Arrhythmia/Electrophysiology

Cellular and Molecular Mechanisms of Atrial Arrhythmogenesis in Patients With Paroxysmal Atrial Fibrillation

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Background—Electrical, structural, and Ca²⁺-handling remodeling contribute to the perpetuation/progression of atrial fibrillation (AF). Recent evidence has suggested a role for spontaneous sarcoplasmic reticulum Ca²⁺-release events in long-standing persistent AF, but the occurrence and mechanisms of sarcoplasmic reticulum Ca²⁺-release events in paroxysmal AF (pAF) are unknown.

Method and Results—Right-atrial appendages from control sinus rhythm patients or patients with pAF (last episode a median of 10–20 days preoperatively) were analyzed with simultaneous measurements of [Ca²⁺] (fluorescent dye) and membrane currents/action potentials (patch-clamp) in isolated atrial cardiomyocytes, and Western blot. Action potential duration, L-type Ca²⁺ current, and Na⁺/Ca²⁺-exchange current were unaltered in pAF, indicating the absence of AF-induced electrical remodeling. In contrast, there were increases in SR Ca²⁺ leak and incidence of delayed after-depolarizations in pAF. Ca²⁺-transient amplitude and sarcoplasmic reticulum Ca²⁺ load (caffeine-induced Ca²⁺-transient amplitude, integrated Na⁺/Ca²⁺-exchange current) were larger in pAF. Ca²⁺-transient decay was faster in pAF, but the decay of caffeine-induced Ca²⁺ transients was unaltered, suggesting increased SERCA2a function. In agreement, phosphorylation (inactivation) of the SERCA2a-inhibitor protein phospholamban was increased in pAF. Ryanodine receptor fractional phosphorylation was unaltered in pAF, whereas ryanodine receptor expression and single-channel open probability were increased. A novel computational model of the human atrial cardiomyocyte indicated that both ryanodine receptor dysregulation and enhanced SERCA2a activity promote increased sarcoplasmic reticulum Ca²⁺ leak and sarcoplasmic reticulum Ca²⁺-release events, causing delayed after-depolarizations/triggered activity in pAF.

Conclusions—Increased diastolic sarcoplasmic reticulum Ca²⁺ leak and related delayed after-depolarizations/triggered activity promote cellular arrhythmogenesis in pAF patients. Biochemical, functional, and modeling studies point to a combination of increased sarcoplasmic reticulum Ca²⁺ load related to phospholamban hyperphosphorylation and ryanodine receptor dysregulation as underlying mechanisms. (Circulation. 2014;129:145-156.)

Key Words: atrial fibrillation ■ calcium ■ computational biology ■ electrophysiology ■ sarcoplasmic reticulum

Atrial fibrillation (AF) is the most common arrhythmia in clinical practice, with an incidence that is rising with aging of the population. AF is associated with increased morbidity and mortality, in particular, attributable to embolic stroke and worsening heart failure. Currently, AF is classified based on its clinical presentation: patients often first show paroxysmal AF (pAF), consisting of self-terminating episodes lasting <7 days, then persistent and finally long-lasting persistent (chronic) states (cAF) that fail to self-terminate. Up to 15% of pAF patients progress to persistent forms annually, likely because of AF-related remodeling. The type of AF also affects clinical outcome, with cAF associated with worse outcomes and less amenable to rhythm-control therapy than pAF.

Clinical Perspective on p 156

The cellular and molecular mechanisms contributing to atrial arrhythmogenesis in cAF have been studied extensively with atrial-tissue samples from cAF patients. Combined with the results from animal models, these studies have highlighted a complex pattern of electrical, structural, and Ca²⁺-handling remodeling, producing a vulnerable substrate for AF maintenance. However, the cellular mechanisms underlying pAF remain elusive.

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Clinical AF initiates when triggers act on arrhythmogenic substrates. The pulmonary veins (PVs) play a particularly important role in pAF patients, and there is evidence that PV cardiomyocytes possess properties predisposing to both Ca\(^{2+}\)-driven focal activity and reentry. Although atrial myocytes from pAF patients undergoing open-heart surgery represent a potentially useful model to study the basic mechanisms underlying AF triggers, studies of the cellular electrophysiological changes that predispose to AF paroxysms in patients are very limited.\(^{13,14}\)

The present study tested the hypothesis that patients with pAF are predisposed to Ca\(^{2+}\)-driven delayed after-depolarizations (DADs), and assessed potential underlying mechanisms with the use of simultaneous measurements of intracellular \([\text{Ca}^{2+}]\) (\([\text{Ca}^{2+}]_{\text{L}}\)) and membrane currents or action potentials (APs, patch-clamp), biochemical analyses, studies of ryanodine receptors (RyR2) in lipid bilayers and computational modeling.

**Methods**

A detailed description of all methods is provided in the online-only Data Supplement.

**Human Tissue Samples and Myocyte Isolation**

Right-atrial appendages were dissected from 73 sinus rhythm (CtI) patients and 47 pAF patients undergoing open-heart surgery. pAF patients had at least 1 documented AF episode that self-terminated within 7 days of onset (for 1 example, Figure I in the online-only Data Supplement). Patient characteristics are provided in Tables I through III in the online-only Data Supplement. AF characteristics were determined based on clinical information in the chart; the last AF episode had terminated a median of 10 to 20 range, 1–72) days preoperatively, and all patients were in sinus rhythm at the time of surgery. No detailed information was available regarding frequency and duration of AF episodes. Experimental protocols were approved by the Medical Faculty Mannheim, Heidelberg University (No. 2011–216 N-MA). Each patient gave written informed consent. After excision, atrial appendages were flash-frozen in liquid N\(_2\) for biochemical/bio-physical studies or were used for myocyte isolation with a previously available protocol.\(^{15,16}\) Isolated cardiomyocytes were suspended in ethylene glycol tetraacetic acid–free storage solution until simultaneous measurement of \([\text{Ca}^{2+}]\) and membrane current/potential.

**Simultaneous Intracellular \([\text{Ca}^{2+}]\) and Patch-Clamp Recording**

\([\text{Ca}^{2+}]\) was quantified with fluo-3-acetoxymethyl (Fluo-3) ester in bath and pipette solution. After deesterification, fluorescence was excited at 488 nm and emitted light (\(>520\) nm) converted to \([\text{Ca}^{2+}]\), assuming

\[
\left[\text{Ca}^{2+}\right] = k_d \frac{F}{F_{\text{max}} - F}
\]

where \(k_d\) is the dissociation constant of Fluor-3 (864 nmol/L), F is Fluor-3 fluorescence, and \(F_{\text{max}}\) is \([\text{Ca}^{2+}]\) saturated fluorescence obtained at the end of each experiment.\(^{17}\)

Membrane currents and APs were recorded at 37°C in whole-cell ruptured-patch configuration by using voltage/current-clamp techniques with simultaneous \([\text{Ca}^{2+}]\) measurement. There was no significant difference in membrane capacitance between pAF (102.0±11.7 pF, n=159 [myocytes/patients]) and CtI (113.6±6.1 pF, n=35/25; P=0.340) myocytes. Currents are expressed as current densities (pA/pF), L-type \([\text{Ca}^{2+}]\)-current (\(I_{\text{Ca,L}}\))-triggered \([\text{Ca}^{2+}]\) transients were recorded simultaneously, as previously described.\(^{15}\)

**Biochemistry**

Protein expression of calmodulin, calsequestrin-2, Ca\(^{2+}\)/calmodulin-dependent protein kinase II (CaMKII), GAPDH, Na+/Ca\(^{2+}\) exchanger (NCX1), phospholamban (PLB), catalytic and regulatory protein kinase A (PKA) subunits, protein phosphatase type-1 and type-2A, ryanodine receptor channels (RyR2), and SR Ca\(^{2+}\)-ATPase (SERCA2a) was quantified by immunoblot, as previously described.\(^{18}\) The phosphorylation state of CaMKII (autophosphorylation site Thr287), PLB (PKA site Ser16; CaMKII site Thr17), and RyR2 (PKA site Ser2808; CaMKII site Ser2814) was assessed with phosphospecific antibodies.

**Computational Modeling**

We developed a novel computational model of the human atrial cardiomyocyte based on work by Grandi et al\(^{20}\) and our recent model extension.\(^{21}\) Our model includes a spatial representation of Ca\(^{2+}\)-handling in the human atrial cardiomyocyte based on longitudinal division into \(\approx 2\)-µm-wide segments and transverse division into \(\approx 1\)-µm-long domains. We recently showed that stochastic channel gating is important for accurate simulation of cardiac dynamics, including Ca\(^{2+}\)-handling abnormalities.\(^{22}\) Accordingly, we included stochastic gating of RyR2 based on experimental single-channel recordings.\(^{23}\) The formulation of several ionic currents was updated to reproduce experimentally observed Ca\(^{2+}\)-handling properties (see online-only Data Supplement). The model was implemented in C++ and compiled by using MinGW (model code available at http://www.uni-du.de/pharmakologie). The effects of tetracaine and caffeine were simulated by reducing RyR2 open probability by 90% and setting the open probability to 100%, respectively.

**Statistical Analysis**

Data were analyzed with multilevel mixed-effects models to take into account correlations between multiple levels of within-patient measurements. The generalized estimating equation approach was performed by the use of the binomial distribution to study the dichotomous spontaneous SR Ca\(^{2+}\)-release event and DAD outcomes. When analyses were performed for multiple cells per patient, the unit used for analysis was the independent variable patient ID. For experiments in which there was only 1 measure per patient, one-way analysis of variance was used to compare the groups. When applicable, heterogeneity of variance was accounted for in the models. All analyses were performed with SAS 9.3 (SAS Institute, Cary, NC). Data are reported as mean±standard error of the mean. When multiple recordings are available from some subjects, sample sizes are given as n/N, where n=cells and N=patients.

**Results**

**Basic Electrophysiological Properties**

AP recordings showed no significant group differences in AP duration (APD) at 20%, 50%, and 90% repolarization (Figure 1A and 1B), indicating the absence of AF-associated electrical remodeling, consistent with the prolonged interval since the last AF episode. Resting membrane potential and AP amplitude were also similar (Figure 1C).

We then simultaneously recorded depolarization-induced \(I_{\text{Ca,L}}\) and Ca\(^{2+}\) transients under voltage-clamp conditions. In agreement with the unaltered APD, we found no significant difference in \(I_{\text{Ca,L}}\) (Figure 2A and 2B). However, we observed an increased Ca\(^{2+}\)-transient amplitude (282.1±39.3 nmol/L versus 183.9±15.2 nmol/L; \(P=0.070\); Figure 2C) and an
accelerated time constant of Ca\textsuperscript{2+} decay (\(\tau=215.3\pm20.6\) ms versus 315.8±26.8 ms; \(P=0.030\); Figure 2D) in pAF (n/N=15/9) versus Ctl (n/N=35/25). These findings suggest a potential role for altered Ca\textsuperscript{2+} handling in pAF pathophysiology.

Incidence of Spontaneous SR Ca\textsuperscript{2+}-Release Events

We assessed the occurrence of abnormal spontaneous SR Ca\textsuperscript{2+}-release events (SCaEs) and DADs/triggered activity under current-clamp conditions in the presence of physiological bath Ca\textsuperscript{2+} concentrations (2.0 mmol/L). SCaEs were defined as unstimulated rises in [Ca\textsuperscript{2+}i] following a 1-minute period of AP-triggered Ca\textsuperscript{2+} transients. Potentially arrhythmogenic DADs were defined as SCaE-induced membrane depolarizations exceeding 20 mV. The susceptibility to DADs (ie, the percentage of cells showing DADs) was significantly increased in pAF (Figure 3A and 3B). The proportion of cells with SCaEs, their intrinsic frequency and their amplitude, were numerically greater, without statistical significance, in pAF (Figure 3C, Left). SCaE-induced membrane depolarizations were significantly larger in pAF (Figure 3C).

SR Ca\textsuperscript{2+} Uptake and Ca\textsuperscript{2+} Content

The increased Ca\textsuperscript{2+}-transient amplitude in pAF despite unaltered triggered \(I_{\text{Cal}}\) suggests either enhanced SR Ca\textsuperscript{2+} load or increased Ca\textsuperscript{2+} sensitivity of RyR2. To assess the possibility of increased SR Ca\textsuperscript{2+} load, we applied caffeine to open RyR2 and release all available Ca\textsuperscript{2+} from the SR. Quantification of the amplitude of caffeine-induced Ca\textsuperscript{2+} transients provides a measure of SR Ca\textsuperscript{2+} content, and was significantly increased in pAF (Figure 4A and 4B).\textsuperscript{17} Consistently, charge carried by NCX1 was also numerically increased (\(P=0.109\); Figure 4B). In contrast, the time constant of caffeine-induced Ca\textsuperscript{2+}-transient decay (a measure of NCX function) was similar (Figure 4C). The slope of the line relating \(I_{\text{NCX}}\) to [Ca\textsuperscript{2+}] (indicating the Ca\textsuperscript{2+}-dependent activation of NCX) (Figure 4D and 4E) showed no differences between groups, confirming unaltered NCX function in pAF. Furthermore, atrial NCX1 protein expression was similar for Ctl versus pAF patients (Figure 4F).

