Small conductance calcium activated K\(^+\) channel inhibitor decreases stretch induced vulnerability to atrial fibrillation

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**Abstract**

**Background:** Atrial dilation is an important risk factor for atrial fibrillation (AF) and animal studies have found that acute atrial dilation shortens the atrial effective refractory period (AERP) and increases the risk of AF. Stretch activated ion channels (SACs) and calcium channels play a role in this. The expression profile and calcium dependent activation makes the small conductance calcium activated K\(^+\) channel (K\(_{\text{Ca}}\)2.x) a candidate for coupling stretch induced increases in intracellular calcium through K\(^+\)-efflux and thereby shortening of atrial refractoriness.

**Objectives:** We hypothesized that K\(_{\text{Ca}}\)2.x channel inhibitors can prevent the stretch induced shortening of AERP and protect the heart from AF.

**Methods:** The effect of K\(_{\text{Ca}}\)2.x channel inhibitor \((N-(pyridin-2-yl)-4-(pyridin-2-yl)thiazol-2-amine (ICA) 1 \mu M)\) was investigated using the isolated perfused rabbit heart preparation. To stretch the left atrium (LA) a balloon was inserted and inflated. AERP and action potential duration (APD) were recorded before and after atrial stretch. AF was induced by burst pacing the LA at different degrees of atrial stretch.

**Results:** Stretching of the LA by increasing the balloon pressure from 0 to 20 mmHg shortened the AERP by 8.6 ± 1 ms. In comparison, the K\(_{\text{Ca}}\)2.x inhibitor ICA significantly attenuated the stretch induced shortening of AERP to 2.5 ± 1.1 ms. Total AF duration increased linearly with atrial balloon pressure. This relationship was not found in the presence of ICA. ICA lowered the incidence of AF induction and total AF duration.

**Conclusion:** The K\(_{\text{Ca}}\)2.x channel inhibitor ICA attenuates the acute stretch induced shortening of AERP and decreases stretch induced vulnerability to AF.

1. Introduction

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia in adults with a prevalence of 2–4\% [1]. Atrial dilation is an important risk factor for AF and can be both consequence and cause of AF (for review see [2]). Animal studies have found that acute atrial dilation shortens the atrial effective refractory period (AERP) and slows the electrical conduction [3–4]. Similar effects have been observed in humans [5–6]. This feedback where mechanical forces on the myocardium and eventually on the cell membrane cause changes in cardiac electrophysiology is known as mechanoelectrical feedback and stretch activated ion channels are key players in conveying the mechanosensitivity. (for review see [7–8]. A role of stretch activated ion channels in the initiation of AF and as potential novel drug targets for preventing AF has been suggested [9–11]. Ion channels that change their open probability in response to cell stretch are divided into cation-non-selective (SAC\(_{\text{NS}}\)) and K\(^+\)-selective channels (SAC\(_{\text{K}}\)). One member of the SAC\(_{\text{K}}\) is the large conductance K\(^+\) channel (K\(_{\text{Ca}}\)1.1), belonging to the family of calcium activated K\(^+\) channels. The family also includes the intermediate conductance (K\(_{\text{Ca}}\)3.1) and small conductance K\(^+\) channel (K\(_{\text{Ca}}\)2.x). In the heart, K\(_{\text{Ca}}\)2.x predominantly exert its effects on the atria. In animal models and in human tissue, pharmacological inhibitors of the K\(_{\text{Ca}}\)2.x channel prolong the atrial action potential duration (APD) and refractory period with small effects on ventricular repolarization, and

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terminate and prevent the induction of AF [12-15]. The open probability of the $\text{K}_{\text{Ca}}2.x$ channel is not affected by stretch, but solely increased by rises in intracellular calcium. The expression profile and calcium dependent activation makes the $\text{K}_{\text{Ca}}2.x$ channel a candidate ion channel for coupling stretch induced increases in intracellular calcium through SAC/Ns or L-type calcium channels to $\text{K}^+$ efflux and thereby shortening of atrial refractoriness. We hypothesized that $\text{K}_{\text{Ca}}2.x$ channel inhibitors can prevent the stretch induced shortening of AERP and protect the heart from AF. To this end we utilized the isolated rabbit heart model, which is a well-known model for studying the acute effect of atrial stretching [3], and the $\text{K}_{\text{Ca}}2$ channel inhibitor ICA [15-16].

