Altered atrial cytosolic calcium handling contributes to the development of postoperative atrial fibrillation

Funsho E. Fakuade 1,2†, Vanessa Steckmeister 1,2†, Fitzwilliam Seibertz 1,2, Judith Gronwald 1,2, Stefanie Kestel 1,2, Julia Menzel 2,3, Julius Ryan D. Pronto 1,2, Karim Tahtah 4,5, Fereshteh Haghighi 2,6, George Kensah 2,6, Charles M. Pearman 7, Felix Wiedmann 8,9, Arco J. Teske 4, Constanze Schmidt 8,9, Katharine M. Dibb 7, Aschraf El-Essawi 2,6,10, Bernhard C. Danner 2,6, Hassina Baraki 2,6, Blanche Schwappach 2,3, Ingo Kutschka 2,6, Fleur E. Mason 1,2, and Niels Voigt 1,2 *

1 Institute of Pharmacology and Toxicology, University Medical Centre Goettingen, Robert-Koch-Straße 40, 37075 Goettingen, Germany; 2 DZHK (German Centre for Cardiovascular Research), Partner Site Goettingen, Germany; 3 Department of Molecular Biology, University Medical Centre, Humboldtallee 23, 37075 Goettingen, Germany; 4 Department of Cardiology, University Medical Centre, Heidelberggärtner 100, 3508 GA Utrecht, The Netherlands; 5 Netherlands Heart Institute, Holland Heart House, Moreelspark 1, 3511 EP Utrecht, The Netherlands; 6 Department of Thoracic and Cardiovascular Surgery, University Medical Centre Goettingen, Robert-Koch-Straße 40, 37075 Goettingen, Germany; 7 Unit of Cardiac Physiology, Division of Cardiovascular Sciences, Manchester Academic Health Science Centre, University of Manchester, Manchester, UK; 8 Department of Cardiology, University Medical Center Heidelberg, Heidelberg, Germany; 9 DZHK (German Center for Cardiovascular Research), Partner Site Heidelberg/Mannheim, Germany; and 10 Department of Thoracic and Cardiovascular Surgery, Klinikum Braunschweig, Braunschweig, Germany

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Aims

Atrial fibrillation (AF) is a commonly occurring arrhythmia after cardiac surgery (postoperative AF, poAF) and is associated with poorer outcomes. Considering that reduced atrial contractile function is a predictor of poAF and that Ca²⁺ plays an important role in both excitation–contraction coupling and atrial arrhythmogenesis, this study aims to test whether alterations of intracellular Ca²⁺ handling contribute to impaired atrial contractility and to the arrhythmogenic substrate predisposing patients to poAF.

Methods and results

Right atrial appendages were obtained from patients in sinus rhythm undergoing open-heart surgery. Cardiomyocytes were investigated by simultaneous measurement of [Ca²⁺] and action potentials (APs, patch-clamp). Patients were followed-up for 6 days to identify those with and without poAF. Speckle-tracking analysis of preoperative echocardiography revealed reduced left atrial contraction strain in poAF patients. At the time of surgery, cellular Ca²⁺ transients (CaTs) and the sarcoplasmic reticulum (SR) Ca²⁺ content were smaller in the poAF group. CaT decay was slower in poAF, but the decay of caffeine-induced Ca²⁺ transients was unaltered, suggesting preserved sodium-calcium exchanger function. In agreement, western blots revealed reduced SERCA2a expression in poAF patients but unaltered phospholamban expression/phosphorylation. Computational modelling indicated that reduced SERCA activity promotes occurrence of CaT and AP alternans. Indeed, alternans of CaT and AP occurred more often and at lower stimulation frequencies in atrial myocytes from poAF patients. Resting membrane potential and AP duration were comparable between both groups at various pacing frequencies (0.25–8 Hz).

Conclusions

Biochemical, functional, and modelling data implicate reduced SERCA-mediated Ca²⁺ reuptake into the SR as a major contributor to impaired preoperative atrial contractile function and to the pre-existing arrhythmogenic substrate in patients developing poAF.

*Corresponding author. Tel: +49 551 3965174; fax: +49 551 3965169, E-mail: niels.voigt@med.uni-goettingen.de
† The first two authors contributed equally to the study.
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1. Introduction

Development of atrial fibrillation (AF) within the immediate postoperative period (poAF) is one of the most frequent complications after cardiac surgery, occurring in up to 50% of cases. In more than 90% of these patients, poAF occurs within the first 6 postoperative days and its occurrence is associated with poorer outcomes, particularly a two- to fourfold increased risk of stroke, bleeding complications, and a two-fold increase in all-cause 30-day mortality. In the USA, the annual healthcare expenditure related to the burden of poAF is estimated at over $1 billion, highlighting poAF as an important socioeconomic problem.

Over the last decades, a great deal has been achieved in understanding pathophysiological mechanisms underlying the initiation, maintenance, and progression of AF in general. Yet the high incidence of AF following cardiac surgery persists and remains poorly understood. Clinical AF is initiated when triggers act on an arrhythmogenic substrate. Cardiac surgery can serve as such a trigger, initiating poAF mediated by adrenergic activation or local inflammation, thereby unmasking the pre-existing pro-arrhythmic substrate. Several studies indicate that poAF is associated with preoperative structural alterations including interstitial fibrosis, cellular hypertrophy, and signs of cellular degeneration, which are thought to be important determinants of the pre-existing atrial arrhythmogenic substrate. However, in contrast to patients with paroxysmal and long-standing persistent AF, in whom alterations of cellular electrophysiology, such as shortening of action potential duration (APD), contribute to the maintenance of the arrhythmia, electrical remodelling does not appear to play a major role in the arrhythmogenic substrate predisposing patients to poAF.