Increased SR Ca\textsuperscript{2+} uptake by SERCA2a could explain the augmentation of SR Ca\textsuperscript{2+} content. SERCA2a protein expression was downregulated in pAF (Figure 5A), which would tend to reduce SR Ca\textsuperscript{2+} uptake. However, PKA phosphorylation (at Ser16) of the SERCA2a inhibitor PLB was significantly increased (Figure 5A), which should relieve PLB-induced SERCA2a inhibition and increase SR Ca\textsuperscript{2+} uptake. We determined expression of PKA catalytic and RIigranular subunits, total and Thr287- autophosphorylated CaMKII, calmodulin and protein phosphatase-type-1 and type-2A to identify potential upstream factors contributing to increased Ser16-PLB phosphorylation, but found no significant differences between Ctl and pAF patients (Figures II and III in the online-only Data Supplement). To assess net functional consequences of the altered protein expression and phosphorylation, we calculated the SERCA2a uptake rate based on the rates of \(I_{\text{Cal}}\)-triggered Ca\textsuperscript{2+}-transient decay (reflecting extrusion by both NCX1 and SERCA2a) and the caffeine-triggered Ca\textsuperscript{2+}-transient decay (reflecting Ca\textsuperscript{2+} extrusion via NCX1), as previously described.\textsuperscript{15,23} We noted a significant increase in SERCA2a-mediated SR Ca\textsuperscript{2+} uptake in pAF (Figure 5B and 5C), suggesting that the increased SR Ca\textsuperscript{2+} load may be at least partly attributable to enhanced SERCA2a function.

RyR2 Function

We next assessed SR Ca\textsuperscript{2+} leak with the tetracaine method of Shannon et al\textsuperscript{14} (Figure 6A), whereby SR Ca\textsuperscript{2+} leak is quantified as the drop in [Ca\textsuperscript{2+}]i when RyR2 channels are blocked with tetracaine in cardiomyocytes clamped at −80 mV and perfused with Na\textsuperscript{+}/Ca\textsuperscript{2+}-free bath solution to prevent transsarcomemmal ion fluxes. Application of caffeine was used to assess SR Ca\textsuperscript{2+} load under the same conditions. SR Ca\textsuperscript{2+} leak was increased in pAF (59.6±8.5 mmol/L, n=6/3) versus Ctl (31.6±6.1 mmol/L, n/N=9/3; \(P=0.023\)). The pAF-related increase in SR Ca\textsuperscript{2+} leak could be attributable to intrinsic RyR2 dysregulation or to increased SR Ca\textsuperscript{2+} load (2051.6±290.8 mmol/L, n=6/3) versus Ctl (1158.4±168.3 mmol/L, n/N=9/3; \(P=0.019\); Figure 6C).

Therefore, we assessed RyR2 expression and phosphorylation levels by Western blotting, and RyR2 single-channel properties in lipid bilayers. RyR2 protein expression was significantly increased in both tissue lysates and isolated SR fractions (Figure 6D), with unaltered Ser2814-RyR2 and slightly reduced Ser2808-RyR2 phosphorylation (in SR fractions only). In addition, we found a significantly increased single-channel open probability of RyR2 from pAF patients (0.007±0.003, Ctl, n=8/4 versus 0.032±0.006, pAF, n/N=8/4; \(P=0.021\); Figure 6E). These data suggest that both increased numbers of channels and enhanced single-channel open-probability could contribute to the increased SR Ca\textsuperscript{2+} leak in pAF.

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**Figure 1.** Atrial cardiomyocyte action potential (AP) characteristics in sinus rhythm (Ctl) or paroxysmal atrial fibrillation (pAF) patients. **A**, Atrial APs recorded at 0.5 Hz. **B**, AP duration at 20%, 50%, and 90% repolarization (APD\textsubscript{20}, APD\textsubscript{50}, and APD\textsubscript{90}). **C**, Resting membrane potential (RMP). **D**, AP amplitude. Comparisons using multilevel mixed-effects models. n/N indicates numbers of myocytes/patients.
Computational Modeling of Atrial Ca²⁺ Handling in Ctl and pAF

To further probe the role of altered RyR2 and SERCA2a functions and associated SR Ca²⁺-load increases in pAF-related Ca²⁺-handling abnormalities, we developed a novel computational model of Ca²⁺ handling in human atrial cardiomyocytes (Figure 7A). The model can be used to simulate patch-clamp and pharmacological protocols used experimentally to assess Ca²⁺-handling properties, and it allows visualization of the spatial distribution of [Ca²⁺]i in movies or line-scan representations (Figure 7B). Parameters of the Ctl model were optimized to reproduce a wide range of human atrial cardiomyocyte Ca²⁺-handling properties, including: I_{Ca,L} amplitude; amplitude and decay time constant of I_{Ca,L}-triggered Ca²⁺ transients; SR Ca²⁺ leak; amplitude of caffeine-induced Ca²⁺ transient; and time constant of caffeine-induced Ca²⁺-transient decay (Figure 7C; Figures IV through VI in the online-only Data Supplement).

Incorporation of pAF-dependent alterations in SERCA2a and RyR2 functions (Table IV in the online-only Data Supplement) reproduced experimentally observed Ca²⁺-handling properties (Figure VII in the online-only Data Supplement).

The control model with stochastic RyR2 gating showed isolated SCaEs when clamped at −80 mV following repeated depolarizing voltage-clamp steps to achieve steady-state SR Ca²⁺ loading (Figure 8A). Incorporating either the pAF-related increase in SR Ca²⁺ uptake or RyR2 dysregulation (increased expression and open probability) increased the incidence of SCaEs. A combination of both alterations in the pAF model resulted in increased SR Ca²⁺ load and a much larger SR Ca²⁺ leak, in agreement with our experimental findings. Our computational modeling indicates that both increased SR Ca²⁺ uptake and RyR2 dysregulation likely contribute to the greater incidence of SCaEs/DADs that we observed in pAF cardiomyocytes. As an initial look at potential therapeutic implications, we simulated RyR2 inhibition by flecainide, which produced a dose-dependent reduction in SCaE incidence (Figures IX and X in the online-only Data Supplement), suggesting that inhibition of RyR2 could contribute to flecainide’s antiarrhythmic properties in pAF.

Discussion

In the present study, we observed increased spontaneous cellular activity in atrial cardiomyocytes from pAF patients and analyzed the underlying cellular and molecular mechanisms. Our data showed an absence of AF-associated electrical remodeling like APD abbreviation or I_{Ca,L} reduction in pAF cardiomyocytes. In contrast, experimental observations revealed an increased incidence of DADs attributable to RyR2 dysregulation and increased SR Ca²⁺ uptake, resulting in enhanced SR Ca²⁺ load. Computational modeling confirmed that these Ca²⁺-handling abnormalities are sufficient to increase the incidence and amplitude of potentially arrhythmogenic DADs leading to...
cellular triggered activity. Together, these data point to Ca\(^{2+}\)-dependent triggered activity underlying atrial arrhythmogenesis in pAF patients and identify potential culprit mechanisms.

**Comparison With Previous Studies of AF-Associated Remodeling**

The very rapid, irregular atrial activation in AF induces electrical remodeling, shortening atrial refractory periods and promoting reentry, contributing to the vicious cycle of AF begets AF.\(^{24}\) Downregulation of \(I_{\text{Ca,L}}\) and upregulation of the inward-rectifier K\(^{+}\)-current \(I_{\text{K1}}\) are major components of the AF-induced electrical remodeling that abbreviates APD. Here, we found no differences in APD between pAF and Ctl patients, indicating the absence of electrical-remodeling indicators in pAF patients. These findings agree with previous work showing unchanged L-type Ca\(^{2+}\)-channel \(\alpha_{1C}\)-subunit expression\(^{25}\) and unchanged \(I_{\text{K1}}\) in right-atrial myocytes of pAF patients.\(^{13}\) Although downregulation of K\(^{-}\)-channel subunits (Kv4.3, Kv1.5, Kir3.1) has been described in atrial tissue from pAF patients, which would be expected to prolong APD,\(^{26}\) these changes may have been attributable to the underlying heart disease rather than pAF per se.\(^{5,26}\) In addition, the longer interval since the last pAF episode in the current study (median, 10–20 days) in comparison with the work of Brundel et al (median of 1.5 days)\(^{26}\) suggests that any AF-induced electrical remodeling changes were reversed by the time of tissue procurement in our study and perhaps not in the Brundel investigation.

We and others have shown that long-standing persistent cAF is associated with pronounced Ca\(^{2+}\)-handling abnormalities.\(^{15,27,28}\) Here, we studied for the first time Ca\(^{2+}\)-handling properties in pAF. Although the incidence of SCaEs is increased in both pAF and cAF patients, the underlying molecular mechanisms appear distinct. In particular, the activity of CaMKII is increased in patients with cAF, resulting in hyperphosphorylation of RyR2.\(^{15,28–30}\) RyR2 hyperphosphorylation increases channel open probability and promotes SR Ca\(^{2+}\) leak and SCaEs. In pAF, we found no increase in RyR2 phosphorylation. Nonetheless, there was an increase in single-channel RyR2 open probability, perhaps as a result of other posttranslational modifications of RyR2 (eg, oxidation, \(S\)-nitrosylation). In addition, the levels of certain RyR2-stabilizing subunits such as calsequestrin-2 and junctophilin-2 are not upregulated in pAF,\(^{14}\) whereas here we noted upregulation of RyR2 expression. The increase in RyR2 without change in the associated regulator proteins calsequestrin-2

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**Figure 3.** Spontaneous SR Ca\(^{2+}\)-release events (SCaEs). A, Intracellular \([Ca^{2+}]_{\text{i}}\) (top) and membrane potential (bottom) in Ctl (left) and pAF (right) cardiomyocytes. SCaEs and delayed after-depolarizations (DADs) were assessed during a 1-minute follow-up period after cessation of 0.5-Hz stimulation in 2.0 mmol/L extracellular \([Ca^{2+}]_{\text{e}}\). B, Prevalence of DADs exceeding 20 mV (left) and SCaEs (right). C, Intrinsic frequency of SCaEs (left), SCaE amplitudes (middle), and corresponding membrane-potential changes (right). \(*P<0.05, **P<0.01\) vs Ctl. Comparisons using multilevel mixed-effects models. Ctl indicates sinus rhythm patients; \(n/N\), numbers of myocytes/patients; pAF, paroxysmal atrial fibrillation; and SR, sarcoplasmic reticulum.
and junctophilin-2 would cause relative depletion of such proteins in the RyR2 complex, potentially enhancing channel activity. SR Ca\(^{2+}\) uptake was increased in pAF (opposite to the decrease in cAF), and the consequent enhancement in SR Ca\(^{2+}\) load promotes greater SR Ca\(^{2+}\) leak and a higher frequency of SCaEs and DADs. In cAF, NCX1 expression is increased, producing larger depolarizing inward current for a given amount of free intracellular Ca\(^{2+}\). In contrast, NCX1 expression and its Ca\(^{2+}\)-dependent activation were unaltered in pAF. These differences in the mechanisms underlying Ca\(^{2+}\)-handling abnormalities in pAF versus cAF suggest that specific molecular signatures characterize the different forms of clinical AF, potentially allowing the development of more specific, patient-tailored therapeutic strategies. Of note, the same phenomenological endpoint (increased SR Ca\(^{2+}\) leak, DADs, and triggered activity) can result from quite distinct pathophysiological mechanism complexes in different forms of AF, emphasizing the importance of understanding the underlying specifics of Ca\(^{2+}\)-handling dysregulation rather than simply studying final common heterostatic manifestations.

Computational modeling has proven useful to elucidate the fundamental mechanisms of atrial arrhythmias. However, most currently available atrial cardiomyocyte models do not consider differences in subcellular structure between atrial and ventricular myocytes. In particular, the absence of a pronounced T-tubular network in atrial cardiomyocytes has a major impact on Ca\(^{2+}\)-wave propagation. Recent models have started to incorporate atrial-specific subcellular structures to analyze Ca\(^{2+}\)-wave propagation. However, none of these models addressed the importance of SR Ca\(^{2+}\) leak or the dynamics of abnormal SR Ca\(^{2+}\) release in human atrial cardiomyocytes. Our newly developed model adds multiple novel components to the recently described model of the human atrial cardiomyocyte developed by Grandi et al:\footnote{10} (1) a subcellular structure able to simulate atrial-specific Ca\(^{2+}\)-wave propagation; (2) stochastic gating of RyR2 channels based on single-channel recordings; and (3) an improved representation of the L-type Ca\(^{2+}\) current, reproducing activation and inactivation properties measured in human atrial cardiomyocytes. Using this novel computational model, we were able to demonstrate that the experimentally observed alterations in SR Ca\(^{2+}\) uptake and RyR2 function account for the alterations in Ca\(^{2+}\) handling and greater incidence of SCaEs that we observed in pAF cardiomyocytes.

