SK Channel Block and Adrenergic Stimulation Counteract Acetylcholine-Induced Arrhythmogenic Effects in Human Atria

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Abstract— **There is increasing evidence on the role of the autonomic nervous system in the pathogenesis of atrial fibrillation. Interventions targeting autonomic modulation of atrial electrical activity have been shown to reduce the incidence of atrial arrhythmias. Additionally, recent investigations have proved that pharmacological therapies inhibiting small-conductance calcium-activated potassium (SK) channels are able to lessen cholinergic effects in the atria.**

In this study we use computational modeling and simulation to test individual and combined effects of SK channel block and adrenergic stimulation in counteracting detrimental effects induced by the parasympathetic neurotransmitter acetylcholine (ACh) on human atrial electrophysiology. Cell and tissue models are built that incorporate descriptions of SK channels as well as of isoproterenol (Iso)- and ACh-mediated regulation of the atrial action potential (AP). Three different cellular AP models, representing a range of physiological AP shapes, are considered and both homogeneous and heterogeneous ACh distributions in atrial tissue are simulated.

At the cellular level, SK channel block is demonstrated to partially revert shortening of AP duration (APD) mediated by ACh at various doses, whereas 1 μM Iso has a variable response depending on the AP shape. The combination of SK block and Iso is in all cases able to take APD back to baseline levels, recovering between 82% and 120% of the APD shortening induced by 0.1 μM ACh. At the tissue level, SK block and Iso alone or in combination do not exert remarkable effects on conduction velocity, but the combination of the two is able to notably prolong the ACh-mediated APD shortening, thus increasing the wavelength for reentry.

In conclusion, the results from this study support the combination of SK channel block and adrenergic stimulation as a potential option to counteract parasympathetically-mediated proarrhythmic effects in the human atria.

I. INTRODUCTION

Atrial fibrillation (AF) is the most common sustained arrhythmia worldwide. Even if AF is not usually life-threatening, it increases 3- to 5-fold the risk for stroke and can eventually lead to heart failure. There is increasing evidence that the autonomic nervous system (ANS) plays a relevant role in the pathogenesis of AF [1]. The parasympathetic neurotransmitter acetylcholine (ACh) activates the outward potassium current I_{KACh}, which shortens the action potential (AP) duration (APD) and hyperpolarizes the resting membrane potential (RMP). These two effects contribute to a reduction in the wavelength (WL), defined as the distance traveled by the depolarization wave during the effective refractory period (ERP), which can in turn facilitate reentrant activity and enhance the susceptibility to the initiation and maintenance of AF. Specifically, since WL is computed as the product of the conduction velocity (CV) and the ERP, ACh release can reduce WL not only by shortening APD but also by diminishing CV. In fact, hyperpolarization of the RMP induced by ACh leads to a reduction in the maximum upstroke velocity (UV) of the AP [2], [3] and this can slow CV and tissue excitability. Importantly, since ACh is rapidly broken down at its release site by acetylcholinesterase, its effects are largely spatially heterogeneous [4], thus additionally contributing to increased AF vulnerability [1].

Based on the important role of the ANS, and particularly of its parasympathetic branch, in modulating atrial electrical activity, therapies targeting the ANS have been explored and shown to reduce the incidence of atrial arrhythmias [1]. Such therapies could be considered individually or in combination with other pharmacological therapies. Although significant advances in AF management have been made during the last decades, important limitations of pharmacological treatments in restoring sinus rhythm have been acknowledged. Recently, *in vivo* and *ex vivo* investigations have shown that inhibition of small-conductance calcium-activated potassium (SK) channels is able to counteract parasympathetically-induced effects in the atria by prolonging APD and ERP [4], [5]. Since SK channels are preferentially expressed in the atria as compared to the ventricles [1], [6], these channels could represent a relatively atrial-selective target.

In this study the ability of different interventions, involving adrenergic stimulation and SK channel inhibition, to restore ACh-induced alterations in atrial electrical activity is tested. We hypothesize that complete SK block combined with administration of the β-adrenergic agonist isoproterenol (Iso) can prolong the APD shortened by ACh and can bring the AP back to its baseline state. To test this hypothesis, *in silico* human atrial cell and tissue models incorporating adrenergic and cholinergic AP modulation are developed. Simulations are run considering both homogeneous and heterogeneous ACh distributions in tissue. This investigation builds on a previous study where adrenergic, cholinergic and SK channel block effects on human atrial myocytes were computationally

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assessed and compared with experimental evidence [15].

II. METHODS

A. Human atrial cell models

The Courtemanche [7] and the Skibsbye [8] human atrial computational AP models were used. For the Courtemanche model, two variants representing two different AP shapes were considered [7]. The default Courtemanche model, denoted by Courtemanche S, presents a spike and dome shape, whereas the other variant obtained by decreasing the maximal conductance of I_{to} by 70%, denoted by Courtemanche R, presents a rectangular shape.