During recent years, cytosolic Ca²⁺ handling abnormalities in atrial myocytes have been suggested to play an important role in initiation and maintenance of AF. However, it is currently unknown whether such abnormalities also contribute to the arrhythmogenic substrate predisposing patients to the development of poAF. Recent studies demonstrate that impaired preoperative left and right atrial contractile function represents an independent predictor of poAF in patients undergoing open-heart surgery. Considering that cytosolic Ca²⁺ handling is a...
major determinant of contractile function, we hypothesize that alterations in cellular Ca\textsuperscript{2+} dynamics contribute to impaired atrial contractility in patients who proceed to develop poAF.

Here we use right atrial myocytes isolated from patients undergoing open-heart surgery to test the hypotheses that Ca\textsuperscript{2+} handling abnormalities contribute to contractile dysfunction and to the atrial arrhythmogenic substrate in patients who develop poAF.

2. Methods

A detailed description of all methods is provided in the Supplementary material online.

2.1 Speckle-tracking echocardiography

Two-dimensional grey-scale images acquired at 50 Hz over three cardiac cycles from a standard apical four-chamber view were obtained from patients in the period prior to cardiac surgery. Using speckle-tracking software (TOMTEC ARENA\textsuperscript{TM} REF TTA2 LOT 31.00), longitudinal deformation of the left atrium (LA) was monitored by point-tracking of six automatically allocated segments of the LA endocardial borders to determine global atrial strain\textsuperscript{14} (Figure 1A and Supplementary material online, Video S1).

2.2 Human tissue samples and myocyte isolation

Right atrial appendages were obtained from 202 patients in normal sinus rhythm undergoing cardiac surgery (Supplementary material online, Tables SI–SVI and Figure S1). During the postoperative period, rhythm was monitored with continuous three-lead electrocardiogram recording for 6 days and stored on a central monitoring system (BeneVision Central Monitoring System, Mindray Medical Germany). The recordings were analysed manually by an experienced clinician, who was blinded to the experimental results. PoAF was detected in 90 patients (45%). Patients were assigned to the poAF group if any episode of AF lasting longer than 30 s was documented. Patients were assigned to the control group (Ctrl) if no episode of AF was observed. Patients with a documented episode of AF at any time before or during cardiac surgery or with AF episodes < 30 s were excluded. Experimental protocols were approved by the ethics committee of the University Medical Center Göttingen (No. 4/11/18) and were performed in accordance with the Declaration of Helsinki. The STROBE checklist used for this study is provided as a Supplementary material online. Each patient gave written informed consent. Excised right atrial appendages were either snap-frozen in liquid nitrogen for biochemical studies or were subjected to a standard protocol\textsuperscript{10,15} for myocyte isolation.

2.3 Intracellular Ca\textsuperscript{2+} measurement and cellular electrophysiology

Only rod-shaped myocytes with clear striations and defined margins were selected for measurements of [Ca\textsuperscript{2+}]i. [Ca\textsuperscript{2+}]i of right atrial myocytes was measured using the fluorescent Ca\textsuperscript{2+} indicator fluo-3 according to our previously published protocol.\textsuperscript{10} Whole-cell ruptured patch-clamp techniques (voltage- and current-clamp) were used to record membrane currents and action potentials (APs) at 37 °C with simultaneous [Ca\textsuperscript{2+}]i measurement.\textsuperscript{10} Currents were related to membrane capacitance and expressed in current density (pA/pF).

2.4 AP and Ca\textsuperscript{2+} transient alternans

Alternans of APs and Ca\textsuperscript{2+} transients (CaTs) occurring at different stimulation frequencies were quantified with a discrete Fourier transform spectral method using modified custom-written software, as previously described.\textsuperscript{16}

2.5 Biochemical studies

Membranes were isolated from frozen tissue by differential centrifugation and solubilized at 1 mg/mL of total protein in solubilization buffer. Expression of key Ca\textsuperscript{2+} handling proteins was determined using immunoblotting techniques and normalized to total protein load as indicated by REVERT\textsuperscript{TM} total protein staining (LI-COR Biotechnology, USA).

The messenger RNA (mRNA) levels of key Ca\textsuperscript{2+} handling proteins were measured by real-time reverse transcriptase-polymerase chain reaction.
2.6 Computational modelling

APs and corresponding CaTs were simulated based on the mathematical model described by Courtemanche et al.\(^\text{17}\). The sarcoplasmic reticulum (SR) \(\text{Ca}^{2+}\) uptake compartment parameter was adjusted by \(+20\%\), \(-20\%\), and \(-40\%\) to simulate altered SR \(\text{Ca}^{2+}\)-ATPase (SERCA) activity.

2.7 Statistical analysis

Summarized data are reported as mean ± standard error of the mean, unless otherwise specified. Continuous data with sample sizes \(n \geq 30\) were assumed to be normally distributed, while the distribution of readouts between \(n = 10–30\) was analysed using the Shapiro–Wilks normality test. Normally distributed data were compared using the paired and unpaired two-tailed Student’s \(t\)-test. Differences between unpaired data with unequal variances were assessed using the Welch’s \(t\)-test, which is indicated in the legends of all affected figures. Non-normally distributed data and all data sets with \(n < 10\) were compared with the Mann–Whitney \(U\) test. The Fisher’s exact test was applied for differences in categorical data. Confounding effects of patient age were determined by the analysis of covariance. Kaplan–Meier curves were compared using the Gehan–Breslow–Wilcoxon test. \(P\)-value < 0.05 was considered statistically significant.