Figure 4. SR Ca\(^{2+}\) content and Na\(^{+}/Ca\(^{2+}\)-exchange (NCX) current (I\(_{\text{NCX}}\)) in atrial myocytes from Ctl and pAF cardiomyocytes. A, Voltage-clamp protocol (top), and resulting I\(_{\text{Ca,L}}\) and caffeine-triggered Ca\(^{2+}\) transients (middle), or membrane currents (I\(_{\text{m}}\), bottom). B, SR Ca\(^{2+}\) load, quantified with caffeine-triggered Ca\(^{2+}\)-transient amplitude (left), or integrated NCX current (right). C, Time constants of caffeine-triggered Ca\(^{2+}\)-transient decay (reflecting Ca\(^{2+}\)-extrusion via NCX). D, I\(_{\text{NCX}}\) as a function of [Ca\(^{2+}\)]\(_{\text{i}}\). E, Ca\(^{2+}\)-dependence of NCX, based on slope of linear fit to the I\(_{\text{NCX}}\)/[Ca\(^{2+}\)]\(_{\text{i}}\) relationship during the decay of the caffeine-triggered Ca\(^{2+}\)-transient. F, Representative Western blots (top) and mean NCX1 protein expression (bottom) in atrial tissue samples. Calsequestrin (CSQ) was loading control. **P<0.01 vs Ctl. Comparisons using multilevel mixed-effects models (B, C, E) or one-way analysis of variance (F). cCaT indicates caffeine-induced Ca\(^{2+}\)-transient; Ctl, sinus rhythm patients; n/N, numbers of myocytes/patients (B, C, E); N, number of tissue samples (F); pAF, paroxysmal atrial fibrillation; and SR, sarcoplasmic reticulum.
Despite substantial progress in our understanding of AF pathophysiology, the arrhythmogenic mechanisms leading to the spontaneously self-terminating AF episodes typical of pAF patients remained elusive. To the best of our knowledge, we provide the first comprehensive study addressing potential Ca2+-related cellular and molecular atrial proarrhythmic mechanisms in pAF patients. Although reentry and focal Ca2+-driven ectopic activity have been postulated to be potential contributors to pAF pathophysiology, direct experimental evidence at the cellular level has been lacking. We did not observe any indices of AF-induced electrical remodeling, such as APD shortening and I\textsubscript{Ca,L} reduction, in pAF patients. In contrast, our data suggest that SCaEs and corresponding DADs associated with enhanced SR Ca2+ uptake and intrinsic RyR2 dysregulation may represent the cellular correlates of the clinically observed focal ectopic (triggered) activity that triggers and may even maintain AF recurrences in pAF patients.

Our previous and current data suggest that increased diastolic SR Ca2+ leak is a common motif in both pAF and cAF, although the underlying molecular basis and the pathophysiological role are likely distinct for each form of the arrhythmia. Patients with pAF do not have atrial tachycardia–induced remodeling and can usually be successfully treated by targeting of PV triggers.12 The mechanism underlying PV trigger activity has been elusive. Our results indicate that in pAF patients, increased SR Ca2+ leak and incidence of diastolic SR Ca2+-release events promote cellular DADs and triggered activity, which, if they occur synchronously in a critical number of cardiomyocytes, might cause triggered activity that underlies AF episodes. Our data also suggest that increased SR Ca2+ load attributable to enhanced SR Ca2+ uptake conspires with RyR2 dysregulation to increase SR Ca2+ leak and cause SCaEs in pAF patients. Because our model indicates that either increased SR Ca2+ uptake or RyR2 dysregulation can also increase incidence of SCaEs individually, pharmacological approaches to pAF may need to target both components, in contrast to cAF, in which targeting RyR2 hyperphosphorylation alone may be sufficient to suppress SCaEs. New pharmacological approaches to Ca2+-handling abnormalities in pAF might allow for more effective targeting in the early paroxysmal stages of the disorder, before the otherwise inexorable progression to more resistant forms occurs.4

Here, we used a newly developed computational model that is the first human atrial cardiomyocyte model able to simulate potentially arrhythmogenic SCaEs. Importantly, the model was extensively validated based on simultaneous ion current and [Ca2+]\textsubscript{i} recordings at physiological temperature in human atrial myocytes.15,16 Taking advantage of these improvements, we also provide the first computational model of atrial cardiomyocytes in pAF that reproduces key pAF-specific alterations in atrial Ca2+-handling properties.

Figure 5. SR Ca\textsuperscript{2+}-ATPase (SERCA2a) and phospholamban (PLB) expression, phosphorylation, and function in Ctl and pAF patients. A, Representative Western blots (top) for total SERCA2a and total PLB protein expression, and Ser16-PLB phosphorylation, Thr17-PLB phosphorylation, and calsequestrin (CSQ) expression. Bottom shows quantification of total SERCA2a and PLB expression and relative Ser16/Thr17 PLB phosphorylation levels. Group data are normalized to CSQ. B, Representative example of a caffeine experiment, highlighting the decay rate of the systolic (I\textsubscript{Ca,L}-triggered) Ca2+ transient (k\textsubscript{syst}) and the decay rate of the caffeine-induced Ca2+ transient (k\textsubscript{caff}). C, Representative rate constants k\textsubscript{syst} (left), k\textsubscript{caff} (middle), and k\textsubscript{SERCA} (right), obtained as the difference between k\textsubscript{syst} and k\textsubscript{caff} in Ctl and pAF patients. Numbers indicate tissue samples per group (A) or myocytes/patients (C). *P<0.05 vs Ctl. Comparisons using 1-way analysis of variance (A) or multilevel mixed-effects models (C). Ctl indicates sinus rhythm patients; NT, normal tyrode solution; pAF, paroxysmal atrial fibrillation; and SR, sarcoplasmic reticulum.
evidence that RyR2 dysregulation can promote reentry through remodeling of Na⁺ channels and intercellular connexins. Abnormal Ca²⁺ handling in cAF may also modulate other ion channels, potentially shortening APD by activating SK channels or favoring the development of constitutive IK,ACh activity, or contributing to repolarization alternans, which has been associated with AF vulnerability in persistent AF. Finally, RyR2 dysregulation has also been associated with worse structural remodeling following cardiac injury, suggesting that cAF-dependent Ca²⁺-handling abnormalities can promote reentry via atrial structural remodeling. Although the potential arrhythmic role of SR Ca²⁺ leak is much more obvious in pAF than cAF, even in pAF cytosolic SR Ca²⁺ leaks could contribute to remodeling and the development of a reentry substrate leading to progression to persistent and long-lasting persistent forms.

Potential Limitations
Because of the limited availability of human tissue, only right-atrial appendages were used in this study. Other atrial regions, notably the peri-PV left atrium, may play a more prominent role in ectopic activity and reentry (with left-to-right dominance of rotor frequencies). Thus, we cannot exclude that other mechanisms may contribute to pAF initiation in these other regions. For example, we previously showed that the inward-rectifier K⁺ current is increased in left, but not right, atrial myocytes from pAF patients. Nevertheless, right-atrial arrhythmogenic sites clearly occur and can represent one-third of all AF generators in AF patients. In addition, there were some small intergroup differences with respect to age and the incidence of diabetes mellitus that should be considered in interpreting our results.

Figure 6. SR Ca²⁺ leak and ryanodine receptor channel (RyR2) function in Ctl and pAF patients. A, Voltage-clamp protocol (top) and [Ca²⁺]i (bottom) in a representative Ctl experiment, illustrating the method for SR Ca²⁺-leak and SR Ca²⁺-content quantification in human atrial myocytes with the use of the tetracaine protocol developed by Shannon et al. B, Total SR Ca²⁺ leak in Ctl and pAF patients. C, SR Ca²⁺ load quantified by using caffeine-triggered Ca²⁺-transient amplitude. D, Representative Western blots showing total RyR2, Ser2808- or Ser2814-phosphorylated RyR2 in tissue homogenates (left) or SR fractions (right). Bottom graphs show total RyR2 expression and Ser2808 and Ser2814 phosphorylation levels (relative to total RyR2 expression) in corresponding Ctl and pAF samples. Data are shown relative to control. E, Representative RyR2 single-channel recordings in lipid bilayers showing channel openings (upward deflections) at 150 nmol/L cytosolic ([cis] [Ca²⁺]) in RyR2 isolated from Ctl or pAF samples (left). RyR2 open probability (Pₒ) in Ctl or pAF samples (right). Numbers indicate myocytes/patients (B, C), tissue samples (D), or channels/patients (E). *P<0.05 vs Ctl. Comparisons using multilevel mixed-effects models (B, C, E) or one-way analysis of variance (D). CaT indicates Ca²⁺-transient amplitude; Ctl, sinus rhythm patients; CSQ, calsequestrin; NT, normal tyrode; pAF, paroxysmal atrial fibrillation; and SR, sarcoplasmic reticulum.
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the absence of any important structural remodeling in our pAF population. Furthermore, recent work demonstrated no increases in global atrial fibrosis in pAF.41 In addition, RyR2 mutations underlying catecholaminergic polymorphic ventricular tachycardia have also been associated with Ca2+-handling abnormalities and pAF in the absence of structural heart disease,42,43 suggesting that SR Ca2+ leak–related DAD/triggered activity mechanisms of the type identified in our study can be sufficient to underlie pAF.

The individuals from whom we obtained tissue samples of necessity included only patients that underwent open-heart surgery for coronary bypass grafting, valve replacement, or both. Such individuals have numerous comorbidities. The phenotype of atrial cardiomyocytes from our Ctl patients may be different from nondiseased controls, and it is unclear whether the pAF mechanisms identified here also apply to pAF patients without any heart disease. Of necessity, information about AF type and timing were obtained from the clinical chart. Although it would be very useful to perform the types of analyses described here in a population with routine prospective long-term rhythm recording before surgery to relate the precise duration and frequency of AF episodes to the ionic and molecular phenotype, such a study is practically extremely difficult and was impossible for us. The limited nature of our AF characterization must be considered in interpreting our results. It has been suggested that at least 2 types of pAF are likely to exist.2 The first is characterized by frequent short-lived episodes that may result from a repetitive ectopic trigger. The second type generally persists longer (>24 hours) and recurs less frequently. The latter type is likely associated with a reentrant mechanism. Whether, as one might expect, the arrhythmogenic mechanisms identified in the present study are associated with particular clinical presentations needs prospective evaluation in future studies. If the phenotype observed here is a common unifying theme in pAF, it will be important to determine the specific underlying molecular abnormalities. In particular, the analysis of the precise molecular mechanisms contributing to increased RyR2 protein expression and greater channel open probability, along with enhanced SERCA2a activity, are beyond the

Figure 7. Computational modeling of human atrial Ca2+ handling. A, Schematic of a single longitudinal segment, highlighting the 18 transverse Ca2+ domains, subcellular Ca2+ diffusion, subcellular compartments (Cyt, cytosol; SR, sarcoplasmic reticulum; subsarcolemmal space), and Ca2+-transport mechanisms (SERCA2a, SR Ca2+-ATPase; RyR2, ryanodine-receptor channel type-2; I_{Ca,L}, L-type Ca2+ current; I_{NCX}, Na+-Ca2+-exchange current; I_{pmCa}, plasmalemmal Ca2+-ATPase). Ca2+ buffers and other ion currents are also incorporated into the model but omitted here for clarity. B, Experimental voltage-clamp protocol (top), along with simulated whole-cell I_{Ca,L}-triggered Ca2+ transient (CaT) and whole-cell caffeine-triggered Ca2+ transient (cCaT; bottom). Inset, Transverse line-scan representation of [Ca2+]i at center of virtual cell, showing Ca2+-wave propagation from subsarcolemma to cell center; color-scale below. C, Comparison between key Ca2+-handling properties in the model (Mdl, black bars) in comparison with experimental data in Ctl myocytes (Exp, white bars, from Figures 2, 4, and 6). Top left, Peak I_{Ca,L} amplitude; Top middle, CaT amplitude; Top right, time constants of CaT decay; Bottom left, SR Ca2+ leak; Bottom middle, SR Ca2+ load (amplitude of cCaT); Bottom right, time constants of cCaT decay. Numbers indicate myocytes/patients (for experimental data). Experimental data are shown as mean±SEM; model simulations produce single value (no error bar relevant).

