

Atrial Fibrillation Is Associated With Increased Spontaneous Calcium Release From the Sarcoplasmic Reticulum in Human Atrial Myocytes

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Background—Spontaneous Ca^{2+} release from the sarcoplasmic reticulum (SR) can generate afterdepolarizations, and these have the potential to initiate arrhythmias. Therefore, an association may exist between spontaneous SR Ca^{2+} release and initiation of atrial fibrillation (AF), but this has not yet been reported.

Methods and Results—Spontaneous Ca^{2+} release from the SR, manifested as Ca^{2+} sparks and Ca^{2+} waves, was recorded with confocal microscopy in atrial myocytes isolated from patients with and those without AF. In addition, the spontaneous inward current associated with Ca^{2+} waves was measured with the use of the perforated patch-clamp technique. The Ca^{2+} spark frequency was higher in 8 patients with AF than in 16 patients without (6.0 ± 1.2 versus 2.8 ± 0.8 sparks/mm per second, $P < 0.05$). Similarly, the spontaneous Ca^{2+} wave frequency was greater in patients with AF (2.8 ± 0.5 versus 1.1 ± 0.3 waves/mm per second, $P < 0.01$). The spontaneous inward current frequency was also higher in 10 patients with AF than in 13 patients without this arrhythmia (0.101 ± 0.028 versus 0.031 ± 0.007 per second, $P < 0.05$, at a clamped potential of -80 mV). In contrast, both the Ca^{2+} released from the SR and the Na^+ - Ca^{2+} exchange rate induced by a rapid caffeine application were comparable in patients with and without AF.

Conclusions—The observed increase in spontaneous Ca^{2+} release in patients with AF probably is due to an upregulation of the SR Ca^{2+} release channel activity, which may contribute to the development of AF. (*Circulation*. 2004;110:1358-1363.)

Key Words: sarcoplasmic reticulum ■ calcium ■ arrhythmia ■ ion channels ■ electrophysiology

Atrial fibrillation (AF) is the most common cardiac arrhythmia in humans. It causes electrophysiological and structural alterations that induce progression and self-maintenance of the cardiac disorder (see Reference 1 for review).¹

Among the electrophysiological alterations associated with AF, cell membrane depolarization² and downregulation of potassium channels I_{TO} and I_{Kur} ,^{3,4} L-type Ca^{2+} current,^{5,6} and protein expression⁷ have been reported in atrial myocytes from patients with AF. Furthermore, electrically induced AF shortened the atrial effective refractory period, and it could be reversed by administration of the L-type Ca^{2+} channel agonist BayY5959, which increased atrial contractility and prolonged atrial refractoriness.^{8,9}

On the other hand, studies in rat ventricular myocytes¹⁰ have shown that depolarization of the cell membrane from -70 to -40 mV favors spontaneous local calcium release from the sarcoplasmic reticulum (SR). These events, called Ca^{2+} sparks and Ca^{2+} waves, induce a local increase in cytosolic Ca^{2+} , and part of this Ca^{2+} is extruded from the cell by the Na^+ - Ca^{2+} exchanger (NCX). This generates an inward Na-Ca exchange

current (I_{NCX}) and a local membrane depolarization. Although a single calcium spark is unable to excite the cell, the concurrence of several calcium sparks may generate a propagating Ca wave and a global membrane depolarization.¹¹

Abnormal depolarizations of the cell membrane (afterdepolarizations) can generate arrhythmias by triggered activity,^{12,13} and a recent study has shown that reinitiation of AF can be caused by early afterdepolarizations.¹⁴ On the other hand, Ca^{2+} sparks can increase the automaticity of latent atrial pacemaker cells.^{15,16} Thus, an increase in the spontaneous Ca^{2+} release from the SR can potentially induce atrial arrhythmias through two different mechanisms: afterdepolarization-induced triggered activity or abnormal automaticity.

The aim of the present study was to determine whether AF is associated with alterations in the Ca^{2+} release from the SR in isolated human atrial myocytes.