3. Results

3.1 Speckle-tracking echocardiography reveals reduced atrial function in poAF patients

Left atrial (LA) function was measured in preoperative patients through cardiac cycle-spanning segmental strain quantification and subsequent global averaging via a speckle-tracking echocardiography technique by an operator blinded to clinical data (Figure 1). Forty-two patients had an eligible preoperative echocardiogram for speckle-tracking analysis, of which 21 developed poAF and 21 did not. Patients who went on to develop poAF exhibited significantly reduced left atrial global contraction strain (LAsct) before cardiac surgery, compared with Ctrl (95.97 ± 5.79 pF, \(n = 35/22\) poAF vs. 78/38 Ctrl, \(P < 0.001\), Figure 2C). The time constant of CaT decay (\(t\)), measured by fitting a single exponential curve to the CaT decay, was found to be significantly higher in poAF vs. Ctrl, equating to a slower rate of decay in poAF (527.38 ± 45.31 vs. 405.44 ± 18.77 ms, \(n = 35/22\) poAF vs. 78/38 Ctrl, \(P < 0.05\), Figure 2D).

\(\beta\)-Adrenergic stimulation has been implicated in the pathogenesis of poAF\(^\text{18}\) and is known to influence excitation–contraction coupling via protein kinase A (PKA)-mediated phosphorylation of related proteins.\(^\text{19}\)

We investigated the response of atrial myocytes to \(\beta\)-adrenergic stimulation using 1 \(\mu\)mol/L isoproterenol. In the presence of isoprenaline, \(I_{\text{Ca,L}}\) and the triggered CaT amplitude were significantly increased in both Ctrl and poAF, while a significant decrease in the time constant (\(t\)) of CaT decay was observed (Supplementary material online, Figure SIV). \(I_{\text{Ca,L}}\) peak amplitude was similar between poAF and Ctrl in both the presence and absence of isoprenaline. However, before the application of isoprenaline, a tendency towards a reduced CaT amplitude was observed in poAF compared to Ctrl and in the presence of isoprenaline, CaT was significantly smaller in poAF. The time constant (\(t\)) of CaT decay was increased in poAF compared to Ctrl, regardless of whether isoprenaline was present.

3.3 Slower SR \(\text{Ca}^{2+}\) uptake contributes to smaller SR \(\text{Ca}^{2+}\) content in atrial myocytes from poAF patients

CaT amplitude is governed by various factors such as \(I_{\text{Ca,L}}\) and SR \(\text{Ca}^{2+}\) content. Considering that \(I_{\text{Ca,L}}\) was similar between groups, the SR \(\text{Ca}^{2+}\) content was subsequently measured. An \(I_{\text{Ca,L}}\)-activating protocol (see above) was applied to atrial myocytes for 3–5 min, after which stimulation was terminated and caffeine (10 mmol/L) was applied to induce complete \(\text{Ca}^{2+}\) release from the SR (Figure 3A). Both the amplitude of the resulting \(\text{Ca}^{2+}\) transient and the resulting charge accumulation [via sodium–calcium exchanger (NCX)-mediated inward current] were significantly smaller in poAF compared to Ctrl, indicating smaller SR \(\text{Ca}^{2+}\) content in poAF (amplitude: 0.63 ± 0.06 vs. 0.97 ± 0.08 \(\mu\)mol/L, \(P < 0.01\), charge: 1.53 ± 0.08 vs. 1.80 ± 0.06 pC/pF, \(P < 0.01\), \(n = 35/22\) poAF vs. 78/38 Ctrl, Figure 3B).

The time constant of decay (\(t\)) of the caffeine-induced CaT (a measure of NCX function), however, was comparable between the two groups (Figure 3C) and we did not detect altered contribution of the plasmalemmal \(\text{Ca}^{2+}\)-ATPase to cytosolic \(\text{Ca}^{2+}\) removal (Supplementary material online, Figure SV). In accordance, the slope of the line relating \(\text{INCX} \pm\) to \(\text{[Ca}^{2+}\]_{i}\) tended to be lower in poAF than Ctrl, indicating lower SR \(\text{Ca}^{2+}\) content during the caffeine-induced CaT decay (Figure 3D). The occurrence of spontaneous CaT release events (Supplementary material online, Figure SW) was not found to be different in poAF vs. Ctrl (Figure 3F and Supplementary material online, Figure SW).

To exclude a role for increased SR \(\text{Ca}^{2+}\) leak in lowering SR \(\text{Ca}^{2+}\) content in poAF, total SR \(\text{Ca}^{2+}\) leak was quantified using the tetracaine method of Shannon et al.\(^\text{20}\) (Figure 4A). SR \(\text{Ca}^{2+}\) leak was not different in the poAF group (Figure 4B). In accordance, occurrence of spontaneous \(\text{Ca}^{2+}\) release events (Supplementary material online, Figure SW), as well as protein and mRNA expression and phosphorylation of ryanodine receptor channels (RYR2, Figure 4C and D and Supplementary material online, Figure SV).
Figure 2  
ICa,L-triggered CaT in atrial myocytes from patients who do not (Ctrl) and who do develop poAF. (A) Voltage-clamp protocol (0.5 Hz, upper), representative simultaneous recordings of ICa,L (middle), and triggered CaT (lower) in Ctrl (left) and poAF (right) myocytes. (B) Mean ± standard error of the mean (SEM) peak ICa,L (left) and integrated ICa,L (right). (C) Mean ± SEM diastolic and systolic [Ca2⁺]i (left) and resulting CaT amplitude (right). (D) Mean ± SEM time constant of decay (τ) of ICa,L-triggered CaT. *P < 0.05, **P < 0.001 vs. Ctrl. n/N = number of myocytes/patients. Comparison using the unpaired t-test with Welch’s correction (C right and D). CaT, Ca2⁺ transients; Ctrl, control; poAF, postoperative atrial fibrillation.

online, Figure SVI), were not found to be different in poAF compared to Ctrl.