2. Methods

All procedures were performed at the Department of Biomedical Sciences, University of Copenhagen, under licenses from the Danish Ministry of justice (licence no. 2017-15-0201-01296) and in accordance with the Danish guidelines for animal experiments according to the European Commission Directive 86/609/EEC. All animals received humane care and were housed in cages with free access to water and food at the animal house facility at room temperature (21 °C) and subjected to a 12 h light/dark cycle.

A total of 44 adult female white rabbits (Charles River, France) (2–3 kg) were included in the study. 9 experiments were excluded because of problems with instrumenting the heart, inability to maintain a constant perfusion pressure or loss of signals. The rabbits were anaesthetized with intramuscular S-ketamine-xylazine (Ketamin 35 mg/kg + xylazin 10 mg/kg I.M). When sedated, heparin (0.5 mL/kg Heparin 1000 IE/mL) and pentobarbital/lidocaine (200 mg/kg)/20 mg/kg were injected through the marginal ear vein. Following euthanasia, the heart was excised through a thoracotomy and immersed into ice-cold Krebs-Henseleit buffer. Then the aorta was cannulated with a metal cannula to a position just above the aortic valve to allow the perfusion fluid to enter through the coronary ostium. Sutures were tied around the aorta-cannula. Once cannulated, the hearts were perfused under a constant pressure of 80 mmHg and submerged in 37 °C Krebs-Henseleit buffer.
containing (mmol/L): 120 NaCl, 25 NaHCO$_3$, 4 KCl, 0.6 MgSO$_4$, 0.6 NaH$_2$PO$_4$, 2.5 CaCl$_2$, 11 glucose. The buffer was continuously gassed with a mixture of 95% oxygen (O$_2$) and 5% carbon dioxide (CO$_2$) via a sintered glass gas distributor.

The Langendorff system was connected to a signal amplifier (Hugo Sachs Elektronik-Harvard Apparatus GmbH, Germany). All data were acquired at 2 KHz using the 16-channel PowerLab system (ADInstru-
m
tments, Oxford, UK), and monitored by LabChart 8 software (ADIn-
struments). Epicardial monophasic action potential (MAP) electrodes (Hugo Sachs Elektronik-Harvard Apparatus GmbH, March-Hugstetten, Germany) were placed on the LA and on both ventricles. A pacing electrode was placed on the LA, and LV.

The heart was stabilized for 10 min before insertion of an intra-atrial balloon. A size ten (0.7 mL) latex balloon-tipped catheter coupled with a pressure transducer and microsyringe (Hugo Sachs Elektronik-Harvard Apparatus GmbH, Germany) was introduced through the pulmonary vein ostia into the left atrium and finally positioned predominantly in the left atrial appendage. This allowed for visual inspection of the location and dilation of the balloon.

The atrioventricular (AV) node was ablated by scarring the area known as the “triangle of Koch” of the right atrium using surgical scissors. During the ablation procedure, MAP recording were used to monitor the ventricular rate.

2.1. Experimental protocol

The AERP was measured by programmed electrical stimulation. The heart was paced at the left atrium for 1 min at 200 ms BCL in order to record atrial monophasic action potentials. At the end, ten S1 stimuli (2 ms) at 200 ms BCL (300 bpm) at 5 times rheobase was applied. An extra-stimuli S2 was imposed every 10th stimulus. The S1-S2 interval was increased until it induced an action potential. The longest S1-S2 interval failing to elicit an action potential was defined as the AERP. AF was induced by LA burst pacing (50 Hz). Runs of AF was defined as fast irregular atrial rhythm lasting more than 2 s. Sustained AF was defined as fast irregular atrial rhythm lasting more than 120 s. To record ventricu-
lar monophasic action potentials, the LV was paced at 200 ms BCL.

The study consisted of two separate parts. In part 1 (Effect of stretch on AERP) two experimental groups were compared (vehicle control vs ICA 1 µM). The experimental protocol consisted of four stages: Baseline,
2.2. Drugs and chemicals

N-(pyridin-2-yl)-4-(pyridin-2-yl)thiazol-2-amine (ICA) was synthesized at Syngene (India). A stock solution of 10 mM ICA in DMSO was used for the experiments and diluted in Krebs-Henseleit buffer to achieve a final concentration of 1 µM. All other chemicals were purchased from Sigma-Aldrich (Munich, Germany).