Methods

Study Population

Clinical data and the pattern of the SR Ca^{2+} release were analyzed in 41 patients undergoing cardiac surgery. Fifteen patients had a history

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of AF; the remaining 26 patients were free of this arrhythmia. Cardiac myocytes from these patients were used for two experimental series. One used confocal microscopy in cells from 8 patients with AF and 16 patients with no AF. The second series used the patch-clamp in cells from 10 patients with AF and 13 patient without AF. In 6 patients (3 of each group), cell yield was large enough to perform both experimental series. Patients treated with Ca^{2+} antagonists were excluded. Two patients with AF received amiodarone and 1 patient with AF received angiotensin receptor blocker.

Cell Isolation

Tissue samples were carefully obtained from the right atrial appendage just before atrial cannulation for cardiopulmonary bypass and were immediately taken to the laboratory. They were rinsed, cut into small pieces in a Ca^{2+} -free solution containing 30 mmol/L butanedione monoxime), and incubated at 35°C in a Ca^{2+} -free solution containing 0.5 mg/mL collagenase (Worthington type 2, 318 units/mg), 0.5 mg/mL proteinase (Sigma type XXIV, 11 units/mg solid). The Ca^{2+} -free solution contained (in mmol): NaCl 88, sucrose 88, KCl 5.4, NaHCO_3 4, NaH_2PO_4 0.3, MgCl_2 1.1, HEPES 10, taurine 20, glucose 10, and sodium pyruvate 5 (pH 7.4 at room temperature). After 45 minutes, the tissue was removed from the enzyme solution and cells were disaggregated in Ca^{2+} -free solution with a Pasteur pipette. The remaining tissue was digested for 15 minutes in a fresh Ca^{2+} -free solution containing 0.4 mg/mL collagenase. This procedure was repeated 3 times. Solutions containing disaggregated cells were centrifuged at 600 rpm for 1 minute. Pellets were resuspended in Ca^{2+} -free solution, and Ca^{2+} was gradually increased to 1 mmol/L. The cell yield varied, depending on the size and quality of the tissue sample. Only elongated cells with clear cross-striations and without granulation were used for experiments.

Although the atrial tissue samples consisted of tissue that would normally be discarded during surgery, permission to study this tissue was obtained from each patient. The study was approved by the ethics committee of our institution.

Spontaneous SR Ca^{2+} Release

Ca^{2+} sparks and Ca^{2+} waves were detected through the use of a laser scanning confocal microscope (Leica TCS SP2 AOBS). The experimental solution contained (in mmol): NaCl 136, KCl 4, NaH_2PO_4 0.33, NaHCO_3 4, CaCl_2 2, MgCl_2 1.6, HEPES 10, glucose 5, and pyruvic acid 5 (pH=7.4). Cells were incubated with 5 $\mu\text{mol/L}$ fluo-3AM for 10 to 20 minutes at 23°C, followed by wash and deesterification for at least 30 minutes. Fluorescence emission was collected between 500 and 650 nm, with the excitation at 488 nm attenuated to 1% to 5%. Ca^{2+} sparks and Ca^{2+} waves were detected at resting conditions during 20.48 seconds. Each scan period consisted of line scan images 512 pixels wide (59.6 μm) and 1024 pixels long, recorded at a scan rate of 1 or 2 kHz. Cells were field-stimulated to verify that cell shortening could be elicited. Ca^{2+} sparks were detected as an increase in the signal mass of a 3- μm section through the center of a Ca^{2+} spark (red arrow in Figure 1A), without any detectable increase in an adjacent 3- μm section (blue arrow in Figure 1A). An increase in the signal mass in 2 or more adjacent 3- μm sections were counted as Ca^{2+} waves (see Figure 1B). The amplitude of each Ca^{2+} spark and its half-life were determined from an exponential fit of the decaying phase of the transient Ca^{2+} spark. The Ca^{2+} spark frequency was determined for each cell and normalized to the scanned cell length.