SERCA function was subsequently calculated to ascertain if any alteration could underlie slower decay of systolic CaT and smaller SR Ca2⁺ content in poAF. The function of SERCA, expressed as the rate constant kSERCA, was calculated by subtracting the rate constant of decay of the caffeine-induced CaT (kCaT, inverse of cCaT) from that of the systolic CaT (kSyst, inverse of systolic CaT, Figure 5A and B), as previously described. kSERCA was found to be significantly lower in poAF vs. Ctrl (1.48 ± 0.17 vs. 2.08 ± 0.15 s⁻¹, n/N = 35/22 poAF vs. 78/38 Ctrl, P < 0.01, Figure 5B). Furthermore, although SERCA2a mRNA levels (ATP2A2) were similar in Ctrl and poAF patients, SERCA2a protein content, analysed by western blot, was revealed to be significantly lower in poAF (Figure 5C). This points to posttranslational modifications of SERCA2a that may contribute to reduced SERCA-mediated Ca2⁺ extrusion in poAF. These results are likely to underlie both the slower decay rate of the systolic CaT and the smaller SR Ca2⁺ load in poAF. mRNA and protein levels and phosphorylation of phospholamban (PLB) and mRNA levels of sarcoplasmic (SLN) were comparable in both groups (Figure 5D and Supplementary material online, Figure SVI), indicating that any reduction of SERCA activity was independent of these regulatory proteins.

In addition to altered activity of Ca2⁺ removal mechanisms, increased intracellular Ca2⁺ buffering has also been shown to reduce both amplitude and the rate of decay of systolic Ca2⁺ transients. NCX-mediated inward current (Supplementary material online, Figure SVIII). NCX current was integrated cumulatively to provide an index of ‘total’ Ca2⁺, which was plotted against the decay phase of the caffeine-induced Ca2⁺ transient (‘free’ Ca2⁺), thereby generating buffer curves which were fitted with a hyperbolic function. The extrapolated Bmax and kᵣ values were not different between poAF and Ctrl.

3.4 Enhanced susceptibility to CaT and AP alternans in atrial myocytes from poAF patients

Reduced SERCA activity has previously been associated with alternans in ventricular myocytes. Alternans describes the observation that, under certain conditions, the shape of the CaT and AP varies on a beat to beat basis between two contrasting states. To investigate whether this is also the case in atrial myocytes, computational modelling was performed
with the atrial-specific Courtemanche model.\textsuperscript{17} A reduction in SERCA activity in this model reduced the stimulation frequency threshold for alternans, both of AP and of CaT, as shown in Supplementary material online, Figure SIX.

In order to further investigate the effect of stimulation frequency on electrophysiology and Ca\textsuperscript{2+} handling, fluo-3-loaded right atrial myocytes from Ctrl and poAF groups were paced at frequencies ranging from 0.25 to 8 Hz in current-clamp to elicit APs and accompanying CaTs. Resting membrane potential (RMP), mean AP duration at 90\% repolarization (APD\textsubscript{90}), maximal slope of AP restitution curve, and diastolic [Ca\textsuperscript{2+}]\textsubscript{i} were not significantly different in poAF vs. Ctrl (Figure 6). CaT amplitude, however, was found to be significantly smaller over the frequencies tested in poAF (Figure 6D), in agreement with the voltage-clamp experiments.

The incidence of alternans was investigated at each stimulation frequency. Figure 7A shows example AP and CaT alternans at 4 Hz stimulation. 54\% of Ctrl myocytes and 63\% of poAF myocytes developed AP alternans. CaT alternans was observed in 17\% and 42\% of myocytes from Ctrl and poAF patients, respectively. Kaplan–Meier analysis over the whole range of frequencies revealed higher susceptibility to AP and CaT alternans in poAF patients (Figure 7B). In addition, and in agreement with results of computational modelling, threshold for AP alternans was significantly lower in the poAF group, i.e. in the presence of reduced SERCA function (2.62 ± 0.52 vs. 5.15 ± 0.68 Hz; n/N = 12/10 poAF vs. 13/12 Ctrl, P < 0.01, Figure 7C). Threshold for CaT alternans showed a tendency to be lower in poAF (Figure 7C).