2.3. Data analysis

APD₉₀ were analyzed in LabChart 8 (AD Instruments, Ltd., Dunedin, New Zealand) using the peak analysis plugin. Statistical data analysis was done by Graphpad Prism 9 (Graph Pad Software, La Jolla, CA, USA). In Figs. 2, 3, and 9 data are presented as the mean ± standard error of the mean. Data in Figs. 5 and 6 are presented as median as they are not normally distributed, and non-parametric multiple comparison Friedman test was used to for statistical analyses. The frequency distribution of the individual AF durations was analyzed by binning the individual AF episodes in bins of 2 s. The relationship between total AF duration and atrial stretch was fitted by simple linear regression, and the slopes of the linear regression for before and after ICA were compared. Effects on AERP and APD₉₀ within each group at different time points were analyzed for statistical differences by one-way ANOVA followed by Tukey’s post hoc test corrected for multiple comparisons. Student’s t-test was used to analyze differences in changes (Δ-values) between the two treatment groups. P < 0.05 was considered statistically significant.

3. Results

3.1. KᵥCa₂.x inhibitor attenuates atrial stretch induced shortening of AERP

22 perfused rabbit hearts instrumented with a balloon to stretch the LA were used for part 1 of the study. AERP was recorded at baseline and after 30 min of perfusion with either vehicle (DMSO) or ICA (1 µM). ICA perfusion resulted in significant prolongation of AERP by 20.5 ± 2.2 ms (from 55.4 ms to 75.9 ms) and atrial APD₉₀ by 9.1 ± 2.7 ms (from 71.5 ms to 80.6 ms) as compared to the changes observed in the vehicle group (see Table 1 and Fig. 3B). ICA had no effects on ventricular repolarization, when recorded during ventricular pacing at 200 ms (Table 1 and Fig. 3D). As expected, in the vehicle group inflation of the LA balloon caused a shortening of the AERP and LA APD₉₀ by 8.6 ± 1 ms (from 59.6 ms to 51 ms) and 9.3 ± 4.1 ms (from 77.8 ms to 68.5 ms) respectively. This effect was reverted by deflating the balloon to 0 mmHg. In comparison, the KᵥCa₂.x inhibitor ICA significantly attenuated the stretch induced shortening of AERP as compared to the vehicle control group (Vehicle: from 59.6 ms to 51 ms and ICA: from 75.9 to 73.4) (Table 1 and Fig. 2).

3.2. KᵥCa₂.x inhibitor prevents induction of AF by burst pacing in the presence of atrial stretch

Dilation of the LA and AERP shortening increased the risk of burst induced AF. No spontaneous AF was detected upon stretch in our experiments, but to investigate if stretching increased the vulnerability of the heart to AF the LA was challenged with 20 times burst pace (50 Hz for 1 s) before and after stretch at 20 mmHg in the two treatment groups. At 0 mmHg 6 of 11 hearts in the control group had individual AF episodes > 2 s upon bursting, and in those 6 hearts the total AF duration had a range of 2.2–122.3 s with a median time of 15 s. During acute atrial dilation 7 of 11 hearts had individual AF episodes lasting more than 2 s upon burst, and of those 7 hearts the total AF duration of each experiment was from 24.3 s to 515.9 s with a median time of 57.8 s. In comparison, in the ICA treatment group two hearts had AF lasting more than 2 s at 0 mmHg and three during stretch. Likewise, the total AF duration of each experiment was smaller in the ICA group with AF durations ranging from 7.9 s to 8.5 s with a median of 8.2 s and from 40.5 s to 92.6 s with a median time of 47.6 s for before and after stretch respectively.

Encouraged by the data we performed additional experiments dedicated to investigate if KᵥCa₂.x channel inhibition could decrease the stretch induced vulnerability to AF. 13 isolated rabbit hearts were subjected to progressively increasing levels of atrial dilation and more forceful burst pacing protocol in order to induce sustained episodes of stretch associated AF. During perfusion with DMSO, a total of 14 episodes of sustained AF was induced in 5 of the 13 hearts (see Fig. 4). In comparison, during the ICA perfusion period, sustained AF only occurred in one rabbit (2 episodes). The median duration of the individual AF episodes was not different when comparing baseline to ICA.
perfusion (Fig. 5). However, the incidence of AF episodes and the % of bursts resulting in AF were significantly increased (Fig. 6). From the individual AF duration distribution (Fig. 7) it can be observed that increasing the balloon pressure shifts the frequency distribution towards longer AF episodes. Interestingly the presence of ICA appears to reduce the number of long lasting AF episodes. The total AF duration recorded at the different levels of atrial stretch induced by the 20 bursts (repeated 5 times) can be seen in Fig. 8. Here it can be observed that total AF duration increases linearly with atrial balloon pressure and that this is not the case in the presence of ICA, where the total AF duration is significantly reduced.

