribution of the I_{SK} current was consistent with the results reported from experiments in isolated atrial myocytes and atrial trabeculae strips from patients in sinus rhythm, where SK channel block increased APD_{90} by around 20% [3].

2.3. Simulation protocols

The impact of cholinergic stimulation on AP was investigated at the following ACh concentrations: 0.001, 0,01, 0,1 and 1 μM. Physiological ACh doses have been reported to be in the range 0.01 – 0.1 μM [11]. The effects of β-adrenergic stimulation were tested on top of cholinergic effects by adding an Isoproterenol (Iso) dose of 1 μM. I_{SK} block was simulated on cholinergically stimulated myocytes as well as on cholinergically and β-adrenergically stimulated myocytes. The following scenarios were investigated:

1) ACh
2) Iso
3) I_{SK} block
4) ACh + I_{SK} block
5) ACh + Iso
6) ACh + Iso + I_{SK} block

Pacing was applied at a fixed cycle length (CL) for 5 minutes to reach steady state. To investigate frequency-dependent effects, CLs varying from 500 to 2000 ms were tested. APD_{90} was measured at 90% repolarization of the AP.

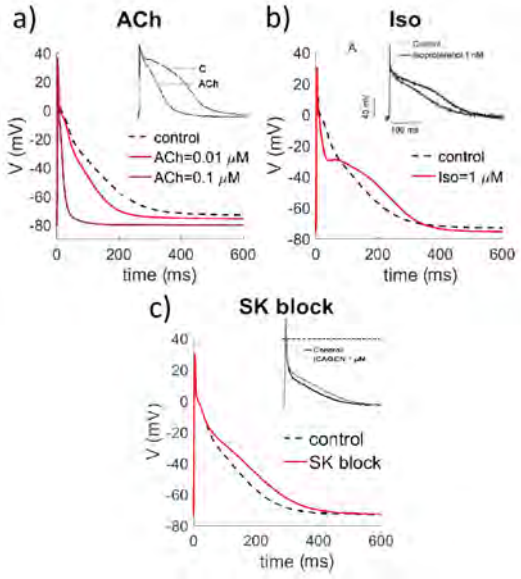

Figure 1. Simulated effects of ACh (at doses of 0.01 and 0.1 μM) (a), Iso (at a 1 μM dose) (b) and SK channel block (c) on human atrial cardiomyocytes paced at a CL of 1000 ms. Simulated results are compared with experimental data reported in [9] (a), [10] (b) and [3] (c).

3. Results

3.1. Individual effects of cholinergic, β-adrenergic and SK channel block

ACh hyperpolarized the resting membrane potential and shortened the APD_{90} in a dose-dependent manner, as can be observed in Figure 1 a), which shows steady-state APs for control as well as after application of ACh at concentrations of 0.01 and 0.1 μM at a CL of 1000 ms. These results are in agreement with reported experimental observations [9], which are shown in Figure 1 a) insets, to facilitate comparison. For the highest tested ACh dose of 1 μM, resting membrane potential was decreased by 7 mV and APD_{90} was shortened over 200 ms (results not shown in figure).

The effects of β-adrenergic stimulation by 1 μM Iso were less prominent than cholinergic effects. As shown in Figure 1 b), 1 μM Iso led to slight AP hyperpolarization and APD_{90} prolongation. Similar effects were reported in some experimental studies [10], which are shown in the inset of the same panel.

SK channel block reduced the repolarization current, leading to prolongation of AP phase 3 and thus increasing APD_{90}. This is illustrated in Figure 1 c), together with reported experimental evidence [3] to confirm the ability of the in silico model to reproduce I_{SK} inhibition action on AP.

3.2. Frequency-dependence of cholinergic, β-adrenergic and SK channel block

Under control conditions, APD_{90} showed a strong frequency dependence, with values varying from 178 ms for a CL of 500 ms to 289 ms for a CL of 2000 ms. Thus, autonomic and SK block actions were analyzed in relative terms, i.e. expressed as percentage with respect to the control case for each given CL.

The relative APD_{90} shortening caused by ACh increased with CL. For an ACh dose of 1 μM, APD_{90} was shortened by 82.7% for a CL of 500 ms up to 88.7 % for a CL of 2000 ms. For an ACh dose of 0.01 μM, the percentages of APD shortening ranged from 21.7% to 36% for CL varying from 500 to 2000 ms.

APD_{90} changes induced by application of 1 μM Iso were also frequency-dependent, with APD_{90} prolongation percentages covering from 20% for a CL of 500 ms to 33% for a CL of 2000 ms.

SK channel block caused frequency-dependent APD_{90} changes, but in this case the effect was stronger for shorter CLs. APD_{90} was prolonged by 40% for CL = 500 ms, 24% for CL = 1000 ms and 23% for CL = 2000 ms.