Patch Clamp

The transient inward I_{NCX} generated by Ca^{2+} waves was recorded in the perforated patch configuration with the use of a software-controlled patch-clamp amplifier (EPC 10, HEKA). The pipette resistance was 2 to 5 M Ω . In some myocytes, I_{NCX} and fluo-3 fluorescence were recorded simultaneously. Experiments were begun when the access resistance was stable and had decreased to <5 times the pipette resistance. The extracellular solution contained (in mmol): NaCl 127, TEA 5, HEPES 10, NaHCO_3 4, NaH_2PO_4 0.33, glucose 10, pyruvic acid 5, CaCl_2 2, and MgCl_2 1.8 (pH=7.4). The

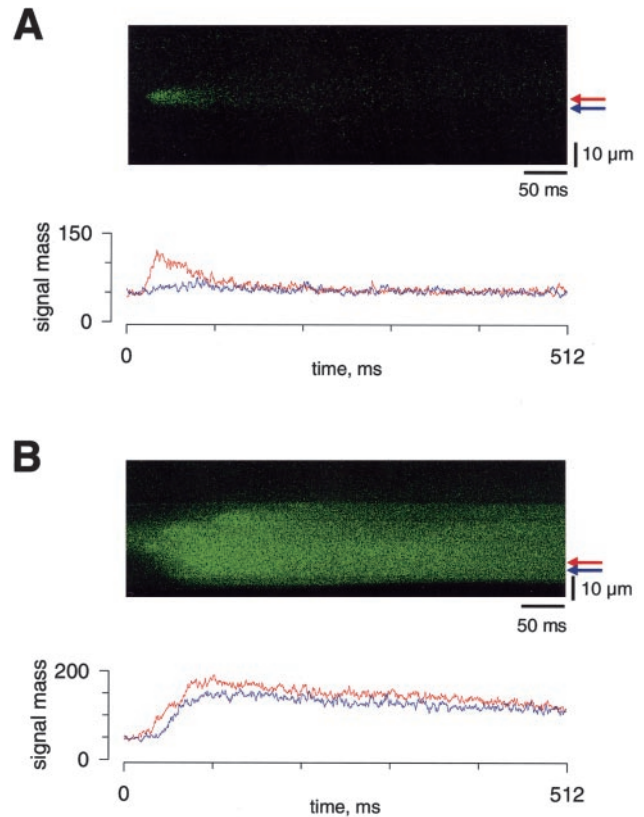


Figure 1. Detection of Ca^{2+} sparks and Ca^{2+} waves in human atrial myocytes with confocal microscopy. A, Line scan of a myocyte shows Ca^{2+} spark (upper panel) and corresponding increase in signal mass (lower panel). Ca^{2+} sparks were identified by local increase in the signal mass in 3- μm -wide section (red arrow) without any increase in a neighboring 3- μm section (blue arrow). B, Line scan shows Ca^{2+} wave and corresponding increase in signal mass. Ca^{2+} waves were identified by an increase in signal mass in at least 2 adjacent 3- μm -wide sections (red and blue arrows).

pipette solution contained (in mmol): aspartic acid 109, CsCl 47, Mg_2 ATP 3, MgCl_2 1, Na_2 phosphocreatine 5, Li_2GTP 0.42, HEPES 10, and 250 $\mu\text{g/mL}$ amphotericin B (pH=7.2).

Data Analysis

Experiments were carried out without knowledge about the clinical data of the patients. The Ca^{2+} sparks and Ca^{2+} waves were recorded in 3 to 10 cells from the same patient and averaged. Average values from each patient were used for statistical analysis and expressed as mean \pm SEM unless otherwise stated. The Student's *t* test and ANOVA were used to test statistical significance and to assess within-patient and between-group differences.

Results

Baseline parameters of the patients are shown in the Table. Only the left atrial diameter differed significantly between groups, being larger in patients with AF.

Ca^{2+} Spark and Ca^{2+} Wave Characterization

Cells from 8 patients with AF had a significantly higher Ca^{2+} spark frequency (6.0 ± 1.2 versus 2.8 ± 0.8 sparks/mm per second) and Ca^{2+} wave frequency (2.8 ± 0.5 versus 1.1 ± 0.3 waves/mm per second, $P < 0.01$) than cells from 16 patients without this arrhythmia (Figure 2). In contrast, the Ca^{2+} spark

Clinical Characteristics of the Study Groups

Variable	Atrial Fibrillation (n=15)	Sinus Rhythm (n=26)
Mean age, y	66.4±11.2	65.5±13.2
Gender (male/female)	6/9	15/11
Mean body surface, m ²	1.68±0.52	1.75±0.78
Hypertension, %	46	34
Mean LA diameter, mm	56.2±7.8	42.6±7.3*
Mean LV ejection fraction, %	61.7±15.2	58.8±15.2
Surgery		
CABG	1	4
AVR	4	12
MVR	3	2
Combination	7	8
Atrial fibrillation (paroxysmal/chronic)	7/8	...