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scope of the current project and should be addressed in future detailed studies.

Conclusions
In this study, we evaluated the cellular and molecular determinants of intracellular Ca\(^{2+}\) handling in pAF patients, and observed an increased incidence of SCaEs attributable to increased SR Ca\(^{2+}\) load and RyR2 dysregulation, causing DADs and triggered activity. The underlying molecular basis appeared to be enhanced SR Ca\(^{2+}\) uptake caused by PLB hyperphosphorylation, and increased expression and open probability of RyR2. The novel experimental and computational insights we obtained into fundamental arrhythmogenic mechanisms in pAF may facilitate the development of safer and more effective mechanism-based therapeutic strategies.

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We thank Claudia Liebetrau, Ramona Nagel, and Katrin Kupser for excellent technical assistance, the cardiac surgeons of Heart Center Heidelberg for kindly providing human atrial tissue samples, and Annik Fortier for superb statistical advice/analysis.

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Disclosures
None.

References


Atrial fibrillation (AF) is the most common arrhythmia in clinical practice. There is substantial information available about cellular electrophysiological abnormalities in patients with long-standing persistent AF, but the atrial-cellular pathophysiology in patients with paroxysmal AF (pAF) is largely unknown. Here, we used simultaneous measurements of intracellular [Ca^{2+}] and membrane current/potential in atrial cardiomyocytes from sinus rhythm and pAF patients (last AF episode a median of 10–20 days preoperatively), together with biochemistry and computational modeling, to define the cellular and molecular mechanisms promoting atrial arrhythmogenesis in pAF. L-type Ca^{2+} currents and action potential durations were unaltered in pAF patients, indicating the absence of typical AF-associated remodeling. However, cardiomyocytes from pAF patients had enhanced diastolic sarcoplasmic reticulum (SR) Ca^{2+} leak and increased susceptibility to spontaneous diastolic SR Ca^{2+}-release events, causing delayed after-depolarizations/triggered activities that promote atrial ectopic (focal) activity. Pharmacological and biochemical studies indicated that the susceptibility to spontaneous cellular activity in pAF was attributable to enhanced SR Ca^{2+} uptake, resulting from phospholamban hyperphosphorylation (removing phospholamban-induced inhibition of SR uptake), and ryanodine receptor (SR Ca^{2+} release) channel dysregulation, including enhanced ryanodine receptor expression and increased single-channel open probability. Simulation studies indicated that both increased SR Ca^{2+} leak and enhanced SR Ca^{2+} uptake likely contribute to aberrant diastolic SR Ca^{2+}-release events. Our findings constitute the first direct evidence for an important role of Ca^{2+}-dependent ectopic activity in atrial arrhythmogenesis of pAF patients and provide insights into underlying mechanisms. The cellular and molecular mechanisms underlying abnormal Ca^{2+} handling in pAF are distinct from those of long-standing persistent AF patients, suggesting possible opportunities to develop tailored therapeutic approaches for pAF.
Cellular and Molecular Mechanisms of Atrial Arrhythmogenesis in Patients With Paroxysmal Atrial Fibrillation

Niels Voigt, Jordi Heijman, Qiongling Wang, David Y. Chiang, Na Li, Matthias Karck, Xander H.T. Wehren, Stanley Nattel and Dobromir Dobrev

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Cellular and Molecular Mechanisms of Atrial Arrhythmogenesis in Patients with Paroxysmal Atrial Fibrillation

Supplemental Material

Niels Voigt, MD*, Jordi Heijman, PhD*, Qiongling Wang, PhD, David Y. Chiang, BSc, Na Li, PhD, Matthias Karck, MD, Xander H.T. Wehrens, MD, PhD, Stanley Nattel, MD, and Dobromir Dobrev, MD

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E-Mail: Dobromir.Dobrev@uk-essen.de
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Patient Characteristics

Right-atrial appendages were dissected from sinus rhythm (Ctl) patients and paroxysmal AF (pAF) patients (defined as patients with normal sinus rhythm during the operation but at least one documented self-terminating AF episode lasting less than 7 days, Online Figure I) undergoing open-heart surgery (Online-Tables I-III). Experimental protocols were approved by the Medical Faculty Mannheim, University of Heidelberg (No. 2011–216N-MA). Each patient gave written informed consent.

Online Table I: Characteristics of patients used for functional studies

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<td>episode, days; median (range)</td>
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Values are presented as mean±SEM, median (range), or number of patients. CAD, coronary artery disease; LAD, left atrial diameter, MVD/AVD, mitral/aortic valve disease; LVEF, left ventricular ejection fraction; ACE, angiotensin-converting enzyme; AT, angiotensin receptor.
**Online Table II: Characteristics of patients used to determine Ca\(^{2+}\)-handling parameters in voltage-clamp experiments**

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<td>Hyperlipidemia, n</td>
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<td>7</td>
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<td>LAD, mm</td>
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Values are presented as mean±SEM, median (range), or number of patients. CAD, coronary artery disease; MVD/AVD, mitral/aortic valve disease; LAD, left atrial diameter; LVEF, left ventricular ejection fraction; ACE, angiotensin-converting enzyme; AT, angiotensin receptor.
### Online Table III: Characteristics of patients used for biochemistry

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<td>Hyperlipidemia, n</td>
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<td>LAD, mm</td>
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<td>43.0±1.0</td>
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<td>LVEF, %</td>
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<td>Lipid-lowering drugs, n</td>
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</table>

Values are presented as mean±SEM, median (range), or number of patients. CAD, coronary artery disease; MVD/AVD, mitral/aortic valve disease; LAD, left atrial diameter; LVEF, left ventricular ejection fraction; ACE, angiotensin-converting enzyme; AT, angiotensin receptor.
Experimental Methods

1. Measurement of Intracellular [Ca\textsuperscript{2+}] and Patch-clamp Experiments

Intracellular [Ca\textsuperscript{2+}] was quantified using Fluo-3-acetoxymethyl ester (Fluo-3 AM; Invitrogen, Carlsbad, CA; 10 µmol/L, 10 min loading and 30 min de-esterification).\textsuperscript{1} Simultaneously, membrane currents or -potentials were recorded in whole-cell ruptured-patch configuration using voltage-clamp or current-clamp techniques, respectively. Borosilicate glass microelectrodes had tip resistances of 2-5 MΩ when filled with pipette solution, containing (in mmol/L): EGTA 0.02, Fluo-3 0.1 (Invitrogen), GTP-Tris 0.1, HEPES 10, K-aspartate 92, KCl 48, Mg-ATP 1, Na\textsubscript{2}-ATP 4; pH=7.2. Seal-resistances were 4-8 GΩ. For voltage-clamp experiments series resistance and cell capacitance were compensated.

During experiments, myocytes were superfused at 37°C with a bath solution containing (in mmol/L): CaCl\textsubscript{2} 2, glucose 10, HEPES 10, KCl 4, MgCl\textsubscript{2} 1, NaCl 140, probenecid 2; pH=7.4. For voltage-clamp experiments, K\textsuperscript{+}-currents were blocked by adding 4-aminopyridine (5 mmol/L) and BaCl\textsubscript{2} (0.1 mmol/L) to the bath solution. pClamp-Software (V10.2, Molecular Devices, Sunnyvale, CA) was used for data acquisition and analysis. Drugs were applied via a rapid solution exchange system (ALA Scientific Instruments, Long Island, NY). Caffeine (10 mmol/L) was used for rapid depletion and quantification of SR Ca\textsuperscript{2+}-content.\textsuperscript{2}

L-type Ca\textsuperscript{2+}-currents (I\textsubscript{Ca,L}) were activated at a rate of 0.5 Hz in voltage-clamp configuration using the following protocol: starting from a holding-potential of -80 mV, fast Na\textsuperscript{+}-currents were inactivated using a 100-ms ramp-pulse to -40 mV. A consecutive 100-ms depolarizing step to +10 mV activated I\textsubscript{Ca,L}, which triggered corresponding [Ca\textsuperscript{2+}]\textsubscript{i}-transients (CaTs). Action potentials were triggered in current-clamp configuration using 1 ms current pulses of 1.2x threshold strength applied at 0.5 Hz.

2. Quantification of SERCA2a Activity

Ca\textsuperscript{2+}-reuptake into the SR mediated by SR Ca\textsuperscript{2+}-ATPase (SERCA2a) and Ca\textsuperscript{2+} extrusion into extracellular space by Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger (NCX1, forward-mode) are the major contributors to the decay of I\textsubscript{Ca,L}-induced Ca\textsuperscript{2+}-transient (CaT).\textsuperscript{3} This decay is commonly described with a mono-exponential equation:

\[
[Ca^{2+}]_i(t) = \left([Ca^{2+}]_{\text{Amplitude}} \times \exp(-k_{\text{syst}} \times t)\right) + [Ca^{2+}]_{\text{diast}}
\]

where [Ca\textsuperscript{2+}]\textsubscript{i}(t), [Ca\textsuperscript{2+}]\textsubscript{Amplitude} and [Ca\textsuperscript{2+}]\textsubscript{diast} represent the Ca\textsuperscript{2+} concentration at the timepoint t, the CaT amplitude and the diastolic Ca\textsuperscript{2+} level, respectively. The rate constant of decay k\textsubscript{syst} largely reflects the rate of combined Ca\textsuperscript{2+} transport by SERCA and NCX and may therefore be expressed as the sum of the two separate rate constants:
Since, the decay of caffeine-induced CaT (cCaT) is almost exclusively due to Ca$^{2+}$ removal via NCX, $k_{NCX}$ can be estimated by analysing the rate of decay of the cCaT ($k_{caff}$):

$$k_{caff} \approx k_{NCX}$$

Using this approach SERCA activity can be estimated by:

$$k_{SERCA} = k_{syst} - k_{caff}$$

### 3. Quantification of Diastolic SR Ca$^{2+}$-Leak with Tetracaine

SR Ca$^{2+}$-leak in intact human atrial myocytes was measured according to Shannon et al.\textsuperscript{4} using Na$^+$- and Ca$^{2+}$-free bath solution (containing, in mmol/L: 4-aminopyridine 5, BaCl$_2$ 0.1, EGTA 10, glucose 10, HEPES 10, KCl 4, LiCl 140, Mg$_2$Cl 1, probenecid 2) and tetracaine (1 mmol/L).

### 4. Immunoblot Analysis

The protein levels of calsequestrin (CSQ, 1:2500; Dianova, Hamburg, Germany), NCX1 (1:1000; Fitzgerald, Concord, MA); total RyR2 (1:500, Affinity BioReagents, Golden, CO), Ser2808- and Ser2814-phosphorylated RyR2 (1:2000 and 1:1000, respectively),\textsuperscript{5,6} SERCA2a (1:2000, Santa Cruz Biotechnology, Santa Cruz, CA), total, Ser16- and Thr287-phosphorylated phospholamban (PLB; all 1:5000, Badrilla Ltd., Leeds, United Kingdom), catalytic PKA-subunit (PKAc, 1:1000; BD Biosciences, Franklin Lakes, NJ); regulatory PKAlu-subunit (PKAlu, 1:500, Santa Cruz Biotechnology), calmodulin (1:1000; abcam, Cambridge, UK), total CaMKII$\delta$ (1:200; Santa Cruz Biotechnology, Santa Cruz, CA), Thr287-phosphorylated CaMKII$\delta$ (1:5000; Promega, Madison, WI), catalytic PP1$\alpha$-subunit (1:500; Biomol, Plymouth Meeting, Pa), and total catalytic PP2A (1:1000, affinity purified; Upstate Biotechnology, Lake Placid, NY), were quantified by Western blotting as described previously.\textsuperscript{7,8} The Ser2808-RyR2 and Ser2814-RyR2 phospho-epitope-specific antibodies were custom generated using the peptide C-RTRRI-(pS)-QTSQV corresponding to the PKA phosphorylation site region at Ser2808 on RyR2 and peptide CSQTSQV-(pS)-VD corresponding to CaMKII phosphorylated RyR2 at Ser2814.