4. Discussion

Atrial dilation is an important risk factor for AF and studies have found that acute atrial dilation increases the risk of AF (for review see 2). The main finding of this study is that the K\text{Ca} channel inhibitor ICA attenuates the acute stretch induced AERP shortening and decreases stretch induced vulnerability to AF.

Similar to other animal studies [3–4] and observations in humans [5–6], we found that acute atrial dilation shortens the AERP. Both voltage- and calcium-gated channels as well as stretch activated non-selective cation channels have been implicated in the stretch induced AF mechanism [9–10,17–18]. In cardiomyocytes voltage-gated calcium channels co-localize with K\text{Ca} channels via their interaction with the cytoskeletal protein \( \alpha \)-actinin-2. This suggests that voltage-gated calcium channels regulate K\text{Ca} channels by providing a local Ca\text{\textsuperscript{2+}} microdomain [19–20]. Moreover, the voltage-dependent calcium channel inhibitor verapamil was found to prevent stretch induced AERP shortening in rabbits and humans [17–18]. Based on this, we speculated if calcium influx via stretch activated cation channels and/or via voltage-dependent calcium channels could activate K\text{Ca} channels and thereby contribute to the shortening of AERP that is observed upon acute atrial stretch. We therefore tested the hypothesis that the K\text{Ca} channel inhibition would prevent the atrial stretch induced shortening of AERP. To this end we used the K\text{Ca} channel inhibitor ICA, which is a K\text{Ca} channel inhibitor (K\text{Ca}2.2 and K\text{Ca}2.3 IC\text{50} = 0.3–0.5 \mu M) with IC\text{50} values > 20 \mu M on a panel of cardiac ion channels [15]. Similar to
previous studies [15, 21] we found that ICA increased the atrial APD and AERP, with no effects on ventricular APD. Interestingly, the \( K_{\text{Ca}2} \) channel inhibitor ICA attenuated the stretch induced shortening of atrial APD/AERP, suggesting a role for \( K_{\text{Ca}2} \) channels in this phenomenon. Acute atrial stretch has been found to increase the number of afterdepolarizations in animals [22–23]. Genetic knock down of \( K_{\text{Ca}2.2} \) in mice was found to increase the incidence of early afterdepolarizations (EAD) recorded in isolated cardiomyocytes [24]. An anti-arrhythmic importance of \( K_{\text{Ca}2} \) channel activity has therefore been put forward. Here \( K_{\text{Ca}2} \) channels are speculated to play a protective role in counteracting afterdepolarizations caused by calcium overload, because the excess calcium activates \( K_{\text{Ca}2} \) channels, which helps to clamp the membrane potential and reduce the incidence of EADs. Contrary to this, many pharmacological large and small animal studies have demonstrated that \( K_{\text{Ca}2} \) channel inhibitors and negative allosteric modulators terminate and protect the heart from AF [12–14, 25–27]. However, these studies did not address AF in the setting of stretch. In the present study we addressed if the \( K_{\text{Ca}2} \) channel inhibitor ICA decreases stretch induced vulnerability to AF. In accordance with earlier findings we observed that stretching the atrium results in increased likelihood and total duration of burst induced AF. Without stretch the atrium can rarely be burst into AF, whereas upon stretch, which lowers the AERP, AF is more readily induced [3]. We observed a positive linear correlation between stretch and the total AF duration. The increase in total AF duration was a result of higher incidence of AF and longer individual episodes of AF as the atrial stretch was increased. Moreover, the occurrence of sustained AF upon bursting also increased with the level of stretch. ICA both prolonged the AERP and limited the incidence of AF, reduced the number of long lasting AF episodes and consequently removed the positive linear correlation between stretch and total AF duration. Interestingly, apart from the reduction in long lasting AF, the major effect of ICA appeared to be a reduction of AF incidence and not the individual AF episode durations, indicating that ICA primarily decreases the vulnerability to stretch induced AF by preventing its induction. Whether the protective effects of ICA translates to the setting of acute atrial stretch in human patients is unknown, but ICA has been found to prolong APDs recorded from human atrial tissue. \( K_{\text{Ca}2} \) channel expression appears to be down-regulated in atria of chronic/permanent AF patients [15, 28–29] and upregulated in severely failing ventricles [28, 30–32], which could limit the clinical use of \( K_{\text{Ca}2} \) channel inhibitors in these patient populations. Based on preclinical observations [13, 15] and the current study, targeting paroxysmal AF in