\section*{4. Discussion}

In the present study, we describe Ca\textsuperscript{2+} handling abnormalities which contribute to the atrial arrhythmogenic substrate predisposing patients to the development of poAF. In addition, this is also likely to lead to impaired atrial contractile function in these patients. Our data show the absence of electrical remodelling at the time of cardiac surgery in atrial myocytes from patients who proceed to develop poAF. In contrast, preoperative echocardiography revealed impaired atrial contractile function in poAF patients and reduction of SR Ca\textsuperscript{2+} release in atrial myocytes, attributable to reduced SR Ca\textsuperscript{2+} load and impaired diastolic SR Ca\textsuperscript{2+}.
uptake. Computational modelling suggests that the latter also predisposes atrial myocytes from poAF patients to CaT and AP alternans. Accordingly, atrial myocytes from poAF patients were found to have higher susceptibility and lower threshold frequencies for alternans both of CaT and of AP. We thereby provide the first evidence that CaT and AP alternans can occur in human atrial myocytes. Together these data point to impaired SR Ca\(^{2+}\) uptake as a common underlying mechanism which contributes both to the impaired pre-existing atrial contractile function as an independent risk factor of poAF and to the arrhythmogenic substrate, which predisposes patients to the development of poAF.
4.1 Comparison with previous studies

It is well accepted that re-entry is the major mechanism of AF maintenance. Re-entry induction requires an appropriate vulnerable substrate and a trigger which initiates re-entry within this substrate. A re-entry-favouring substrate is determined by slow conduction and short refractoriness. Long-standing persistent AF, but not paroxysmal AF (pAF), is associated with electrical remodelling of various ion currents, e.g., reduced \( I_{\text{Ca,L}} \) and increased \( I_{\text{K1}} \), leading to re-entry-promoting shortening of the atrial APD. Slow impulse propagation due to fibrosis or impaired electrical coupling between myocytes facilitates re-entry by allowing more time for tissue to regain excitability, contributing to the arrhythmogenic substrate.

Spatially discordant electrical alterations in excitability (alternans) cause electrical heterogeneity which favours re-entry, thus promoting AF maintenance. Although the presence of a pre-existing AF-maintaining substrate in patients developing pAF has been shown, its molecular basis is largely unknown. Several studies indicate that development of pAF is associated with preoperative alterations in extracellular matrix, such as increased interstitial fibrosis, impaired connexin expression, and cellular remodelling.

**Figure 5** SR Ca\(^{2+}\) ATPase (SERCA2a) and PLB expression, phosphorylation and function in atrial myocytes from patients who do not (Ctrl) and who do develop poAF. (A) Representative caffeine experiment, indicating the decay rate constant of the systolic (\( I_{\text{Ca,L}} \)-triggered) Ca\(^{2+}\) transient (\( k_{\text{sys}} \)) and the decay rate constant of the caffeine-induced Ca\(^{2+}\) transient (\( k_{\text{caf}} \)). (B) Respective rate constants \( k_{\text{sys}} \) (left), \( k_{\text{caf}} \) (middle), and \( k_{\text{SERCA}} \) (right), calculated as the difference between \( k_{\text{sys}} \) and \( k_{\text{caf}} \) in Ctrl and poAF (mean ± SEM). (C) Representative western blots (upper) showing the expression of SERCA2a (green) against total protein in the same gel area (red). Lower panel shows quantification of SERCA2a expression normalized against total protein (mean ± SEM). (D) Representative western blots (upper) showing the expression of PLB, PLB-Ser16 and PLB-Thr17 normalized to SERCA2a. Lower panel shows quantification of PLB, PLB-Ser16 and PLB-Thr17 normalized to SERCA2a. * \( P < 0.05 \), ** \( P < 0.01 \) vs. Ctrl. **

**Figure 6** Combined measurements of action potentials (APs) and Ca\(^{2+}\) transients (CaTs) in atrial myocytes from patients who do not (Ctrl) and who do develop poAF. (A) Representative traces of simultaneous AP (upper) and CaTs (lower) recorded at various frequencies from a patient proceeding to develop poAF. (B) Mean ± standard error of the mean (SEM) of resting membrane potential (RMP) at increasing pacing frequencies. (C) Mean ± SEM of APD\(_{90}\) at increasing pacing frequencies (left) and diastolic intervals (right, AP restitution). (D) Mean ± SEM frequency-dependent effects on diastolic calcium (left) and CaT amplitude (right) in myocytes from Ctrl and poAF patients. A single decay curve was fitted when no significant difference between groups was detected with an extra sum-of-squares F test. Two curves imply a global significant difference between both groups. ** \( P < 0.001 \).**

\( n/N = \) number of myocytes/patients. APD, action potential duration; Ctrl, control; poAF, postoperative atrial fibrillation.
Only one previous report has demonstrated increased I_{Ca,L} in poAF. Previous work showing unchanged expression and activity of depolarizing sodium and Ca^{2+} currents, as well as unchanged repolarizing potassium currents, suggesting comparable APD and RMP, only one previous report has demonstrated increased I_{Ca,L} in poAF patients. The reasons for this discrepancy are unclear but may include differences in clinical characteristics and experimental approaches.

Long-standing persistent AF (‘chronic’: cAF) and pAF are associated with pronounced Ca^{2+} handling abnormalities which promote ectopic firing. In both cAF and pAF, there is evidence for increased incidence of Ca^{2+}-driven delayed afterdepolarizations (DADs), leading to cellular triggered activity. These are thought to be caused by increased SR Ca^{2+} leak with enhanced incidence of spontaneous SR Ca^{2+} release events (SCaEs). In the present study, we did not find evidence for increased SR Ca^{2+} leak or increased incidence of SCaEs in myocytes from poAF patients at the time of surgery. This is in accordance with our data showing unaltered RyR2 expression and phosphorylation in poAF patients (Figure 4 and Supplementary material online, Figure SVI) and with a previous report documenting unaltered RyR2 mRNA levels. Taken together, a preoperative increase in the incidence of cellular Ca^{2+}-dependent DADs and triggered activity does not appear to contribute to the arrhythmogenic substrate in patients developing poAF.