Appropriate peroxidase-conjugated goat anti-rabbit (Sigma-Aldrich) and goat anti-mouse (Sigma-Aldrich) were used as secondary antibodies and visualized by chemiluminescence (GE Healthcare, Chalifont St. Giles, UK). Chemiluminescence was quantified using a LAS1000 chemiluminescence detection system (FujiFilm, Düsseldorf, Germany) and AIDA software (Raytest, Sprockhövel, Germany). Protein expression was normalized to CSQ, which was unchanged in paroxysmal AF compared to sinus rhythm (Ctl) samples (Online Figure III), in agreement with previous findings.\textsuperscript{9}
5. Preparation of Sarcoplasmic-reticulum (SR) Fractions

Atrial tissues were homogenized on ice in a solution containing 10 mM HEPES, 500 mM sucrose and 5 mM EDTA supplemented with 20 mM NaF, 1 mM Na₃VO₄, and protease inhibitor and phosphatase inhibitor cocktails (cOmplete, Mini and PhosSTOP from Roche Applied Science, Indianapolis, IN). The homogenates were then subjected to three sequential steps of centrifugation. First, the homogenates were centrifuged at 3,800 g for 15 min at 4°C. Second, the supernatants were collected and centrifuged at 27,900 g for 15 min at 4°C. Third, the supernatants were ultracentrifuged at 110,000 g for 1 hour at 4°C. The supernatants were removed and the pellets resuspended in 50 µl of the same solution as for homogenization but without the EDTA. The resultant suspensions were used for single channel recording and Western blot analysis.

6. Quantification of RyR2 Expression and Phosphorylation in SR-fractions

Sarcoplasmic reticulum preparations were incubated with 2x Laemmli Sample Buffer (Bio-Rad, Hercules, CA) at room temperature for 30 min before being subjected to electrophoresis on 5 and 10% stacked acrylamide gels, and transferred onto polyvinyl difluoride membranes overnight at 4°C. Antibodies against the following targets were used to probe the membranes: RyR2 (MA3-916; Thermo Fisher Scientific, Waltham, MA), RyR2 pS2808 (custom), RyR2 pS2814 (custom), and calsequestrin (CSQ; PA1-913; Thermo Fisher Scientific). Membranes were then incubated with secondary anti-mouse and anti-rabbit antibodies conjugated respectively to Alexa-Fluor 680 (Molecular Probes, Eugene, OR) and IR800Dye (Rockland Immunochemicals, Gilbertsville, PA). Bands were quantified using Image J.

7. RyR2 Single-channel Recordings

Single-channel recordings of ryanodine-receptor channels (cardiac subtype; RyR2) were obtained under voltage-clamp conditions at 0 mV, as previously described.¹⁰ Atrial SR membrane-preparations were incorporated into lipid-bilayer membranes comprised of a mixture of phosphatidylethanolamine and phosphatidylserine at a 3:1 ratio (Avanti Polar Lipids, Alabaster, AL) dissolved in n-decane (25 mg/ml). Bilayers were formed across a 150 µm aperture of a polystyrene cuvette. The trans chamber (corresponding to the luminal side of the SR) contained (in mmol/L) HEPES 250, KCl 50 and Ca(OH)₂ 53. The cis chamber (corresponding to the cytosolic side of the SR) contained (in mmol/L) HEPES 250, Tris-base 125, KCl 50, EGTA 1, CaCl₂ 0.5, pH=7.35. Ryanodine (5 µmol/L) was applied to the cis chamber to confirm identity of RyR2 channels at the end of each experiment. Data were collected using Digidata 1322A (Molecular Devices, Sunnyvale, CA) and Warner Bilayer Clamp Amplifier BC-535 (Warner Instruments, Hamden, CT) under voltage-clamp conditions. Cytosolic free \([\text{Ca}^{2+}]\) was calculated with WinMax32. Data were analyzed from digitized current recordings using pCLAMP-9.2 software (Molecular Devices).
Definition of Computational Model

1. Ca\(^{2+}\)-buffers and Simulation of EGTA

The Ca\(^{2+}\)-buffers in the cytosol (low-affinity troponin-C sites, high-affinity Ca\(^{2+}\) and Mg\(^{2+}\) troponin-C sites, calmodulin, myosin, and SR-membrane sites), in the SR (calsequestrin) and in the subsarcolemmal compartments (low- and high-affinity sarcolemmal binding sites) were incorporated into every Ca\(^{2+}\)-domain \(d\) of each segment \(s\) based on their first-order implementation in the model by Grandi et al.\(^{11}\) Unless specified otherwise, all simulations were performed with normal, intact (non-Ca\(^{2+}\)-buffered) Ca\(^{2+}\) handling ([EGTA]\(_{\text{max}}\) = 0 mmol/L). In order to reproduce experiments performed in the presence of EGTA in the pipette solution, we included EGTA as a potential Ca\(^{2+}\)-buffer in a subset of simulations. EGTA was included as a Ca\(^{2+}\)-buffer in the cytosolic and subsarcolemmal compartments with a maximum concentration identical to that specified in the experimental conditions and with first-order on and off rates based on the work of Smith et al.:\(^{12}\)

\[
\begin{align*}
\frac{d[\text{EGTA}]_{\text{bound,cyt}}}{dt} &= k_{\text{on,EGTA}} \times [\text{Ca}^{2+}]_\text{cyt}^{5d} \times ([\text{EGTA}]_{\text{max}} - [\text{EGTA}]_{\text{bound,cyt}}) \\
- k_{\text{off,EGTA}} \times [\text{EGTA}]_{\text{bound,cyt}} \\
k_{\text{on,EGTA}} &= 5.0 \text{ (mmol/L)}^{-1}\text{ms}^{-1}, \quad k_{\text{off,EGTA}} = 7.5 \times 10^{-4} \text{ ms}^{-1} \\
\end{align*}
\]

\[
\begin{align*}
[\text{Ca}^{2+}]_{\text{buf,ca,cyt}} &= \frac{d[\text{TnC}_{\text{low}}]_{\text{bound}}}{dt} + \frac{d[\text{TnC}_{\text{high,Ca}}]_{\text{bound}}}{dt} + \frac{d[\text{TnC}_{\text{high,Mg}}]_{\text{bound}}}{dt} + \frac{d[\text{CaM}]_{\text{bound}}}{dt} \\
&+ \frac{d[\text{MyosinCa}]_{\text{bound}}}{dt} + \frac{d[\text{MyosinMg}]_{\text{bound}}}{dt} + \frac{d[\text{SR}]_{\text{bound}}}{dt} + \frac{d[\text{EGTA}]_{\text{bound,cyt}}}{dt} \\
\end{align*}
\]

2. Model Structure

2.1. Cellular Compartmentation

The virtual model cell was divided into 50 longitudinal segments (\(N_{\text{Seg}} = 50\)). Each segment contains two membrane domains (including subsarcolemmal space) with all transmembrane ion channels, and 18 subcellular Ca\(^{2+}\)-domains (\(N_{\text{Dom}} = 18\)). Each domain contains a cytosolic space, sarcoplasmic reticulum (SR), and SR Ca\(^{2+}\)-release space (SRS; representing a microdomain around the SR in which neighboring ryanodine receptor (RyR2) channels can activate each other. In addition to RyR2 channels, each Ca\(^{2+}\)-domain contains formulations for SR Ca\(^{2+}\)-uptake by a SERCA2a/PLB complex.

The volume of each subcellular compartment was similar to that in Grandi et al.,\(^{11}\) with the volume of the newly introduced SRS compartment set at 0.1% of cell volume:

\[
\begin{align*}
v_{\text{cell}} &= 33 \text{ pL} \\
v_{\text{cyt}} &= 0.65 \times v_{\text{cell}} \quad v_{\text{SR}} = 0.035 \times v_{\text{cell}} \\
v_{\text{SRS}} &= 0.001 \times v_{\text{cell}} \quad v_{\text{subsl}} = 0.02 \times v_{\text{cell}}
\end{align*}
\]
2.2. Ca\textsuperscript{2+}-Diffusion

For every segment $s$, Ca\textsuperscript{2+} can diffuse from a given Ca\textsuperscript{2+}-domain $d$ to its left neighbor $d-1$ and its right neighbor $d+1$ (Figure 7A). This diffusion is implemented for all three compartments (albeit with different diffusion rates). The left subsarcolemmal space is considered the left neighbor of Ca\textsuperscript{2+}-domain 0 (the leftmost domain). Similarly, the right subsarcolemmal space is considered the right neighbor of Ca\textsuperscript{2+}-domain 17 (the rightmost domain). The diffusion between segments occurs similarly, with Ca\textsuperscript{2+} diffusing from segment $s$, domain $d$ to domain $d$ in segments $s-1$ and segment $s+1$.

For every segment $s$ (between 0 and 49, inclusive) and domain $d$ (between 0 and 17, inclusive), Ca\textsuperscript{2+} diffusion is then determined as follows (with $[\text{Ca}^{2+}]_{X}^{-1,d} = [\text{Ca}^{2+}]_{X}^{0,d}$, $[\text{Ca}^{2+}]_{X}^{50,d} = [\text{Ca}^{2+}]_{X}^{49,d}$, $[\text{Ca}^{2+}]_{X}^{s-1} = [\text{Ca}^{2+}]_{X}^{s,0}$, and $[\text{Ca}^{2+}]_{X}^{s,18} = [\text{Ca}^{2+}]_{X}^{s,17}$ for all three compartments):

\[
\begin{align*}
\tau_{\text{diff.dom.cyt}} &= 0.60 \text{ ms}, & \tau_{\text{diff.seg.cyt}} &= \tau_{\text{diff.dom.cyt}} \\
\tau_{\text{diff.dom.SR}} &= 15 \text{ ms}, & \tau_{\text{diff.seg.SR}} &= \tau_{\text{diff.dom.SR}} \\
\tau_{\text{diff.dom.SRS}} &= 0.125 \text{ ms}, & \tau_{\text{diff.seg.SRS}} &= 0.30 \text{ ms} \\
\tau_{\text{diff.SRS}} &= 12 \text{ ms} \\
\end{align*}
\]

\[
\begin{align*}
J_{\text{diff.cyt}}^{s,d} &= \left( [\text{Ca}^{2+}]_{\text{cyt}}^{s,d-1} + [\text{Ca}^{2+}]_{\text{cyt}}^{s,d+1} - 2 \times [\text{Ca}^{2+}]_{\text{cyt}}^{s,d} \right) / \tau_{\text{diff.dom.cyt}} \\
&\quad + \left( [\text{Ca}^{2+}]_{\text{cyt}}^{s-1,d} + [\text{Ca}^{2+}]_{\text{cyt}}^{s+1,d} - 2 \times [\text{Ca}^{2+}]_{\text{cyt}}^{s,d} \right) / \tau_{\text{diff.seg.cyt}} \\
J_{\text{diff.SR}}^{s,d} &= \left( [\text{Ca}^{2+}]_{\text{SR}}^{s,d-1} + [\text{Ca}^{2+}]_{\text{SR}}^{s,d+1} - 2 \times [\text{Ca}^{2+}]_{\text{SR}}^{s,d} \right) / \tau_{\text{diff.dom.SR}} \\
&\quad + \left( [\text{Ca}^{2+}]_{\text{SR}}^{s-1,d} + [\text{Ca}^{2+}]_{\text{SR}}^{s+1,d} - 2 \times [\text{Ca}^{2+}]_{\text{SR}}^{s,d} \right) / \tau_{\text{diff.seg.SR}} \\
J_{\text{diff.SRS}}^{s,d} &= \left( [\text{Ca}^{2+}]_{\text{SRS}}^{s,d-1} + [\text{Ca}^{2+}]_{\text{SRS}}^{s,d+1} - 2 \times [\text{Ca}^{2+}]_{\text{SRS}}^{s,d} \right) / \tau_{\text{diff.dom.SRS}} \\
&\quad + \left( [\text{Ca}^{2+}]_{\text{SRS}}^{s-1,d} + [\text{Ca}^{2+}]_{\text{SRS}}^{s+1,d} - 2 \times [\text{Ca}^{2+}]_{\text{SRS}}^{s,d} \right) / \tau_{\text{diff.seg.SRS}} \\
J_{\text{diff.SRS-cyt}}^{s,d} &= \left( [\text{Ca}^{2+}]_{\text{SRS-cyt}}^{s,d} - [\text{Ca}^{2+}]_{\text{cyt}}^{s,d} \right) / \tau_{\text{diff.SRS-cyt}} \\
\end{align*}
\]

Similarly, Ca\textsuperscript{2+} diffuses between segments in both left and right subsarcolemmal compartments ($m$):

\[
\begin{align*}
\tau_{\text{diff.seg.subs1}} &= 3.4 \text{ ms} \\
J_{\text{diff.subs1}}^{s,m} &= \left( [\text{Ca}^{2+}]_{\text{subs1}}^{s-1,m} + [\text{Ca}^{2+}]_{\text{subs1}}^{s+1,m} - 2 \times [\text{Ca}^{2+}]_{\text{subs1}}^{s,m} \right) / \tau_{\text{diff.subs1}} \\
\end{align*}
\]