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**Fig. 7. Frequency distribution of AF episode durations.** Relative frequencies of the AF (>2 s) episodes before (A, black) and after ICA 1 \( \mu \)M (B, red). X-axis is divided in 2 s bins, except for the last bin which includes all AF episodes longer than 10 s. n = 13. mmHg refers to LA balloon pressure. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**Fig. 8. AF duration increases with stretch.** Total AF duration recorded at the different stretch levels before (black) and after ICA 1 \( \mu \)M (red). A positive linear correlation was found for the effect of stretching the left atrium and the AF duration (insert formula) at baseline. The two linear regressions (stretch and AF duration (baseline) vs stretch and AF duration (ICA)) are significantly different. n = 13. mmHg refers to LA balloon pressure. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
non-valvular patients and valvular patients with enlarged atria could potentially be of interest. A phase II study evaluating the efficacy of a K_\text{Ca}2 channel inhibitor for cardioversion of recent onset AF is currently ongoing (Clinical Trial identifier: NCT04571385).

In conclusion, stretching of the left atrium was associated with shortening of the atrial ERP, and with increased vulnerability to AF. These effects were attenuated in the presence of K_\text{Ca}2 channel inhibitor ICA. This points to a role of K_\text{Ca}2 channels in the setting of atrial stretch.

5. Limitations

Occurrence of short runs of AF in isolated rabbit hearts does not directly translate into the clinical manifestation of AF in patients. Because AF does not readily occur in atria of rabbits under normal conditions, we defined AF as episodes > 2 s, this is much shorter than the long lasting episodes observed in patients with AF. Future studies in animals with larger atria exposed to experimental conditions mimicking human conditions with stretched atria (e.g. sleep apnea/negative airway pressure) will aid the translation to humans.

Sex differences in beta-adrenergic activation of ventricular K_\text{Ca}2 channels have been reported [33]. However, as female rabbits can be housed together, only female rabbits were used in the study, and therefore we do not know if similar effects of K_\text{Ca}2 channel inhibition would have been observed in male rabbits. The anti-arrhythmic effect of K_\text{Ca}2 channel inhibition upon atrial stretch was investigated in healthy rabbits. Whether, similar effects are observed in a setting of atrial fibrosis or atrial enlargement is unknown. Another K_\text{Ca}2 channel inhibitor has demonstrated anti-arrhythmic effects in an atrial tachycyphag pig model [13], which shows fibrosis and increased left atrial volume [34].

We used a balloon inserted in the LA to induce acute stretching of the atrium, as has been done before [35]. The pressures do therefore not correspond directly to the pressures in the left atrium, but only reflects the pressure of the balloon. Therefore we cannot make a direct comparison to the atrial pressures observed in patients under acute stretch. To this end, future preclinical studies using ligation of vena cavae and pulmonales and precisely control of the atrial pressure would be valuable. We applied unphysiologically high intra-atrial pressures (> 20 mmHg). These pressures are not clinically relevant but were used in this preclinical setting to investigate the correlation between stretch and AF.

From the conducted experiments it cannot be concluded whether K_\text{Ca}2 channels were activated by influx via voltage-dependent calcium channels and/or stretch activated cation channels. Moreover, it can be argued that the marked increase in AERP by ICA is responsible for the reduction in stretch-induced AERP shortening, and that no link to activation of K_\text{Ca}2 channels by stretch occurs. However, prolongation of AERP by K\textsuperscript+ channel inhibition does not always result in reduction in stretch-induced AERP shortening, as illustrated by the effect of glibenclamide in rabbit atria [18]. Further experiments will be done to clarify this interesting aspect.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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