Values are mean±SD.

LA indicates left atrium; LV, left ventricle; CABG, coronary artery bypass graft; AVR, aortic valve replacement; and MVR, mitral valve replacement.

* $P<0.001$.

amplitude (F/F_0) and its half-life were similar in both patient groups. Indeed, F/F_0 was 1.53 ± 0.02 in patients without AF and 1.47 ± 0.04 in patients with AF, whereas the half-life was 45 ± 4 ms and 51 ± 9 ms, respectively.

Preincubation of cells for at least 30 minutes with $30\ \mu\text{mol/L}$ of the SR Ca^{2+} pump inhibitor cyclopiazonic acid abolished Ca^{2+} sparks and Ca^{2+} waves, confirming that they were due to Ca^{2+} release from the SR (data not shown).

Spontaneous Inward NCX Current

The perforated patch-clamp technique was used to confirm that Ca^{2+} waves were always associated with an inward I_{NCX} (Figure 3A) and cell contraction (Figure 3B).

To test the influence of membrane depolarization on spontaneous I_{NCX} , the holding potential was switched between -80 mV and -50 mV every 30 seconds. Figure 4 shows that the I_{NCX} frequency was higher when the holding potential was kept at -50 mV. Indeed, 2-way ANOVA showed that the holding potential significantly affected the spontaneous I_{NCX} frequency ($P<0.001$, $n=23$). Switching the holding potential from -80 to -50 mV increased the I_{NCX} frequency from

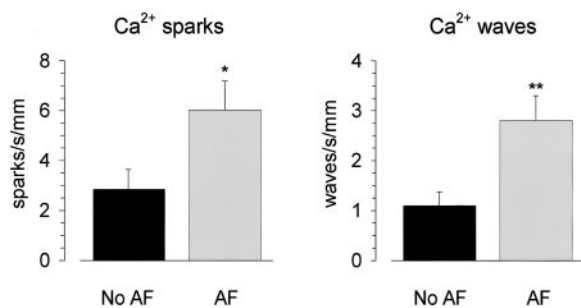


Figure 2. Frequency of Ca^{2+} sparks and Ca^{2+} waves in atrial myocytes from 16 patients without episodes of AF (no AF) and from 8 patients with AF. Patients with AF showed a higher frequency of Ca^{2+} sparks (* $P<0.05$) and Ca^{2+} waves (** $P<0.01$) than patients without AF.

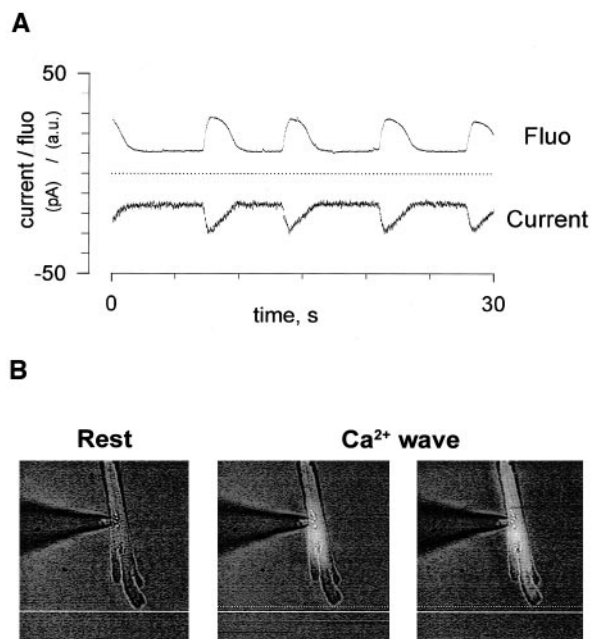


Figure 3. Recordings of intracellular transient Ca^{2+} , membrane current, and cell shortening. A, Simultaneous recordings of Fluo-3 fluorescence (Fluo) and membrane current (Current) in a human atrial myocyte clamped to -50 mV shows concurrence of the two signals. B, Video recordings of fluorescence and cell length in the same cell during a Ca^{2+} wave. Solid and dashed white lines indicate cell length at rest and during the Ca^{2+} wave, respectively.