2.3. Changes in [Ca\textsuperscript{2+}]

For all domains (with the exception of the leftmost and rightmost) in every segment, (i.e., $d$ between 1 and 16, inclusive), intracellular Ca\textsuperscript{2+} concentrations were updated as follows:
For both subsarcolemmal compartments (m) in each segment:
\[ I_{Ca,subsl,tot}^{s,m} = I_{Ca,b,subsl}^{s,m} + I_{Ca,S,R}^{s,m} - 2 \times I_{NCX,subsl}^{s,m} \]

\[ k_{on,SL,Low} = 100 \text{ (mmol/L)}^{-1}\text{ms}^{-1}, \quad k_{off,SL,Low} = 1.3 \text{ ms}^{-1} \]
\[ k_{on,SL,High} = 100 \text{ (mmol/L)}^{-1}\text{ms}^{-1}, \quad k_{off,SL,High} = 0.03 \text{ ms}^{-1} \]

\[ [SL,Low]_{subsl,max} = 37.4 \cdot 10^{-3} \times \frac{v_{cyl}}{v_{subsl}} \]
\[ [SL,High]_{subsl,max} = 13.4 \cdot 10^{-3} \times \frac{v_{cyl}}{v_{subsl}} \]

\[ \frac{d[Ca^{2+}]^{s,d}_{cyt}}{dt} = -j_{up}^{s,d} \times \frac{v_{SR}}{v_{cyl}} - j_{Buffer, Ca,cyl}^{s,d} + j_{diff,cyl}^{s,d} + j_{diff,SRS-cyt}^{s,d} \]

\[ \frac{d[Ca^{2+}]^{s,d}_{SRS,tot}}{dt} = j_{rel}^{s,d} \times \frac{v_{SR}}{v_{SRS}} + j_{diff,SRS}^{s,d} - j_{diff,SRS-cyt}^{s,d} \times \frac{v_{cyl}}{v_{SRS}} \]

\[ \frac{d[Ca^{2+}]^{s,d}_{SR,tot}}{dt} = j_{up}^{s,d} - j_{rel}^{s,d} + j_{diff,SR}^{s,d} \]

\[ \frac{d[EGTA]^{s,m}_{bound,subsl}}{dt} = k_{on,EGTA} \times [Ca^{2+}]^{s,m}_{subsl} \times ([EGTA]^{s,m}_{max} - [EGTA]^{s,m}_{bound,subsl}) - k_{off,EGTA} \times [EGTA]^{s,m}_{bound,subsl} \]

\[ \frac{d[SL,Low]^{s,m}_{bound,subsl}}{dt} = k_{on,SL,Low} \times [Ca^{2+}]^{s,m}_{subsl} \times ([SL,Low]^{s,m}_{subsl,max} - [SL,Low]^{s,m}_{bound,subsl}) - k_{off,SL,Low} \times [SL,Low]^{s,m}_{bound,subsl} \]

\[ \frac{d[SL,High]^{s,m}_{bound,subsl}}{dt} = k_{on,SL,High} \times [Ca^{2+}]^{s,m}_{subsl} \times ([SL,High]^{s,m}_{subsl,max} - [SL,High]^{s,m}_{bound,subsl}) - k_{off,SL,High} \times [SL,High]^{s,m}_{bound,subsl} \]

\[ j_{Buffer, Ca,subsl} = \frac{d[EGTA]^{s,m}_{bound,subsl}}{dt} + \frac{d[SL,Low]^{s,m}_{bound,subsl}}{dt} + \frac{d[SL,High]^{s,m}_{bound,subsl}}{dt} \]

For the first, leftmost domain (d=0) connected to membrane 0:
\[ j_{diff,cyl->subsl}^{s,0} = k_{Ca,cyl->subsl} \times ([Ca^{2+}]^{s,0}_{subsl} - [Ca^{2+}]^{s,0}_{cyl}), \quad k_{Ca,cyl->subsl} = 6.5486 \cdot 10^{-12} \]
\[ j_{diff,SRS->subsl}^{s,0} = k_{Ca,SRS->subsl} \times ([Ca^{2+}]^{s,0}_{subsl} - [Ca^{2+}]^{s,0}_{SRS}), \quad k_{Ca,SRS->subsl} = 8.2413 \cdot 10^{-15} \]
\[ I_{Ca,S,R}^{s,0}_{tot} = I_{Ca,L}^{s,0} + I_{Ca,B,R}^{s,0} + I_{pCa,SRS}^{s,0} - 2 \times I_{NCX,SRS}^{s,0} \]

\[ \frac{d[Ca^{2+}]^{s,0}_{cyl}}{dt} = -j_{up}^{s,0} \times \frac{v_{SR}}{v_{cyl}} - j_{Buffer, Ca,cyl}^{s,0} + j_{diff,cyl->subsl}^{s,0} \times \frac{0.5}{v_{cyl}/N_{Dom}} + j_{diff,cyl}^{s,0} + j_{diff,SRS->cyl}^{s,0} \]
For the last, rightmost domain ($d=17$) connected to membrane $1$:

$$j_{\text{diff,cyt\ right}}^{5,1} = k_{Ca, cyt \rightarrow \text{subsl}} \times ([Ca^{2+}]_{\text{subsl}}^{5,1} - [Ca^{2+}]_{\text{cyt}}^{5,1})$$

$$j_{\text{diff,SRS\ right}}^{5,1} = k_{Ca,SRS \rightarrow \text{subsl}} \times ([Ca^{2+}]_{\text{subsl}}^{5,1} - [Ca^{2+}]_{\text{SRS}}^{5,1})$$

$$I_{Ca,tot}^{5,1} = I_{CaL}^{5,1} + I_{Ca,b,SRS}^{5,1} + I_{pCaSRS}^{5,1} - 2 \times I_{NCX,SRS}^{5,1}$$

The ‘free’ concentration of $[Ca^{2+}]_{SRS}$ and $[Ca^{2+}]_{SR}$ was calculated based on the total concentrations and the known $Ca^{2+}$-buffer properties in each compartment, using quasi-steady-state assumptions:

$$\text{Buffer}^{s,d}_{Ca,SRS,b} = [SLLow]_{SRS,\text{max}} + [SLLow]_{SRS,\text{max}} - [Ca^{2+}]_{SRS,\text{tot}}^{s,d} + K_{m,SLlow} + K_{m,SLhigh}$$

$$\text{Buffer}^{s,d}_{Ca,SRS,c} = K_{m,SLlow} \cdot K_{m,SLhigh} \cdot [Ca^{2+}]_{SRS,\text{tot}}^{s,d} \cdot (K_{m,SLlow} + K_{m,SLhigh}) + [SLLow]_{SRS,\text{max}} \cdot K_{m,SLhigh} + [SLLow]_{SRS,\text{max}} \cdot K_{m,SLlow}$$

$$\text{Buffer}^{s,d}_{Ca,SRS,d} = -K_{m,SLlow} \cdot K_{m,SLhigh} \cdot [Ca^{2+}]_{SRS,\text{tot}}^{s,d}$$

$$K_{m,SLlow} = \frac{k_{off,SLlow}}{k_{on,SLlow}}, \quad K_{m,SLhigh} = \frac{k_{off,SLHigh}}{k_{on,SLHigh}}$$
3. Ryanodine Receptor Channel

We employed the 4-state Markov model of the human atrial action potential (AP) model by Grandi et al.\textsuperscript{11} to simulate RyR2 gating (Online Figure IV A). The model structure reflects activation (left and right halves of the Markov model), predominantly by an increase in [Ca\textsuperscript{2+}] in the SRS, and regulation by SR Ca\textsuperscript{2+} (top and bottom halves of the Markov model). The mechanisms controlling termination of Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+}-release and subsequent refractoriness of RyR2 remain an area of intensive investigation.\textsuperscript{13} Recent research has suggested several potential contributing mechanisms. Although direct inactivation of RyR2 appears unlikely, there is strong evidence for regulation of RyR2 activity by luminal [Ca\textsuperscript{2+}].\textsuperscript{14} The lower half of the model reflects such a non-conducting ‘inactivated’ state which is controlled by luminal Ca\textsuperscript{2+}. Model parameters were optimized such that the model operates around a steady-state SR Ca\textsuperscript{2+}-load that is consistent with experimental observations and is able to reproduce key properties of single-channel RyR2 recordings obtained in bilayer experiments with RyR2 from human atrial samples (Online Figure IV B, C).\textsuperscript{15} For the pAF model, RyR2 open probability at diastolic [Ca\textsuperscript{2+}] (150 nmol/L) was increased 4 fold, in line with experimental findings (Online Figure V; Online Table IV). Stochastic gating of single RyR2 was simulated as recently described for a stochastic model of the canine ventricular myocyte.\textsuperscript{16} Briefly, for every state in the Markov model the number of channels making a transition to every other state within the time period $\Delta t$ (in ms) is determined using a random number from a Binomial distribution based on the number of channels in this state and the individual transition rates ($k_{x,y}$ in ms$^{-1}$) to neighboring states.\textsuperscript{16}

For the RyR2 in segment $s$ and domain $d$, the transition rates and release flux are determined as follows:
\[
P[0]_{\text{RYR2}} = \begin{cases} 
0.2, & \text{(Ctl)} \\
0.4, & \text{(pAF)} 
\end{cases} 
\quad P[1]_{\text{RYR2}} = \begin{cases} 
0.22, & \text{(Ctl)} \\
0.24, & \text{(pAF)} 
\end{cases} 
\quad P[2]_{\text{RYR2}} = 12 
\]

\[
P[3]_{\text{RYR2}} = 1.0, \quad P[4]_{\text{RYR2}} = 20 
\]

\[
P[5]_{\text{RYR2}} = \begin{cases} 
0.0035, & \text{(Ctl)} \\
0.0105, & \text{(pAF)} 
\end{cases} 
\quad P[6]_{\text{RYR2}} = 0.63 
\]

\[
P[7]_{\text{RYR2}} = 9.0 \cdot 10^{-4}, \quad P[8]_{\text{RYR2}} = 0.8 \cdot 10^{-4}, \quad P[9]_{\text{RYR2}} = 1.75 
\]

\[
P[10]_{\text{RYR2}} = 6.3, \quad P[11]_{\text{RYR2}} = 0.007 \ (0.014 \text{ in the presence of caffeine}) 
\]

\[
\text{Inact}_{\infty}^{s,d} = \frac{P[0]_{\text{RYR2}}}{1 + \left(\frac{[\text{Ca}^{2+}]_{\text{SR}}^{s,d}}{4}\right)^4} + \frac{1 - P[0]_{\text{RYR2}}}{P[2]_{\text{RYR2}}} 
\]

\[
\tau_{\text{Inact}}^{s,d} = P[3]_{\text{RYR2}} + \frac{P[4]_{\text{RYR2}}}{1 + \left(\frac{P[1]_{\text{RYR2}}}{[\text{Ca}^{2+}]_{\text{SR}}^{s,d}}\right)^P[2]_{\text{RYR2}}} 
\]

\[
\frac{dI_{\text{rel}}^{s,d}}{dt} = j_{\text{rel}}^{s,d} - \frac{0.1}{1 + \exp\left((j_{\text{rel}}^{s,d} - 1.5 \cdot 10^{-3})/1.0 \cdot 10^{-4}\right)} \times I_{\text{rel}}^{s,d} 
\]

\[
C_{\text{O}R_{YR}}^{s,d} = \frac{1}{1 + \left(I_{\text{rel}}^{s,d}/0.5\right)^8} 
\]

\[
\text{Act}_{\infty}^{s,d} = C_{\text{O}R_{YR}}^{s,d} \times \left(P[5]_{\text{RYR2}} + \frac{P[6]_{\text{RYR2}}}{1 + \exp\left( ([\text{Ca}^{2+}]_{\text{SR}}^{s,d} - P[7]_{\text{RYR2}})/P[8]_{\text{RYR2}}\right)}\right) 
\]

\[
\text{Act}_{\infty, \text{low}}^{s,d} = C_{\text{O}R_{YR}}^{s,d} \times \left(P[5]_{\text{RYR2}} + \frac{P[6]_{\text{RYR2}}}{1 + \exp\left( ([\text{Ca}^{2+}]_{\text{SR}}^{s,d} - P[7]_{\text{RYR2}} \times P[9]_{\text{RYR2}})/P[8]_{\text{RYR2}}\right)}\right) 
\]

\[
\tau_{\text{Act}}^{s,d} = P[10]_{\text{RYR2}} 
\]

\[
k_{\text{C-O}}^{s,d} = \frac{\text{Act}_{\infty}^{s,d}}{\tau_{\text{Act}}^{s,d}}, \quad k_{\text{O-C}}^{s,d} = \frac{1 - \text{Act}_{\infty}^{s,d}}{\tau_{\text{Act}}^{s,d}} 
\]

\[
k_{\text{Cl-OI}}^{s,d} = \frac{\text{Act}_{\infty, \text{low}}^{s,d}}{\tau_{\text{Act}}^{s,d}}, \quad k_{\text{O1-CI}}^{s,d} = \frac{1 - \text{Act}_{\infty, \text{low}}^{s,d}}{\tau_{\text{Act}}^{s,d}} 
\]

\[
k_{\text{O0-0I}}^{s,d} = \frac{\text{Inact}_{\infty}^{s,d}}{\tau_{\text{Inact}}^{s,d}}, \quad k_{\text{01-CI}}^{s,d} = \frac{1 - \text{Inact}_{\infty}^{s,d}}{\tau_{\text{Inact}}^{s,d}} 
\]

\[
k_{\text{Cl-CI}} = 0.1 \times k_{\text{O1-CI}}, \quad k_{\text{C-CI}}^{s,d} = \frac{k_{\text{C-O}} \times k_{\text{O0-0I}} \times k_{\text{O1-CI}} \times k_{\text{Cl-CI}}}{k_{\text{Cl-OI}} \times k_{\text{O1-CI}} \times k_{\text{O-C}}} 
\]
4. L-type Ca\(^{2+}\)-channel

We updated the L-type Ca\(^{2+}\)-current (I\(_{Ca,L}\)) formulation of the model to the Markov model developed by Decker et al.\(^{17}\) (Online Figure VIA) in order to simulate an appropriate balance between voltage- and Ca\(^{2+}\)-dependent inactivation. This model incorporates channel activation (left-to-right transitions) and inactivation (front-to-back transitions) in the absence (lower tier) or presence of Ca\(^{2+}\) (upper tier). The model is able to reproduce voltage dependence of I\(_{Ca,L}\) activation (Online Figure VIB) and steady-state inactivation (Online Figure VIC) recorded in human atrial myocytes in the presence of EGTA.\(^{18,19}\)

We have previously performed simultaneous measurements of I\(_{Ca,L}\) and Ca\(^{2+}\)-transients in response to a depolarization to +10 mV in atrial myocytes isolated from patients in sinus rhythm in the absence of EGTA. The model was able to reproduce the amplitude and integral of I\(_{Ca,L}\) under these conditions (Online Figure VID).