4.2 Novel findings and potential clinical implications

To the best of our knowledge, we provide the first comprehensive study investigating pre-existing arrhythmogenic alterations in atrial Ca^{2+} homeostasis in patients developing poAF.

Here we propose that the impaired SR Ca^{2+} uptake associated with increased cellular susceptibility to CaT and AP alternans contributes to atrial arrhythmogenesis in patients developing poAF by creating an arrhythmogenic substrate. In addition, impaired diastolic Ca^{2+} reuptake into the SR leads to reduced SR Ca^{2+} load and a subsequent reduction in systolic Ca^{2+} release, thereby providing a cellular correlate for the clinical observation that atrial contractile dysfunction is an independent risk factor for the development of poAF. Based on our findings we propose that reduced SERCA activity is a common cellular mechanism underlying both impaired contractile function and atrial arrhythmogenesis.

In the current study, we provide the first demonstration of CaT and AP alternans in human atrial myocytes. Within atrial tissue, spatially discordant electrical alterations in excitability and electrical heterogeneity can be caused by cellular AP alternans. This favours re-entry and promotes AF maintenance. Accordingly, patients with a history of cAF and pAF exhibit alternans of monophasic APs of greater magnitude and at lower stimulation frequencies. We demonstrate here that right atrial myocytes from patients undergoing open-heart surgery and developing poAF are more susceptible to AP alternans at the time of surgery. The canonical mechanism of AP alternans suggests that it arises because of the extent to which APs shorten in response to changes in the preceding diastolic interval. More recent work suggests that CaT alternans drives AP alternans, i.e. beat to beat alternations in the cytosolic Ca^{2+} concentration, for example, increased SR Ca^{2+} release and by factors reducing Ca^{2+} uptake associated with factors increasing SR Ca^{2+} release, thereby providing a cellular correlate for the clinical observation that atrial contractile dysfunction is an independent risk factor for the development of poAF. Based on our findings we propose that reduced SERCA activity is a common cellular mechanism underlying both impaired contractile function and atrial arrhythmogenesis.

Figure 7 Occurrence of alternans in atrial myocytes from patients who do not (Ctrl) and who do develop poAF. (A) Representative traces of concordant alternans in AP (amplitude and repolarization alternans; upper) and CaT (amplitude and diastolic alternans; lower) at 4 Hz measured from a patient proceeding to develop poAF. (B) The first occurrence of frequency-dependent alternans as a Kaplan–Meier plot in AP (upper panel) and CaT (lower panel). (C) Alternans threshold frequency. Data are mean ± standard error of the mean. Kaplan–Meier curves compared with the Gehan–Breslow–Wilcoxon test. **p < 0.01 vs. Ctrl. n/N = number of myocytes/patients. Alternans threshold frequency compared using the Mann–Whitney U test. AP, action potentials; CaT, Ca^{2+} transients; Ctrl, control; poAF, postoperative atrial fibrillation.
corroborating the role of reduced expression of SERCA2a in the patients compared to Ctrl regardless of isoprenaline presence, further revealing improved systolic function. These approaches could therefore guide future studies to prevent poAF in patients undergoing open-heart surgery while slowing of the decay of the Ca\textsuperscript{2+} transient can occur due to factors other than SERCA, our data do not support roles for SR Ca\textsuperscript{2+} leak (Figure 4), cytosolic Ca\textsuperscript{2+} buffering (Supplementary material online, Figure SIII), or altered t-tubule density (Supplementary material online, Figure SII). Again, we cannot exclude local alterations on a subcellular level within the specific RyR2 microdomain. In addition, since RyR2 expression and phosphorylation were evaluated only in a subset of patients, we cannot exclude that statistical power was not sufficient and alterations in these values may be detected in a larger cohort.

In the present work, we largely focus on Ca\textsuperscript{2+} handling abnormalities contributing to the pre-existing cellular substrate predisposing patients to the development of poAF. However, activation of the autonomic nervous system and inflammation are the most accepted acute factors associated with cardiac surgery in triggering poAF. β-Adrenoceptor stimulation and pro-inflammatory cytokines such as tumour necrosis factor alpha and interleukin-1β have been shown to increase the incidence of SCaEs in ventricular and atrial myocytes and may thereby contribute to the initiation of poAF. The impact of inflammatory mediators on cytosolic Ca\textsuperscript{2+} handling in patients with and without the development of

**4.3 Potential limitations**

In the present study, only the right atrial appendages from patients undergoing open-heart surgery were used because of limited availability of human atrial tissue. Our findings may therefore not apply fully to other atrial regions. Furthermore, in our cohort we analysed atrial strain only in the LA due to unfavourable sonographic conditions in the right atrium. However, a recent study demonstrated altered right atrial strain parameters in patients developing poAF, suggesting that impaired atrial contractile function is a general preoperative phenomenon in both atria of poAF patients and that the molecular alterations we observed in right atrial appendages are likely to be similar in left atrial regions. Nevertheless, future studies investigating both left and right atrial strain in patients developing poAF are necessary.

Here, we identified potential arrhythmogenic mechanisms in isolated atrial cardiomyocytes which may contribute to poAF development. There are additional factors (genetic, type of surgery, autonomic nervous system, inflammation) predisposing patients to the development of poAF and we do not claim that the properties studied here account fully for the arrhythmogenic phenotype in poAF patients.