0.031 ± 0.007 to 0.057 ± 0.009 per second ($P<0.001$) in patients without AF and from 0.101 ± 0.028 to 0.199 ± 0.039 per second ($P<0.001$) in those with AF.

Thus, it appears that a loss of the membrane potential could lead to an increase in the frequency of spontaneous SR Ca^{2+} release. Notice, however, that patients with AF had a higher I_{NCX} frequency both at -80 mV ($P<0.05$) and at -50 mV ($P<0.01$). Indeed, 2-way ANOVA confirmed that a previous history of AF significantly affected the I_{NCX} frequency ($P<0.001$).

To compare the frequency of spontaneous Ca^{2+} waves measured by confocal microscopy and the frequency of such events measured as I_{NCX} with the patch-clamp, data obtained with these two techniques were expressed as events per second. With a holding potential of -80 mV, the I_{NCX} frequency was similar to Ca^{2+} wave frequency in patients with AF (0.093 ± 0.017 versus 0.101 ± 0.028 per second, $P>0.8$) and in patients without AF (0.052 ± 0.015 versus 0.031 ± 0.007 per second, $P>0.2$).

ANOVA showed that treatment of patients with ACE inhibitors (7 patients with AF and 6 without) did not affect spontaneous Ca^{2+} release from the SR.

Sarcoplasmic Reticulum Ca^{2+} Content and Na^+ - Ca^{2+} Exchange Rate

Because cell size, SR Ca^{2+} content, and the activity of the NCX could affect spontaneous SR Ca^{2+} release, we assessed these parameters by using a rapid application of $10\ \text{mmol/L}$ caffeine. Cell capacitance was similar in patients without and with AF (58.0 ± 7.8 versus 61.2 ± 4.4 pF, respectively). Figure

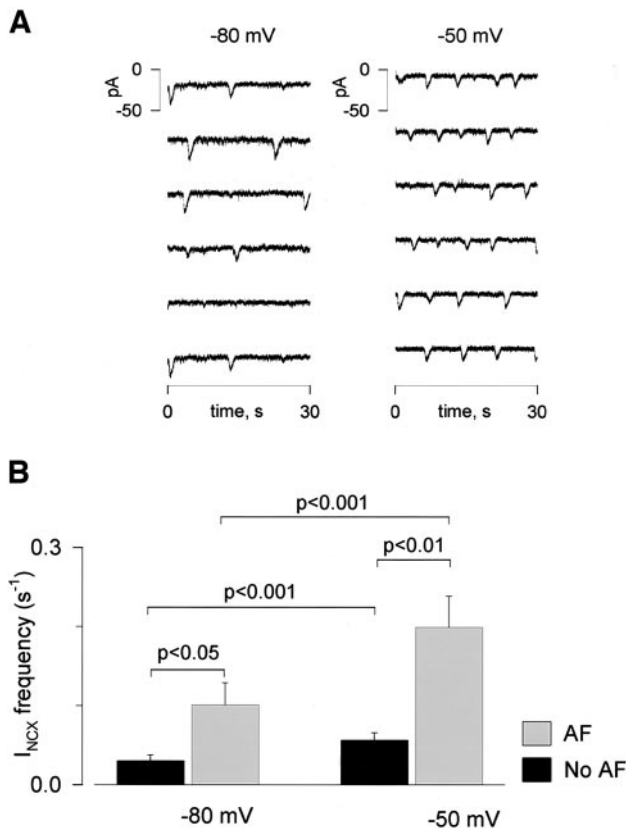


Figure 4. Effect of holding potential on spontaneous I_{NCX} frequency. A, Switching holding potential from -80 to -50 mV increased the number of spontaneous I_{NCX} . B, Average I_{NCX} frequencies at -80 and -50 mV in the two patient groups. Patients with AF had more spontaneous I_{NCX} than those without AF.