In both Courtemanche R and S models, parasympathetic effects were described by including an acetylcholine-activated potassium current, I_{KACh}, as defined in [9] with the updates proposed in [10]. In the Skibsbye model, I_{KACh} was described as proposed in [11].

In all AP models, sympathetic effects were modeled by considering the activation of the β-adrenergic signaling cascade by Iso through protein kinase A-dependent phosphorylation of cellular substrates, as proposed in [12]. Specifically, the maximum conductances of the L-type calcium current (g_{CaL}) and of the slow delayed rectifier potassium current (g_{Ks}) were increased by 250% and 58%, respectively, and the maximum conductance of the transient outward current (g_{to}) was reduced by 38% to simulate the effects of 1 μM Iso.

The effect of blocking SK channels was additionally tested. The Skibsbye model already included a description for the I_{SK} current [8]. Since Courtemanche S and R models did not include the I_{SK} current, it was introduced by following the formulation developed by Engel et al [13] for neurons, with its conductance g_{SK} updated to match the experimentally observed 20% increase in APD after I_{SK} block in atrial myocytes from sinus rhythm patients [4].

B. Human atrial tissue models

Square tissues of 5x5 cm with a diffusion coefficient of 0.0055 cm^2/ms and a transversal-to-longitudinal diffusion coefficient ratio of 0.5 were considered. Since combined SK block and Iso rendered qualitatively similar results in the three analyzed AP models, results in tissue are only presented for the Courtemanche S model. Both a homogeneous spatial ACh distribution and a heterogeneous ACh distribution with circular patches of 1 cm radius were tested.

C. Simulation protocols

Cell and tissue simulations were run with a temporal resolution of 0.005 ms. For tissue simulations, a space step of 0.02 cm was considered.

ACh concentrations of 0, 0.001, 0.01, 0.1 and 1 μM were tested to cover values below and above the reported physiological range of 0.01–0.1 μM [14]. In tissue, only the maximal physiological dose of 0.1 μM was investigated.

Individual and combined effects of SK channel block and Iso were tested on top of ACh. In tissue, SK block and Iso were considered as uniformly applied all over the tissue, independently of whether ACh was homogeneously or heterogeneously distributed.

To reach steady state, single cells were paced at a fixed cycle length (CL) of 1000 ms for 1 minute. The values of the models' state variables at steady-state were used to initialize tissue simulations. APD_{90} was measured at 90% AP repolarization. Activation time (AT) was computed as the time instant associated with maximum UV during phase zero of each AP, while total activation time (TAT) was computed as the time lapse between the first and last activation across the tissue.

Cellular simulations were performed using MATLAB while tissue simulations were performed with ELECTRA, an in-house software implementing the Finite Element and Meshfree Mixed Collocation methods for the solution of the monodomain model. In this work we used the Finite Element implementation.

III. RESULTS

A. Parasympathetic, sympathetic and SK channel block effects in single cells

ACh slowed down the UV of the AP and shortened the APD in a dose dependant manner, as shown in Fig. 1 and Fig. 2. In particular, 0.1 μM ACh reduced the UV by 5.2% in the Courtemanche S and R models and by 5.0% in the Skibsbye model. Additionally, 0.1 μM ACh shortened the APD_{90} by 36.2% in Courtemanche S, by 25.01% in Courtemanche R and by 41.7% in Skibsbye models.

Sympathetic stimulation by 1 μM Iso was highly dependent on the AP shape. For Courtemanche models, Iso shortened APD_{90} by 25.1% in the S model and by 23.9% in the R model. For the Skibsbye model, APD_{90} was slightly prolonged by Iso. This variability in the AP response to Iso is in line with reported experimental evidence, some of which point out to APD_{90} prolongation [12], whereas others report APD_{90} shortening [16].

Fig. 1 and Fig. 2 show the results for individual and combined effects of SK channel block and Iso on top of ACh. Regarding SK block, it could be observed that, for physiologically moderate ACh doses (0.01 muM), I_{SK} block was able to completely counteract the effects of ACh. For higher physiological ACh doses (0.1 μM ACh), the effects were less prominent, particularly for Skibsbye and Courtemanche S models, where SK block only prolonged APD_{90} by 28.57% and 19.48% of the APD reduction caused by ACh (Fig. 2 (c) and (a)).

Results for adrenergic stimulation on top of ACh exhibited a strong model dependence. In the case of the Skibsbye model, 1 μM Iso was able to attenuate the effects of parasympathetic stimulation, while in both Courtemanche variants Iso caused further APD_{90} shortening.