3.3. Combined cholinergic, β-adrenergic and SK channel block

Figure 2 presents results corresponding to cholinergic stimulation applied individually as well as in combination with β-adrenergic stimulation and/or I_{SK} block, for a fixed CL of 1000 ms. Adrenergic stimulation by 1 μM Iso partially attenuated the effects of cholinergic stimulation, but only when ACh doses were below 0.01 μM. For cholinergically stimulated cardiomyoctes with the lowest physiological ACh dose of 0.01 μM, Iso prolonged APD_{90} by recovering 42% of the lost APD value, this being in line with experimentally reported results [11]. I_{SK} block was able to partially counteract the effects of cholinergic activation but, again, only for moderate ACh doses (by recovering 21% of the lost APD value under 0.01 μM ACh), while for the upper physiological limit of 0.1 μM Ach, the

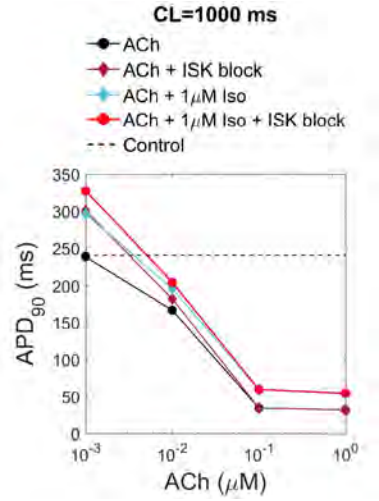

Figure 2. APD_{90} vs ACh dose for human atrial myocytes paced a CL of 1000 ms at four different tested scenarios comprising cholinergic stimulation individually and in combination with β-adrenergic stimulation and/or I_{SK} block.

effects were negligible. The combination of SK block and 1 μM Iso potentiated the individual actions for moderate cholinergic stimulation. For an ACh dose of 0.01 μM, the combined action led to a recovery as much as 58% of the lost APD, while for the upper physiological limit of 0.1 μM ACh, the effect was the same as due solely to Iso.

Figure 3 presents absolute (top panel) and relative (bottom panel) changes, expressed as % recovery of the lost APD, induced by combined cholinergic, β-adrenergic and/or I_{SK} block actions for a fixed ACh dose of 0.01 μM and CL varying from 500 to 2000 ms. Relative APD_{90} prolongation by Iso and Iso + SK block applied to of cholinergically stimulated myocytes was relatively constant upon CL variations, whereas isolated SK block led to relative APD_{90} prolongations remarkably stronger for short CLs (60% prolongation for CL = 500 ms, 13% for CL = 2000 ms).

4. Discussion and conclusion

This study has investigated the effects of SK channel inhibition on autonomically stimulated human atrial cardiomyocytes. For myocytes under moderate cholinergic stimulation, corresponding to application of a physiological ACh dose of 0.01 μM/L, I_{SK} inhibition has shown the ability to partially antagonize cholinergic effects, particularly when in combination with β-adrenergic stimulation. Those effects were more manifest for high pacing frequencies, above 1 Hz, whereas a remarkable reduction in its effectivenes was seen for frequencies around 0.5 Hz. For highly cholinergically stimulated myocytes, corresponding

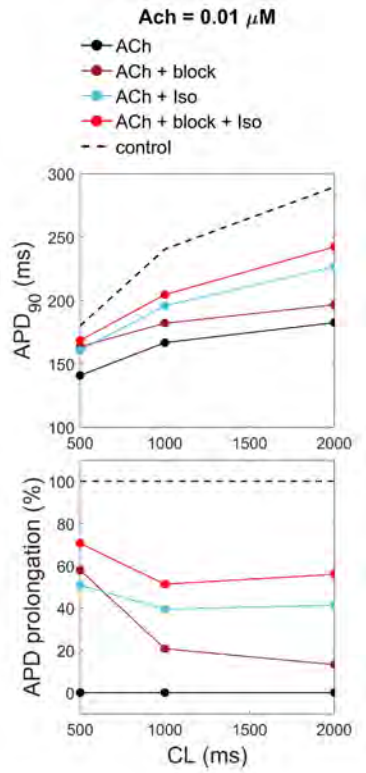

Figure 3. Top panel: represents, for an ACh dose of 0.01 μM, APD_{90} vs CL for the different simulated scenarios. Bottom panel: Percentage of APD_{90} prolongation recovery calculated with respect to the difference between the APD_{90} of the control case (100%) and ACh only (zero reference) vs the CL for the different simulated scenarios.

to application of a physiological ACh dose of 0.1 μM/L, the effects of SK inhibition were negligible. Thus, the results from this study do not support SK channel block as an option always valid to counteract the effects of cholinergic stimulation, although they confirm the ability of SK channel block to potentiate the effects of β-adrenergic stimulation, particularly for low frequencies where the isolated effect of the SK block was weaker.