5A shows that caffeine temporarily abolished spontaneous activity, confirming that spontaneous I_{NCX} requires SR Ca^{2+} loading. Figure 5B shows that both the peak of the NCX rate during a caffeine application and its half-life (1.12 ± 0.22 and 1.10 ± 0.14 seconds) were comparable in patients with and without AF. Figure 5C shows that the time integral of the caffeine-induced I_{NCX} , used as an estimate of the SR Ca^{2+} content, was also comparable in patients with and without AF (8.3 ± 1.5 versus 8.3 ± 1.2 amol/pF).

Discussion

Main Findings

The novel finding of this study is that isolated right atrial myocytes from patients with episodes of AF exhibit a more frequent spontaneous SR Ca^{2+} release than myocytes from patients free of this arrhythmia. This was true both for a local, nonpropagated Ca^{2+} release from the SR (Ca^{2+} sparks) and for a more extensive spontaneous SR Ca^{2+} release (Ca^{2+} waves). Two pathophysiological considerations regarding this finding must be discussed. One is the mechanism that makes patients with AF more prone to present spontaneous sarcoplasmic calcium release and the other is whether these calcium release events may favor the genesis of AF.

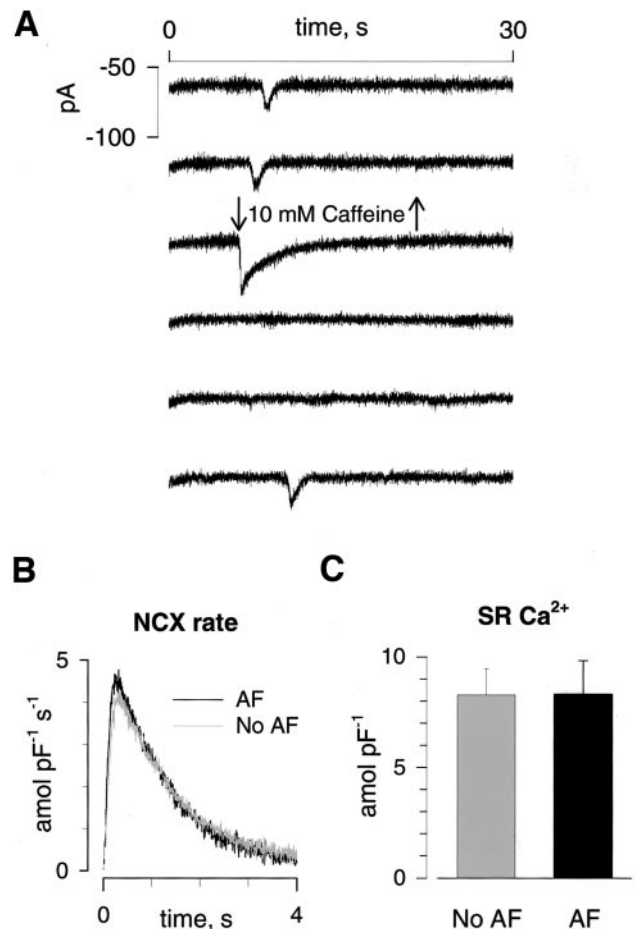


Figure 5. Assessment of NCX rate and SR Ca^{2+} content in human atrial myocytes. A, Spontaneous I_{NCX} are temporarily abolished by clearance of the SR Ca^{2+} content with transient rapid caffeine application (arrows). B, Average NCX rate (V_{NCX}) during rapid caffeine application. C, Average caffeine-releasable SR Ca^{2+} content was comparable in the two patient groups.