The combination of SK block and 1 μM Iso counteracted ACh effects in all cases. For the maximal physiological ACh dose of 0.1 μM, the combined action led to a recovery of baseline APD_{90}, with prolongation being 92% of the the ACh-induced APD_{90} shortening for Skibsbye, 120% for Courtemanche R and 82.6% for Courtemanche S models.

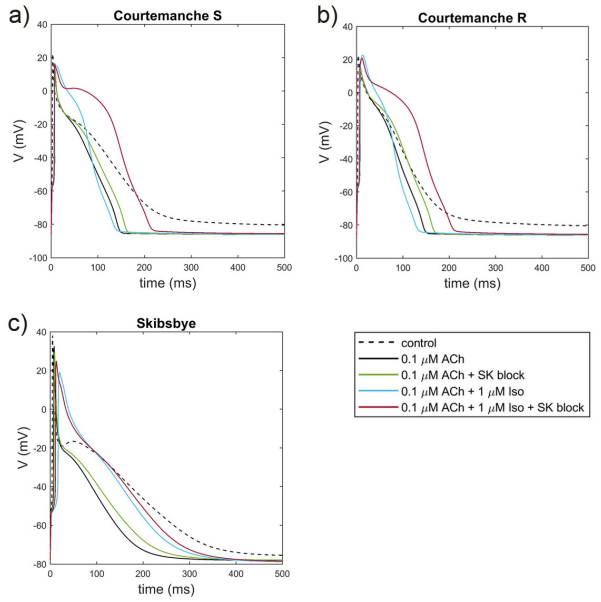

Fig. 1. Simulated APs at baseline and under 0.1 μM ACh, individually and in combination with 1 μM Iso and/or SK channel block on human atrial cardiomyocytes paced at a CL of 1000 ms, for Courtemanche S (a), Courtemanche R (b) and Skybsbye (c) AP models.

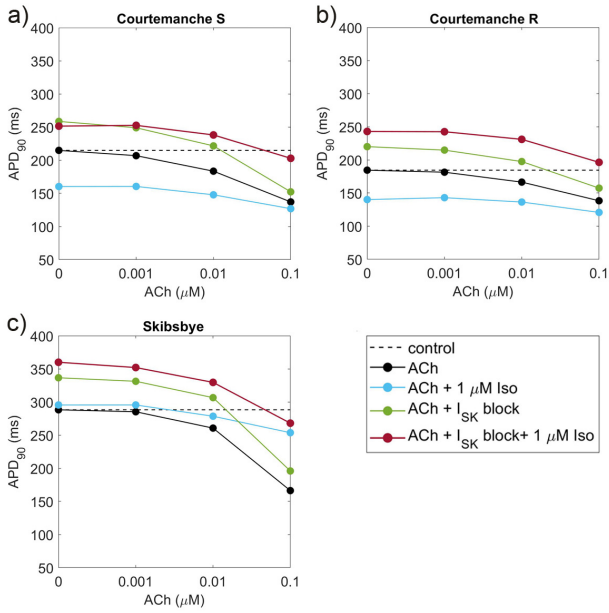

Fig. 2. APD_{90} vs ACh dose for human atrial myocytes paced at a CL of 1000 ms at baseline and under ACh, individually and in combination with 1 μM Iso and/or SK channel block, for Courtemanche S (a), Courtemanche R (b) and Skybsbye (c) AP models.

B. Parasympathetic, sympathetic and SK channel block effects in tissue

ACh concentration of 0.1 μM slowed down the propagation of the electrical wavefront in tissue, slightly increasing TAT by 4 ms for homogeneous ACh distribution and by 2 ms for heterogeneous ACh distribution. The mean longitudinal CV decreased from 95.9 cm/s to 88.9 cm/s for homogeneous ACh, which is in agreement with experimental results [10]. Interventions involving SK channel block, Iso or the combination of the two did not modify the reduced CV induced by ACh, with TAT values remaining the same as under ACh, both when ACh was considered as distributed homogeneously and heterogeneously in the tissue.

Results in terms of APD_{90} were, however, remarkably altered by all tested interventions. While mean APD_{90} across the tissue was 244.26 ms at baseline, such value was shortened by 110.74 ms when ACh was homogeneously added. SK block on top of ACh prolonged mean APD_{90} by 67.35 ms, while Iso had the opposite effect, causing further shortening by 18.22 ms. The combination of Iso and SK block was able to prolong mean APD and restore it to a value of 200.86 ms. The corresponding results for heterogeneous ACh distribution are presented in Fig. 3 (a), which shows APD_{90} maps for baseline, heterogeneous ACh as well as individual and combined SK block and Iso on top of ACh. Fig. 3 (b) summarizes median, lower and upper quartiles of APD_{90} values. As can be observed from the figures, the heterogeneous addition of ACh shortened mean APD by 58.01 ms. SK channel block on top of ACh prolonged mean APD_{90} by 33.06 ms while Iso caused further shortening by 45.64 ms. As in the homogeneous case, the combination of Iso and SK block was able to prolong the mean APD and restore it to a value of 236.6 ms, which is very close to the baseline value.