L-type Ca\(^{2+}\)-channels were located in both left and right membrane domains \(m\) of each segment \(s\). All L-type Ca\(^{2+}\)-channels were assumed to sense and modulate the [Ca\(^{2+}\)] in the nearest junctional SR Ca\(^{2+}\)-release space, providing a microdomain for the initiation of Ca\(^{2+}\)-induced Ca\(^{2+}\)-release and the subsequent propagation of the Ca\(^{2+}\) wave towards the cell center.

\[
I_{rel}^{s,d} = P[11]_{RYR2} \times N_{domains} \times n_0^{s,d} \times \left( \frac{N_{RYR}}{N_{domains} \times N_{segments}} \right) \times \left( [Ca^{2+}]_{SR}^{s,d} - [Ca^{2+}]_{SR,SS}^{s,d} \right)
\]

\[
\frac{[Ca^{2+}]_{obs}^{s,m}}{[Ca^{2+}]_{SR,SS}^{s,m}} = \begin{cases} 
[Ca^{2+}]_{SR,SS}^{s,0}, & \text{if } m = \text{left membrane} \\
[Ca^{2+}]_{SR,SS}^{s,N_{Dom}^{-1}}, & \text{if } m = \text{right membrane}
\end{cases}
\]

\[
ACT_t = 0.59 + 0.8 \cdot \frac{\exp(0.052 \cdot (V_m + 13))}{1 + \exp(0.132 \cdot (V_m + 13))}
\]

\[
ACT_\infty = \frac{1}{\left(1 + \exp \left(-\frac{V_m - 13.56}{9.45}\right) \right) \cdot \left(1 + \exp \left(-\frac{V_m + 25}{5}\right) \right)}
\]

\[
\alpha_{Ca,L} = \frac{ACT_\infty}{ACT_t}, \quad \beta_{Ca,L} = \frac{1 - ACT_\infty}{ACT_t}
\]

\[
I_{V,\infty} = \frac{1}{1.2474} \cdot \left(0.2474 + \frac{1}{1 + \exp((V_m + 17.5)/3)} \right)
\]

\[
I_{SV,\infty} = \frac{1}{1.001} \cdot \left(0.001 + \frac{1}{1 + \exp((V_m + 17.5)/3)} \right)
\]

\[
I_{V,\tau} = \frac{1}{70.0 \cdot \left(1 + \exp((-V_m + 49.10)/10.349) \right) + 26.553 \cdot \left(1 + \exp((-V_m + 0.213)/10.807) \right)}
\]
\[ I_{s,m}^{s,m} = 13.825 - \frac{6.3836}{1 + \left( \frac{3.500 \cdot 10^{-4}}{[Ca^{2+}]_{obs}^{s,m}} \right)^4} \]

\[ I_{s,m}^{v_C,\tau} = \frac{1}{70.0 \cdot \left( 1 + \exp\left( \frac{(V_m + 49.10)/10.349}{1 + \exp\left( -(V_m + 0.213)/10.807 \right) } \right) \right)} + I_{s,m}^{c,\tau} = 1 + \exp\left( -(V_m + 0.213)/10.807 \right) \]

\[ x_{Ca,L} = \frac{I_{v,\infty}}{I_{v,\tau}}, \quad y_{Ca,L} = \frac{1 - I_{v,\infty}}{I_{v,\tau}} \]

\[ x_{s,m}^{Ca,L} = \frac{I_{s,v,\infty}}{I_{s,v,\tau}}, \quad y_{s,m}^{Ca,L} = \frac{1 - I_{s,v,\infty}}{I_{s,v,\tau}} \]

\[ \delta_{Ca,L}^{s,m} = \frac{14.9186}{1 + \left( \frac{1.100 \cdot 10^{-3}}{[Ca^{2+}]_{obs}^{s,m}} \right)^4}, \quad \theta_{Ca,L} = 1 \]

\[ \theta_{I_{Ca,L}} = 1.0 \cdot 10^{-6}, \quad \delta_{I_{Ca,L}}^{s,m} = \frac{x_{Ca,L} \cdot y_{s,m}^{Ca,L} \cdot \delta_{Ca,L}^{s,m}}{\theta_{Ca,L} \cdot x_{s,m}^{Ca,L} \cdot \theta_{Ca,L}} \]

\[ \frac{dc_{Ca,L}}{dt} = -\left( \alpha_{Ca,L} + \delta_{Ca,L}^{s,m} + y_{Ca,L} \right) \cdot C_{Ca,L}^{s,m} + \beta_{Ca,L} \cdot C_{Ca,L}^{s,m} + \theta_{Ca,L} \cdot C_{Ca,L}^{s,m} + x_{Ca,L} \cdot Cl_{Ca,L}^{s,m} \]

\[ \frac{do_{Ca,L}}{dt} = -\left( \beta_{Ca,L} + \delta_{Ca,L}^{s,m} + y_{Ca,L} \right) \cdot O_{Ca,L}^{s,m} + \alpha_{Ca,L} \cdot C_{Ca,L}^{s,m} + \theta_{Ca,L} \cdot O_{Ca,L}^{s,m} + x_{Ca,L} \cdot O_{Ca,L}^{s,m} \]

\[ \frac{dc_{s,m}^{Ca,L}}{dt} = -\left( \alpha_{Ca,L} + \theta_{Ca,L} + y_{s,m}^{Ca,L} \right) \cdot C_{s,m}^{Ca,L} + \delta_{s,m}^{Ca,L} \cdot C_{s,m}^{Ca,L} + \beta_{Ca,L} \cdot O_{s,m}^{Ca,L} + \theta_{Ca,L} \cdot C_{s,m}^{Ca,L} + x_{s,m}^{Ca,L} \cdot Cl_{s,m}^{Ca,L} \]

\[ \frac{do_{s,m}^{Ca,L}}{dt} = -\left( \beta_{Ca,L} + \theta_{Ca,L} + y_{s,m}^{Ca,L} \right) \cdot O_{s,m}^{Ca,L} + \delta_{s,m}^{Ca,L} \cdot O_{s,m}^{Ca,L} + \alpha_{Ca,L} \cdot C_{s,m}^{Ca,L} + \theta_{Ca,L} \cdot O_{s,m}^{Ca,L} + x_{s,m}^{Ca,L} \cdot O_{s,m}^{Ca,L} \]

\[ \frac{dc_{Cl,m}^{Ca,L}}{dt} = -\left( \alpha_{Ca,L} + \theta_{Ca,L} + x_{Ca,L} \right) \cdot Cl_{s,m}^{Ca,L} + \delta_{Cl,m}^{Ca,L} \cdot Cl_{s,m}^{Ca,L} + \beta_{Ca,L} \cdot Cl_{s,m}^{Ca,L} + \theta_{Ca,L} \cdot C_{Cl,m}^{Ca,L} + x_{s,m}^{Ca,L} \cdot Cl_{s,m}^{Ca,L} \]

\[ \frac{do_{Cl,m}^{Ca,L}}{dt} = -\left( \beta_{Ca,L} + \theta_{Ca,L} + x_{Ca,L} \right) \cdot Cl_{s,m}^{Ca,L} + \delta_{Cl,m}^{Ca,L} \cdot Cl_{s,m}^{Ca,L} + \alpha_{Ca,L} \cdot O_{s,m}^{Ca,L} + \theta_{Ca,L} \cdot O_{s,m}^{Ca,L} + x_{s,m}^{Ca,L} \cdot O_{s,m}^{Ca,L} \]

\[ \frac{dc_{s,m}^{Ca,L}}{dt} = -\left( \alpha_{Ca,L} + \theta_{Ca,L} + x_{s,m}^{Ca,L} \right) \cdot Cl_{s,m}^{Ca,L} + \delta_{s,m}^{Ca,L} \cdot Cl_{s,m}^{Ca,L} + \beta_{Ca,L} \cdot Cl_{s,m}^{Ca,L} + \theta_{Ca,L} \cdot C_{s,m}^{Ca,L} + x_{Ca,L} \cdot Cl_{s,m}^{Ca,L} \]

\[ \frac{do_{s,m}^{Ca,L}}{dt} = -\left( \beta_{Ca,L} + \theta_{Ca,L} + x_{s,m}^{Ca,L} \right) \cdot Cl_{s,m}^{Ca,L} + \delta_{s,m}^{Ca,L} \cdot Cl_{s,m}^{Ca,L} + \alpha_{Ca,L} \cdot O_{s,m}^{Ca,L} + \theta_{Ca,L} \cdot O_{s,m}^{Ca,L} + x_{s,m}^{Ca,L} \cdot O_{s,m}^{Ca,L} \]

\[ p_{Ca,L} = 1.7 \cdot 10^{-4} \text{ cm}^3 s^{-1}, \quad y_{Ca,i} = 1.0, \quad y_{Ca,o} = 0.341 \]

\[ \Gamma_{Ca,L}^{s,m} = \frac{p_{Ca,L} \cdot (z_{Ca})^2 \cdot V_m \cdot F^2}{R \cdot T} \cdot \frac{y_{Ca,i} \cdot [Ca^{2+}]_{obs}^{s,m} \cdot \exp\left( z_{Ca} \cdot V_m \cdot \frac{F}{R \cdot T} \right) - y_{Ca,o} \cdot [Ca^{2+}]_o}{\exp\left( z_{Ca} \cdot V_m \cdot \frac{F}{R \cdot T} \right) - 1} \]
5. SR Ca\(^{2+}\)-Uptake

We updated SR Ca\(^{2+}\)-uptake formulation from the Grandi et al.\(^{11}\) model to reproduce experimentally observed Ca\(^{2+}\)-handling properties in every segment \(s\) and domain \(d\):

\[
I_{Ca,L}^{s,m} = I_{Ca,L}^{s,m} \cdot (O_{Ca,L}^{s,m} + O_{Ca,L}^{s,m})
\]

\[
J_{up}^{s,d} = J_{up,max} \times \left( \left( \frac{[Ca^{2+}]_{cyt}^{s,d}}{K_{m,up,f}} \right)^{1.787} - \left( \frac{[Ca^{2+}]_{SR}^{s,d}}{K_{m,up,r}} \right)^{1.787} \right)
\]

\[
K_{m,up,f} = \begin{cases} 
6.25 \cdot 10^{-4} \text{mmol/L (SR)} \\
3.125 \cdot 10^{-4} \text{mmol/L (pAF)}
\end{cases}, \quad K_{m,up,r} = \begin{cases} 
1.0 \text{mmol/L (SR)} \\
1.5 \text{mmol/L (pAF)}
\end{cases}
\]

6. Miscellaneous

The maximum conductance of the background Ca\(^{2+}\)-current \(I_{Ca,b}\) was increased by 85% compared to the Grandi et al.\(^{11}\) based on experimentally observed diastolic \([Ca^{2+}]\) levels. Similarly, the maximum rate of Ca\(^{2+}\)-extrusion via the Na\(^+\)-Ca\(^{2+}\)-exchange current \(I_{NCX}\) was increased by 35% based on the rate of caffeine-induced Ca\(^{2+}\)-transient decay. Simulations were performed using fixed intracellular [Na\(^+\)] of 8.5 mmol/L.