Pre-existing fibrosis, for example, has been identified as an important contributor to the re-entry-promoting substrate in patients developing poAF. Interestingly, neither left atrial diameter (Supplementary material online, Tables SII-SVII) nor left atrial conduit strain (Figure 1) was significantly different between poAF and Ctrl, suggesting the absence of any important structural remodelling in our poAF population. Furthermore, age has been identified as an important risk factor for poAF, and here, patients used for voltage-clamp experiments and strain analysis were significantly older (Supplementary material online, Tables SII and SIII). However, further analysis revealed that CaT amplitude and decay, as well as strain parameters, did not correlate with age in these experiments (Supplementary material online, Figures SX and SXI), suggesting that impaired SR Ca\textsuperscript{2+} release and subsequent reduction in atrial contractile function are independent risk factors for poAF development.

The methods applied in the present study are mainly designed to detect alterations in global cytosolic Ca\textsuperscript{2+} handling. Based on our experiments we conclude that reduced SERCA2a expression is a major contributor to slower Ca\textsuperscript{2+} reuptake into the SR. However, we cannot exclude that reduced cytosolic Ca\textsuperscript{2+} within the specific SERCA2a microdomain may also contribute to this observation. Furthermore, whether atrial contractile dysfunction due to reduced SERCA activity (atrial cardiomyopathy) predisposes to stroke, independent of AF development, remains to be investigated.

Targeting impaired SERCA2a function may represent an important therapeutic approach to improve atrial contractile function and prevent development of poAF and related negative outcomes. Several concepts to increase ventricular SERCA2a expression and function have been investigated in patients with heart failure. In particular, the effects of adeno-associated viral SERCA2a gene transfer and pharmacological activation of SERCA2a with istaroxime were investigated in phase-2 trials, revealing improved systolic function. These approaches could therefore guide future studies to prevent poAF in patients undergoing open-heart surgery or improve atrial contractile function in patients with atrial cardiomyopathy.
poAF is beyond the scope of the current project and should be addressed in future detailed studies.

5. Conclusions

In this study, we evaluated pre-existing Ca\(^{2+}\) handling abnormalities underlying impaired atrial contractility and atrial arrhythmogenesis in poAF patients. Reduced SERCA activity appears to be an important cause of impaired excitation–contraction coupling and contributes to the arrhythmogenic substrate in these patients. Our data will help to develop new patient-tailored preventive and therapeutic strategies, which could target not only the immediate postoperative period but also provide long-term protection of patients exhibiting specific risk factors such as impaired atrial contractility.

Data availability

All available data are incorporated into this article and its online supplementary material.

Supplementary material

Supplementary material is available at Cardiovascular Research online.

Authors’ contributions

F.E., F.E.M., and N.V. designed the studies. F.E., V.S., F.S., J.G., S.K., J.M., J.R.D.P., K.T., F.H., G.K., C.M.P., A.J.T., K.M.D., B.S., F.E.M., and N.V. performed the research and analysed the data. F.W., C.S., A.E.-E., B.C.D., H.B., and I.K. provided expertise about human heart samples and clinical data analysis. F.E.F., F.S., F.E.M., and N.V. wrote the manuscript, and all authors contributed to the final version.

