Proarrhythmic effect of blocking the small conductance calcium activated potassium channel in isolated canine left atrium

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BACKGROUND Small conductance calcium activated potassium (SK_{Ca}) channels are voltage insensitive and are activated by intracellular calcium. Genome-wide association studies revealed that a variant of SK_{Ca} is associated with lone atrial fibrillation in humans. Roles of SK_{Ca} in atrial arrhythmias remain unclear.

OBJECTIVE To determine roles of SK_{Ca} in atrial arrhythmias.

METHODS Optical mapping using the isolated canine left atrium was performed. The optical action potential duration (APD) and induction of arrhythmia were evaluated before and after the addition of specific SK_{Ca} blockers—apamin or UCL-1684.

RESULTS SK_{Ca} blockade significantly increased APD_{80} (188 $\pm$ 19 ms vs 147 $\pm$ 11 ms; $P < .001$). The pacing cycle length thresholds to induce 2:2 alternans, and wave breaks were prolonged by SK_{Ca} blockade. Increased APD heterogeneity was observed after the SK_{Ca} blockade, as measured by the difference between the maximum and the minimum APD (39 $\pm$ 4 ms vs 26 $\pm$ 5 ms; $P < .05$), by standard deviation (12.43 $\pm$ 2.36 ms vs 7.49 $\pm$ 1.47 ms; $P < .001$), or by coefficient of variation (6.68% $\pm$ 0.97% vs 4.90% $\pm$ 0.84%; $P < .05$). No arrhythmia was induced at baseline by an S1-S2 protocol. After SK_{Ca} blockade, 4 of 6 atria developed arrhythmia.

CONCLUSIONS SK_{Ca} blockade promotes arrhythmia and prolongs the pacing cycle length threshold of 2:2 alternans and wave breaks in the canine left atrium. The proarrhythmic effect could be attributed to increased APD heterogeneity in the canine left atrium. This study provides supportive evidence of genome-wide association studies showing association of *KCNN3* and lone atrial fibrillation.

KEYWORDS Atrial arrhythmia; SK_{Ca}; Action potential duration; Repolarization; Optical mapping

ABBREVIATIONS **AF** = atrial fibrillation; **APD** = action potential duration; **LAA** = left atrium appendage; **PCL** = pacing cycle length; **SD** = standard deviation; **SK_{Ca}** = small conductance calcium activated potassium; **SNP** = single nucleotide polymorphism

Introduction

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia and contributes to significant morbidity and mortality.[1] Efficient pharmacological treatment for AF is currently limited, and many approaches come with considerable risk of adverse effects. Numerous factors are involved in the pathogenesis of AF.[2,3] Specifically, genetic variations in ion channels that predispose to AF have been reported.[2,3] The structural and ionic remodeling during AF further leads to the complexity of AF studies. This raises the importance of understanding the role of ion channels in the genesis of AF.

The small conductance calcium activated potassium (SK_{Ca}) channel is voltage independent and is activated by submicromolar concentrations of intracellular calcium.[4] The SK family was first cloned by Adelman and his colleagues in 1996.[5] It consists of 3 members—SK1, SK2, and SK3—and is characterized by the specific blockade by apamin.[6] Although the calcium sensitivity (K_d = 0.6–0.7 μM) and conductance (9.2–9.9 pS) are similar among 3 subtypes, the sensitivity to pharmacological modulation and tissue distribution differs.[6–9] It has been shown that SK1 and SK2 are predominantly abundant in mouse atria while SK3 expresses equally in atria and ventricles.[8] In humans, the distribution is different. SK2 and SK3 are more abundant than SK1 in atria.[9] In the heart, SK_{Ca} plays an important role in the action potential duration (APD), contributing to the cardiac repolarization current.[7,8] Despite the abundance of SK_{Ca} in the atrium, it remains unclear whether SK_{Ca} plays a role in atrial arrhythmogenesis.

Genome-wide association studies showed 2 genetic variations in *KCNN3* (gene encoding SK3) to be associated with lone AF.[10,11] One of those variations is an intronic single nucleotide polymorphism (SNP), and the other is a

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synonymous SNP. Although no amino acid is changed by these identified SNPs, it is possible that they are linked to an unidentified nonsynonymous SNP in *KCNN3* that leads to increased susceptibility to lone AF. Physiological evidence of a role for SK_{Ca} in atrial arrhythmia comes from studies on SK2.[12,13] A potential relationship between SK2 expression and atrial remodeling in AF has been shown. For example, intermittent burst pacing in rabbit hearts increases SK2 mRNA and protein, and the trafficking of SK2 to membrane is increased, which leads to increased apamin-sensitive current and shortened APD.[12] Moreover, SK2 knockout mice show prolonged APD and increased AF inducibility.[13] To extend these previous studies to large animal models, we used normal canine atria to clarify the role of SK_{Ca} in atrial arrhythmogenesis. We hypothesize that SK_{Ca} plays a protective role in the structurally normal atrium and that SK_{Ca} blockade promotes arrhythmia.