Further studies, including tissue simulations, will help to shed more light and elucidate the mechanisms underlying the experimentally reported beneficial effects of SK channel block in human atria, particularly following cholinergic and/or β-adrenergic stimulation. Of note, this study focused on patients in sinus rhythm, where the SK current has been reported to be more highly expressed than in AF remodelling [6]. Subsequent investigations could expand this research to assess SK channel inhibition effects on remodeled atria from chronic AF patients.

References

[1] P. S. Chen, L. S. Chen, M. C. Fishbein "Role of the Autonomic Nervous System in Atrial Fibrillation: Pathophysiology and Therapy," *Circ. Res.*, vol. 114, pp. 1500-1515, 2014.

[2] P. Coumel "Autonomic Influences in Atrial Tachyarrhythmias," *J Cardiovasc Electrophysiol*, vol. 7, pp. 999-1007, Oct. 1996.

[3] L. Skibsbye, C. Poulet, J.G. Dines, B.H. Bentzen, L. Yuan, U. Kappert, K. Matschke, E. Wettwer, U. Ravens, M. Grunnet, T. Christ, T. Jespersen, "Small Conductance Calcium Activated Potassium (SK) Channels Contribute to Action Potential Repolarisation in Human Atria," *Cardiovasc Res*, vol. 103, pp. 156–167, May. 2014.

[4] L. Skibsbye, J. G. Diness, U. S. Sorensen, R. S. Hansen, M. Grunnet " The Duration of Pacing Induced Atrial Fibrillation is Reduced In Vivo by Inhibition of Small Conductance Ca2+ Activated K+ Channels," *J Cardiovasc Pharmacol*, vol. 57, pp. 672–681, 2011.

[5] S. Nattel" Calcium-Activated Potassium Current: a Novel Ion Channel Candidate in Atrial Fibrillation," *J Physiol*, vol. 587, no. 7, pp. 1385–1386, 2009.

[6] N. Ozgen, W. Dun, E. A. Sosunov, E. P. Anyukhovsky, M. Hirose, H. S. Duffy, P. A. Boyden, M. R. Rosen"Early Electrical Remodeling in Rabbit Pulmonary Vein Results from Trafficking of Intracellular SK2 Channels to Membrane Sites," *Cardiovasc Res*, vol.75, no. 4, pp. 758–769, Sept. 2007.

[7] E.Grandi, S.V.Pandit, N.Voigt, A.J.Workman, D.Dobrev, J.Jalife,D. M. Bers "Human Atrial Action Potential and Ca2+ Model: Sinus Rhythm and Chronic Atrial Fibrillation," *Circ Res.*, vol.109, no. 9, pp. 1055-1066, xx. 2011.

[8] J. Engel, H.A.Schultens, D.Schild "Small Conductance Potassium Channels cause an Activity-Dependent Spike Frequency Adaptation and make the Transfer Function of Neurons Logarithmic," *Biophysical journal*, vol.76, no. 3, pp. 1310-1319, Oct. 1999.

[9] E. A. Sosunov, E. P. Anyukhovsky, R. R. Rosen "Adrenergic-Cholinergic Interaction that Modulates Repolarization in the Atrium is Altered with Aging," *J Cardiovasc Electrophysiol*, vol.13, no. 4, pp. 374-379, Apr. 2002.

[10] M. Gonzales de la Fuente, A. Barana, R. Gomez, I. Amoro, P. Dolz-Gaito, S. Sacrista, F. Atienza, A. Pita, A. Pinto, F. Fernandez-Avile, R. Caballero, J. Tamargo, E. Delpo "Chronic atrial fibrillation up-regulates β1-Adrenoceptors affecting repolarizing currents and action potential duration," *Cardiovasc. Res.*, vol.97, pp. 379–388, Oct. 2012.

[11] Y. Song, J. C. Shryock, L. Belardinelli "Potentiating Effect of Acetyichoiine on Stimulation by Isoproterenol of L-Type Ca2+ Current and Arrhythmogenic Triggered Activity in Guinea Pig Ventricular Myocytes," *J Cardiovasc Electrophysiol*, vol.9, pp. 718-726, Jul. 1999.

Address for correspondence:

Chiara Celotto
Universidad de Zaragoza, Campus Río Ebro, Edif. I+D, D.5.1.01, C/ Poeta Mariano Esquillor, s/n, 50.018 Zaragoza
chiaracelotto@posta.unizar.es