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References

1. Dobrev D, Aguilar M, Heijman J, Guichard JB, Nattel S. Postoperative atrial fibrillation: mechanisms, manifestations, and management. Nat Rev Cardiol 2019;16:417–436.
2. Swartz MF, Fink GW, Lutz CJ, Taffet SM, Berensfeld O, Viskstrom KL, Kasprowicz K, Bhartia I, Puksas F, Kailis J, Jafie J. Left versus right atrial difference in dominant frequency. K\(^{-}\) channel transcripts, and fibrosis in patients developing atrial fibrillation after cardiac surgery. Hear Rhythm 2009;6:1415–1422.
3. Wang GD, Shen LH, Wang L, Li HW, Zhang YC, Chen H. Relationship between integrated backscatter and atrial fibrosis in patients with and without atrial fibrillation who are undergoing coronary bypass surgery. Clin Cardiol 2009;32:E56–E60.
4. Tinca G, Mocanu V, Zugen-Eloxe F, Butcovan D. Clinical and histological predictive risk factors of atrial fibrillation in patients undergoing open-heart surgery. Exp Ther Med 2015;10:2299–2304.
5. Heijman J, Voigt N, Nattel S, Dobrev D. Cellular and molecular electrophysiology of atrial fibrillation initiation, maintenance, and progression. Circ Res 2014;114:1483–1499.
6. Wagoner DV, Pond AL, Lamorgese M, Rossie SS, McCarthy PM, Nerbonne JM. Atrial L-type Ca\(^{2+}\) currents and human atrial fibrillation. Circ Res 1999;85:428–436.
7. Brands MC, Priebe L, Böhle T, Südkamp M, Beckelmann DJ. The ultra-rapid and the transient outward K\(^{+}\) current in human atrial fibrillation. Their possible role in post-operative atrial fibrillation. J Mol Cell Cardiol 2000;32:1885–1896.
8. Dobrev D, Wettwer E, Kortner A, Knaut M, Schuler S, Ravens U. Human inward rectifier potassium channels in chronic and postoperative atrial fibrillation. Cardiovasc Res 2002;54:397–404.
9. Workman AJ, Pau D, Redpath CJ, Marshall GE, Russell JA, Kane KA, Norrie J, Rankin AC. Post-operative atrial fibrillation is influenced by beta-blocker therapy but not by pre-operative atrial cellular electrophysiology. J Cardiovasc Electrophysiol 2006;17:1230–1238.
10. Voigt N, Li N, Wang Q, Wang W, Trafford AW, Abu-Taha I, Sun Q, Wieland T, Ravens U, Nattel S, Wehrens XHT, Dobrev D. Enhanced sarcoplasmic reticulum Ca\(^{2+}\) Leak and increased Na\(^{+}\)-Ca\(^{2+}\) exchanger function underlie delayed afterdepolarizations in patients with chronic atrial fibrillation. Circulation 2012;125:2059–2070.
11. Voigt N, Heijman J, Wang Q, Chiang DY, Li N, Karck M, Wehrens XHT, Nattel S. Dobrev D. Cellular and molecular mechanisms of atrial arrhythmogenesis in patients with paroxysmal atrial fibrillation. Circulation 2014;129:145–156.
12. Verdejo HE, Becerra E, Zalaquett R, Campo AD, Garcia L, Troncoso R, Chiong M, Marin A, Castro PF, Lavandero S, Gabrielli L, Corbáln A. Atrial function assessed by speckle tracking echocardiography is a good predictor of postoperative atrial fibrillation in elderly patients. EchoCARDIOGRAPHY 2016;33:242–248.
13. Akus U, Kalkan K, Gulcu O, Alskakal E, Oztürk M, Topçu S. The role of the right atrium in development of postoperative atrial fibrillation: a speckle tracking echocardiography study. J Clin Ultrason 2019;47:470–476.
14. Badano LP, Kolas TJ, Muraru D, Abraham TP, Aurigemma G, Edwardsen T, D’Hooge J, Donal E, Fraser AG, Marwick T, Mertens L, Popescu BA, Sengupta PP, Lancelotti P, Thomas JD, Voigt JU. Standardization of left atrial, right ventricular, and right atrial deformation imaging using two-dimensional speckle tracking echocardiography: a consensus document of the EACVI/ASE/Industry Task Force to standardize deformation imaging. Eur Heart J Cardiovasc Imaging 2018;19:591–600.
15. Voigt N, Zhou XB, Dobrev D. Isolation of human atrial myocytes for simultaneous measurements of Ca\(^{2+}\) transients and membrane currents. J Vis Exp 2013;37:e50235.
16. Pearman CM, Madders GW, Radcliffe EJ, Kirkwood GJ, Watkins A, Smith AM, Trafford AW, Eisner DA. Isoproterenol does not enhance Ca-dependent Na/Ca exchanger function in human atrial myocytes. Pflügers Arch 2003;445:947–952.
17. Ravens U, Nattel S, Wehrens XHT, Dobrev D. Enhanced sarcoplasmic reticulum Ca\(^{2+}\) Leak and increased Na\(^{+}\)-Ca\(^{2+}\) exchanger function underlie delayed afterdepolarizations in patients with chronic atrial fibrillation. Circulation 2012;125:2059–2070.
18. Maesen B, Nijs J, Maessen J, Alesse M, Schotten U. Post-operative atrial fibrillation: a maze of mechanisms. Europace 2012;14:159–174.
19. Ginsburg KS, Bers DM. Isoproterenol does not enhance Ca-dependent Na/Ca exchange current in intact rabbit ventricular myocytes. J Mol Cell Cardiol 2005;39:972–981.
20. Shannon TR, Ginsburg KS, Bers DM. Quantitative assessment of the SR Ca\(^{2+}\) leak-load relationship. Circ Res 2002;91:594–600.
21. Walden AP, Dibb KM, Trafford AW. Differences in intracellular calcium homeostasis between atrial and ventricular myocytes. J Mol Cell Cardiol 2009;46:463–473.
22. Diaz ME, Trafford AW, Eisner DA. The role of intracellular Ca buffers in determining the shape of the systolic Ca transient in cardiac ventricular myocytes. Pflügers Arch 2001;442:96–100.
23. Trafford AW, Diaz ME, Eisner DA. A novel, rapid and reversible method to measure Ca buffering and time-course of total sarcoplasmic reticulum Ca content in cardiac ventricular myocytes. Pflügers Arch 1999;437:501–503.
24. Weiss JN, Nivala M, Garfinkel A, Qiu Z. Alternans and arrhythmias: from cell to heart. Circ Res 2011;108:98–112.
25. Wagoner DV, Pond AL, McCarthy PM, Trimmer JS, Nerbonne JM. Outward K\(^{-}\) current densities and Kv1.5 expression are reduced in chronic human atrial fibrillation. Circ Res 1997;80:772–781.
Altered calcium handling in postoperative AF

Translational perspective

Development of atrial fibrillation (AF) within the immediate postoperative period (poAF) represents one of the most frequent complications after cardiac surgery and is associated with poorer outcomes. Our results suggest that reduced Ca\textsuperscript{2+} uptake into the sarcoplasmic reticulum (SR), associated with increased cellular susceptibility to Ca\textsuperscript{2+}-transient and action potential alternans, contributes to the arrhythmogenic substrate predisposing patients to the development of poAF. Therefore, modulation of SERCA activity may represent a novel mechanistic target to prevent the development of poAF. Furthermore, we show that the impaired SR Ca\textsuperscript{2+} uptake contributes to reduced systolic Ca\textsuperscript{2+} release and impaired atrial contractility in poAF patients. Atrial contractility may therefore represent an important factor for identification of patients at risk for poAF development.