Methods

The animal protocol was approved by the Institutional Animal Care and Use Committee of the Indiana University School of Medicine and the Methodist Research Institute, Indianapolis, IN, and it conforms to the guidelines of the American Heart Association.

Canine left atrial tissue preparation

Male mongrel dogs (weighing 25–30 kg) were used in this study ($n = 9$ for optical mapping and $n = 5$ for Western blotting). They were fully anesthetized with isoflurane and were euthanized to obtain hearts by thoracotomy. The atrial tissue preparation was performed as previously described but with some modification.[14] Briefly, the heart was harvested and perfused with cardioplegic solution. The cardioplegic solution was composed of 129 mM NaCl, 12 mM KCl, 0.9 mM NaH_2PO_4, 20 mM $NaHCO_3$, 1.8 mM $CaCl_2$, 0.5 mM $MgSO_4$, and 5.5 mM glucose. The left coronary artery was then cannulated through its aortic orifice. Subsequently, the right atria and the ventricles were surgically removed. All open vessels on the border of the remaining left atrial tissue were ligated. Afterward, oxygenated Tyrode's solution was perfused through the left circumflex. The compositions of Tyrode's solution was as follows: 125 mM NaCl, 4.5 mM KCl, 0.5 mM $MgCl_2$, 24 mM $NaHCO_3$, 1.8 mM NaH_2PO_4, 1.8 mM $CaCl_2$, 5.5 mM glucose, and 2% bovine serum albumin, equilibrated with 95% O_2 and 5% CO_2 to maintain a pH of 7.4.

Optical mapping system

The perfusate was maintained at 37°C with a flow rate around 30 mL/min. After stabilizing the preparation, 10 μM of the voltage-sensitive dye RH237 was added to the perfusate along with 15 μM of the excitation-contraction uncoupler blebbistatin. A laser light at 532 nm was used to excite the stained atrial preparation, and fluorescence was collected with a CMOS camera (BrainVision, Tokyo, Japan). It recorded voltage fluorescence through a 710-nm long-pass filter. Fluorescence image was captured at a frame rate of 2 ms/frame and at 100 × 100 pixels with a spatial resolution of 0.35 × 0.35 mm^2/pixel for 4 seconds. To determine the rhythm of the atrium, a pseudo-electrocardiogram was used. It was obtained with a widely spaced bipolar electrode. The signals were filtered from 0.05 to 100 Hz and digitized at 1 kHz with AxoScope software (Molecular Devices, Sunnyvale, CA).

Pacing protocol and induction of atrial arrhythmia

A bipolar pacing lead was placed at the apex of the appendage, with an output at 2.5 times the diastolic threshold. Optical recording was performed after 40 beats of stable pacing at each pacing cycle length (PCL). The PCL was progressively shortened from 1500 ms (1500, 1000, 800, 500, 450, 400, 350, 300, 280, 250, 220, 200, 190, 180, 170, 160 ms, etc) until loss of capture. After 5-minute recovery, arrhythmia inducibility was evaluated by an S1-S2 protocol, consisting of continuous 9 S1 beat stimuli followed by an additional S2 beat. An episode with more than 2 extra beats that resulted from reentry was regarded as a successful arrhythmia induction. The same procedure was repeated after 30 minutes of perfusion with 100 nM of apamin (Tocris Bioscience, Minneapolis, MN) or 100 nM of UCL-1684 (Tocris Bioscience). In this study, 5 atria were treated with apamin and 4 were treated with UCL-1684, respectively. The arrhythmia induction protocol was performed in 6 atria among the 9 atria that were optically mapped. Two atria were treated with apamin and 4 with UCL-1684, respectively.

Proteins extraction

Left atrium appendage (LAA) was cut into 8 predefined pieces and homogenized by POLYTRON in RIPA buffer containing protease inhibitors (50 mM Tris buffer [pH 8.4], 150 mM NaCl, 1% NP40, 0.5% sodium deoxycholate, 1 mM PMSF, 2 μg/mL leupeptin, 1 μg/ml pepstatin A, and 5 μg/mL aprotinin). Homogenates were incubated on ice for 30 minutes and then centrifuged at 14,000 rpm for 15 minutes. After the collection of the supernatant, the protein concentration was quantified by Bradford assay (Bio-Rad, Hercules, CA) using bovine serum albumin as the standard.

Western blotting

Protein electrophoresis was done by using a Bio-Rad mini gel system (Bio-Rad) and transferred to the polyvinylidene difluoride membrane (Millipore, Billerica, MA). The membrane was bathed in TBS with 5% milk for 1 hour and probed with anti-KCNN2 antibody (Abcam, ab83733, 1:2500) or anti-glyceraldehyde 3-phosphate dehydrogenase antibody (Pierce, MA1-22670, 1:5000) overnight. Following primary antibody incubation, the membrane was incubated with horseradish peroxidase-conjugated secondary antibodies (Sigma 1:5000) for 30 minutes. Finally, the Luminata Crescendo HRP substrate (Millipore, WBLUR100) was added to the membrane according to manufacturer's instructions.