7. Simulation of pAF

We developed a computational model of the human pAF myocyte by incorporating the molecular and functional experimental data obtained in pAF myocytes into the control model (Online Table IV, all other parameters remained unaltered). The pAF model was subsequently validated using whole-cell Ca\(^{2+}\)-handling properties of pAF myocytes (Online Figure VII).
## Online Table IV: Model alterations to simulate pAF

<table>
<thead>
<tr>
<th>Element</th>
<th>Parameter</th>
<th>Change from Ctl</th>
<th>Underlying Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>SERCA2a</td>
<td>$K_{m,\text{up},f}$</td>
<td>-50%</td>
<td>Increased Ser16-PLB phosphorylation (Figure 5A), used to match SR Ca(^{2+}) load</td>
</tr>
<tr>
<td></td>
<td>$K_{m,\text{up},r}$</td>
<td>+50%</td>
<td>Increased Ser16-PLB phosphorylation (Figure 5A), used to match increased Ca(^{2+})-transient decay</td>
</tr>
<tr>
<td></td>
<td>$J_{\text{up,max}}$</td>
<td>-25%</td>
<td>Reduced SERCA2a protein expression (Figure 5A)</td>
</tr>
<tr>
<td></td>
<td>$N_{\text{RyR}}$</td>
<td>+64%</td>
<td>Increased RyR2 protein expression in SR fractions (Figure 6D)</td>
</tr>
<tr>
<td></td>
<td>$P[11]_{\text{RyR}}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$P[0]_{\text{RyR}}$</td>
<td>+100%</td>
<td>Altered RyR2 property to balance open probability (Online Figure V) and SR Ca(^{2+}) leak (Online Figure VII)</td>
</tr>
<tr>
<td>RyR2</td>
<td>$P[1]_{\text{RyR}}$</td>
<td>+9%</td>
<td>Altered RyR2 property to balance open probability (Online Figure V) and Ca(^{2+})-transient amplitude (Online Figure VII)</td>
</tr>
<tr>
<td></td>
<td>$P[5]_{\text{RyR}}$</td>
<td>+200%</td>
<td>Increased RyR2 single-channel open probability (Figure 6E, Online Figure V)</td>
</tr>
<tr>
<td>Other</td>
<td>$\tau_{\text{diff,dom,cyt}}$</td>
<td>+200%</td>
<td>Slowed [Ca(^{2+})] diffusion to slightly slow the decay of caffeine-induced Ca(^{2+})-transient (Figure 4C)</td>
</tr>
</tbody>
</table>
8. Simulation of Flecainide Effects on RyR2

The effects of flecainide on RyR2 were simulated by extending the RyR2 Markov model with a blocked tier, representing channels with a flecainide molecule bound. Channels can transition between the two tiers through the open state, in line with the open-state block of RyR2 by flecainide identified by Hilliard et al.\textsuperscript{20} (Online Figure IXA). The model was parameterized using RyR2 single-channel recordings in lipid bilayers.\textsuperscript{21} In both experiments and model, RyR2 were activated using high cytosolic [Ca\textsuperscript{2+}] (100 μmol/L) and the relative reduction in RyR2 open probability at various concentrations of flecainide was reproduced (Online Figure IXB,C). Importantly, reduced RyR2 open probability was due to a reduction in open time (and not due to prolongation of closed times as is for example the case with tetracaine; Online Figure IXC), thereby reproducing a key characteristic of flecainide-induced RyR2 inhibition.\textsuperscript{21}

Transition rates between states were the same in the blocked and non-blocked tiers. Transition rates between the two tier depended on flecainide concentration, as follows:

\[
FlecB_{\infty}^{x, d} = \frac{1}{1 + \left( \frac{50}{[\text{Flecainide}]} \right)^{1.5}}
\]

\[
\tau_{s,d}^{\text{Flec}} = 0.5 \text{ ms}
\]

\[
k_{5,d}^{O-OB} = \frac{FlecB_{\infty}^{x, d}}{\tau_{s,d}^{\text{Flec}}}, \quad k_{5,d}^{O-OB} = \frac{1 - FlecB_{\infty}^{x, d}}{\tau_{s,d}^{\text{Flec}}}
\]

The effects of different concentrations of flecainide on spontaneous SR Ca\textsuperscript{2+}-release events (SCaEs) were subsequently evaluated in the Ctl and pAF models (Online Figure X).
Online Figures

Online Figure I. ECG-holter recording of spontaneous AF conversion to sinus rhythm (red box) in a patient with paroxysmal AF. The total duration of the AF episode was about 5 hours. Each line represents 30 s of a continuous ECG-holter recording.
Online Figure II. Representative Western blots and quantification of protein levels in right-atrial samples of patients with sinus rhythm (Ctl) or paroxysmal AF (pAF). A. Total and Thr287-phosphorylated Ca^{2+}/calmodulin-dependent protein kinase-II (CaMKII). B. Calmodulin (CaM). C. Protein kinase-A (PKA) catalytic and regulatory IIα-subunit. D. Protein phosphatase type-1 (PP1) catalytic subunit. E. Protein phosphatase type-2A (PP2A) catalytic subunit. Data were normalized to calsequestrin (CSQ) or GAPDH and are shown relative to Ctl. Dot-plots were used for experiments with fewer than 10 samples. Numbers within columns or above symbols indicate number of samples. Comparisons using one-way ANOVA.
Online Figure III. Representative Western blots and quantification of protein levels of calsequestrin (CSQ; left panel) and GAPDH (middle panel) in right-atrial samples of patients with sinus rhythm (Ctl) or paroxysmal AF (pAF). Right panel shows quantification of CSQ normalized to GAPDH. Data are shown relative to Ctl expression levels. Numbers within columns indicate number of tissue samples. Comparisons using one-way ANOVA.
Online Figure IV. Validation of RyR2 single-channel properties. A. RyR2 Markov model structure. B. Single-channel recordings of RyR2 isolated from human atrial myocytes of patients in sinus rhythm (left panel, experimental data from Voigt et al.\textsuperscript{15}) or stochastic simulation of single RyR2 (right panel) at different concentrations of (cytosolic) cis-[Ca\textsuperscript{2+}]. C. Average open time of single RyR2 (left panel) and open probability (right panel) at increasing concentrations of cis-[Ca\textsuperscript{2+}] in experiments\textsuperscript{15} (white bars / symbols) and stochastic RyR2 model (black bars / line).
Online Figure V. Validation of relative increase in RyR2 open probability ($P_o$) in pAF compared to Ctl in model and experiment at diastolic cytosolic $\text{cis-}[\text{Ca}^{2+}]_i$ (150 nmol/L)
Online Figure VI. Validation of $I_{\text{Ca,L}}$ properties. A. Markov model structure of the $I_{\text{Ca,L}}$ model, based on Decker et al. B. Validation of peak $I_{\text{Ca,L}}$ I-V relationship in the presence of 10 mmol/L EGTA and 3.0 mmol/L extracellular [Ca$^{2+}$]. Experimental data from Christ et al. C. Validation of $I_{\text{Ca,L}}$ inactivation in the presence of EGTA. Experimental data from Van Wagoner et al. D. Validation of peak and integrated $I_{\text{Ca,L}}$ in the Ctl model compared to experimental recordings in Ctl patients obtained in the absence of EGTA.
Online Figure VII. Validation of paroxysmal AF (pAF) model properties. A. Depolarization-induced and caffeine-induced Ca\textsuperscript{2+}-transient (CaT and cCaT, respectively) in Ctl (black line) and pAF (blue line) models. B. Validation of the rate of decay of the systolic CaT (reflecting SR Ca\textsuperscript{2+}-uptake by SERCA2a and Ca\textsuperscript{2+}-extrusion by NCX), cCaT (reflecting predominantly Ca\textsuperscript{2+}-extrusion by NCX) and the derived rate of SERCA2a SR Ca\textsuperscript{2+}-uptake in Ctl and pAF models. C. Validation of I\textsubscript{Ca,L} amplitude, CaT amplitude, SR Ca\textsuperscript{2+}-leak and cCaT amplitude (SR Ca\textsuperscript{2+}-load) in Ctl and pAF models. Results were obtained in the absence of spontaneous Ca\textsuperscript{2+}-release events.
Online Figure VIII. Whole-cell $[\text{Ca}^{2+}]_i$ and total membrane current in the control (Ctl) and paroxysmal AF (pAF) models for the simulations shown in Figure 8.
Online Figure IX. Simulation of RyR2 inhibition by flecainide. A. RyR2 Markov model structure with group of blocked states accessible via the open state. B. Stochastic simulation of single RyR2 at different concentrations of flecainide. C. Validation of average RyR2 open probability (left panel) and RyR2 open time (right panel) at increasing concentrations of flecainide in experiments\textsuperscript{21} (white bars) and stochastic RyR2 model (black bars).
Online Figure X. Effect of simulated flecainide on spontaneous SR Ca\textsuperscript{2+}-release events (SCaEs).  

A. Voltage-clamp protocol, and whole-cell [Ca\textsuperscript{2+}]\textsubscript{i} in the absence (top panel) or presence (bottom panel) of 50 μmol/L flecainide in the sinus rhythm control model (Ctl). The insets show a transverse line-scan representation of [Ca\textsuperscript{2+}] at the center of the virtual cell. B. Similar to panel A for the paroxysmal AF (pAF) model. C. Quantification of the effect of 20 or 50 μmol/L flecainide on SCaE incidence, Δ[Ca\textsuperscript{2+}]\textsubscript{i} (amplitude) of SCaEs, and SCaE-induced membrane current (top to bottom) in Ctl and pAF models.

Flecainide causes a dose-dependent decrease in incidence of SCaEs in both models. In the Ctl model, this is accompanied by a reduction in the amplitude of SCaEs and the corresponding depolarizing membrane inward current. In the pAF model, the reduced incidence of SCaEs in the presence of flecainide allows more time for Ca\textsuperscript{2+}-uptake into the SR, thereby producing lower diastolic [Ca\textsuperscript{2+}]\textsubscript{i} levels, which tend to increase amplitude of SCaEs. The flecainide concentrations employed in our simulations are supratherapeutic, but were necessary to observe an effect due to the relatively low affinity (IC\textsubscript{50} ≈ 55 μmol/L) observed in lipid-bilayer experiments when RyR2 channels were activated using high cytosolic [Ca\textsuperscript{2+}]\textsubscript{i} which were used for the parameterization of our model. The differences in the affinity of flecainide towards RyR2 following different activation protocols is at present incompletely understood and therefore not incorporated in our model. We therefore interpret our findings as showing that flecainide concentrations at or below the IC\textsubscript{50} for RyR2 inhibition can reduce the incidence of SCaEs.

Although the present results suggest that RyR2-inhibition by flecainide has antiarrhythmic properties in pAF, it should be noted that in vivo the effects of flecainide are complex, involving inhibition of Na\textsuperscript{+}-channels and modulation of intracellular Ca\textsuperscript{2+}-handling via altered intracellular [Na\textsuperscript{+}], in addition to direct RyR2 inhibition.\textsuperscript{22} As such, flecainide may have better antiarrhythmic properties in vivo than suggested by our simulations of RyR2-inhibition alone. On the other hand, it has been shown that Ca\textsuperscript{2+}-overload reduces the efficacy of flecainide against SCaEs,\textsuperscript{22} in agreement with our findings that the incidence of SCaEs in the pAF model with flecainide was still greater than in the Ctl model in the absence of flecainide.
Online Videos

Online Video I. Animation of membrane potential, whole-cell Ca$^{2+}$-transient and spatial [Ca$^{2+}$]$_i$ distribution over time in the Ctl model. Protocol corresponds to the line-scan image shown in Figure 8A, top panel.

Online Video II. Animation of membrane potential, whole-cell Ca$^{2+}$-transient and spatial [Ca$^{2+}$]$_i$ distribution over time in the Ctl model with increased SR Ca$^{2+}$-uptake. Protocol corresponds to the line-scan image shown in Figure 8A, second panel.

Online Video III. Animation of membrane potential, whole-cell Ca$^{2+}$-transient and spatial [Ca$^{2+}$]$_i$ distribution over time in the Ctl model with RyR2 dysfunction. Protocol corresponds to the line-scan image shown in Figure 8A, third panel.

Online Video IV. Animation of membrane potential, whole-cell Ca$^{2+}$-transient and spatial [Ca$^{2+}$]$_i$ distribution over time in the pAF model combining increased SR Ca$^{2+}$-uptake and RyR2 dysfunction. Protocol corresponds to the line-scan image shown in Figure 8A, bottom panel.
References