IV. DISCUSSION AND CONCLUSION

This study has investigated the effects of SK channel inhibition and adrenergic stimulation on parasympathetically stimulated human atrial cardiomyocytes and tissue. For myocytes under physiological ACh doses, I_{SK} inhibition showed the ability to antagonize parasympathetic effects, whereas the effects of Iso were highly dependent on the AP shape, in agreement with experimental results. Combined SK block and Iso were in any case able to revert ACh effects, prolonging APD_{90} to values close to baseline levels. At the tissue level, the combination of Iso and SK block did not have any remarkable effects on CV, but, similarly to the cell level, it was able to bring mean APD_{90} back to baseline values.

This study focused on patients in sinus rhythm, where the SK current has been reported to be more highly expressed than in AF remodelling [17]. Future investigations could expand this research to assess SK channel inhibition effects on remodeled atrial tissues representative of chronic AF patients. Additional characterizations on the ability of SK channel inhibition and adrenergic stimulation to revert arrhythmogenic behaviors induced by ACh could be undertaken in further studies, e.g. aimed at testing the capacity of such interventions to stop reentrant activity [18].

In conclusion, the results from this study support SK channel block, in combination with adrenergic stimulation, as an option to counteract potential arrhythmogenic effects of

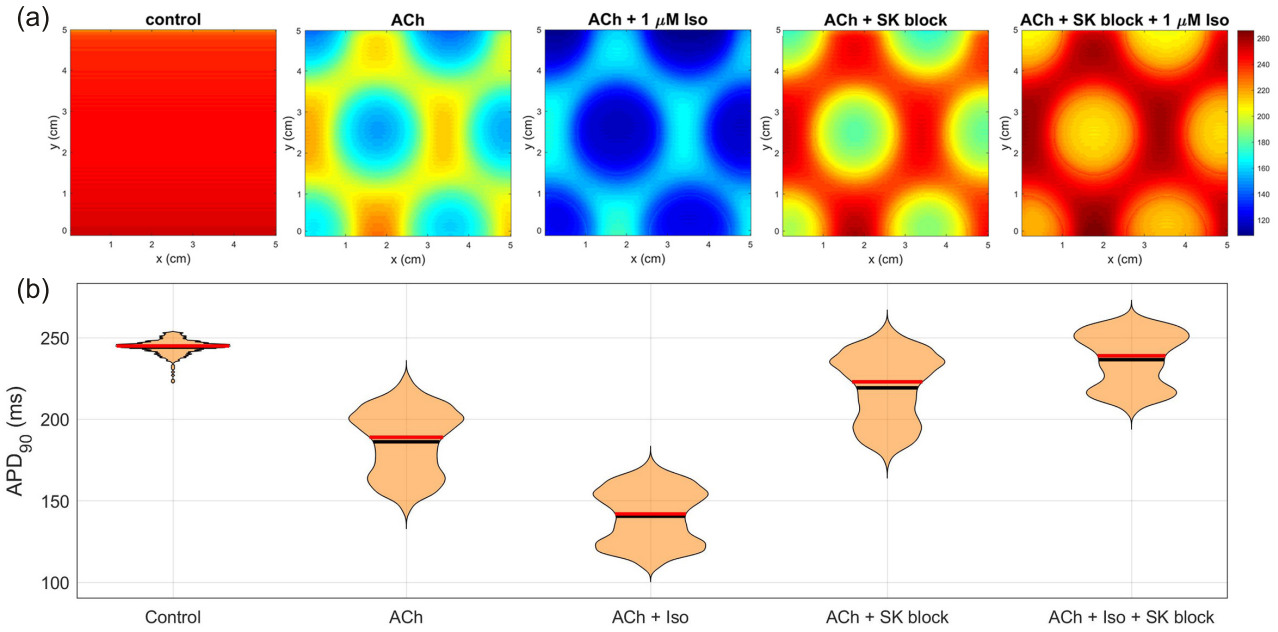

Fig. 3. APD_{90} maps (a) and violin plots (b) at baseline and under ACh, individually and in combination with 1 μM Iso and/or SK channel block, for APs defined by the Courtemanche S model. In the violin plots, black lines represent the mean APD_{90} while red ones, the median APD_{90}.

parasympathetic stimulation in human atria, even if further investigations are needed to confirm this statement.

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