Spontaneous Sarcoplasmic Reticulum Ca^{2+} Release in Atrial Fibrillation

The electrophysiological remodeling induced in the fibrillating atria and its molecular basis have been extensively reviewed,^{4,17} and studies of isolated human atrial myocytes have shown that the I_{Ca} density is lower in patients with persistent AF than in patients without this arrhythmia.^{5,6} Moreover, a reduction in both L-type Ca^{2+} channel mRNA and protein has been reported in patients with persistent AF.^{18,19} A downregulation of calcium channels (secondary to arrhythmia-induced calcium overload) appears to be responsible for the reduction in I_{Ca} density.⁶

A reduction of the I_{Ca} density in patients with persistent AF is expected to diminish the SR calcium loading. Moreover, reduced levels of SERCA mRNA^{18,19} or protein²⁰ observed in patients with AF would be expected to favor a lowering of the releasable SR Ca^{2+} in these patients. In contrast to this assumption, our data show a greater number of spontaneous Ca^{2+} sparks and Ca^{2+} waves in patients with AF, whereas a comparable SR Ca^{2+} content was observed in patients with and without a history of AF. Thus, it appears that the activity of the SR Ca^{2+} release channel is upregulated in patients with

AF. In agreement with this assumption, continuous application of a low dose of caffeine (250 $\mu\text{mol/L}$), which increases the open probability of the SR Ca^{2+} release channel, was found to increase the frequency of spontaneous SR Ca^{2+} release despite a lower SR Ca^{2+} content in isolated rat myocytes.²¹

A constant electrophysiological feature in isolated atrial myocytes and cardiac preparations from patients with AF is the finding of membrane depolarization, low amplitude, and short duration of the transmembrane action potential.^{2,22,23} In our study, the effect of membrane potential on spontaneous SR Ca^{2+} release was addressed by comparing the effect of a normal holding potential (-80 mV) and a depolarized potential (-50 mV) on the frequency of spontaneous inward I_{NCX} . The higher I_{NCX} frequency found at -50 mV suggests that membrane depolarization could at least partly account for the increased frequency of Ca^{2+} sparks and Ca^{2+} waves. However, at a given holding potential, the spontaneous I_{NCX} frequency continued to be higher in patients with AF than in patients without this arrhythmia, suggesting that a direct alteration in the SR Ca^{2+} release channel is responsible for the increased number of Ca^{2+} sparks and Ca^{2+} waves in patients with AF.

Alternatively, the more frequent spontaneous SR Ca^{2+} release in patients with AF could be due to a lower-than-normal Ca^{2+} extrusion by the NCX.^{24,25} However, this would be expected to result in a larger Ca^{2+} spark amplitude (F/F_0) and a longer half-life of the Ca^{2+} sparks,²⁶ but none of these features were observed in the present study. Furthermore, direct assessment of the NCX rate during a rapid caffeine application gave comparable peak NCX rates and half-lives in the two groups of patients.

Because patients with enlarged atria are more prone to development of AF,²² it is possible that atrial enlargement itself favors spontaneous SR Ca^{2+} release. In this respect, alterations in SR Ca^{2+} handling have been reported in patients with enlarged failing hearts,^{27,28} and, in agreement with our findings, these patients also showed upregulation of the SR Ca^{2+} release channel.²⁸ However, since we did not encounter any patient with AF and normal atrial size, we cannot determine whether the increased SR Ca^{2+} release is a consequence of the arrhythmia itself or the result of the atrial enlargement. On the other hand, cell hypertrophy is unlikely to account for the observed increase in spontaneous SR Ca^{2+} release in our patients with AF because the capacitance of the cells studied was comparable in patients with and those without AF.

Spontaneous Sarcoplasmic Calcium Release and Atrial Arrhythmogenesis

Cellular electrophysiological studies in atrial tissue have shown that abnormal automaticity²² and triggered activity are major mechanisms leading to atrial arrhythmias in humans.²³ In this respect, Ca^{2+} sparks have been reported to activate latent pacemaker cells in cat atrial myocytes.^{15,16} We do not know to what extent this mechanism may apply in our model. If part of our cell population were in fact latent pacemaker cells, they would be expected to show rhythmic Ca^{2+} transients, but only one cell showed rhythmic Ca^{2+} transients in

our study. On the other hand, the increased frequency of spontaneous SR Ca^{2+} release observed in patients with AF is expected to augment the number of afterdepolarizations,¹³ thereby favoring the induction of triggered activity. Moreover, because the high heart rate in AF can induce cellular Ca^{2+} overload,²⁵ a resulting enhancement of spontaneous SR Ca^{2+} release may occur^{25,29} and thereby contribute to a further elevation of the spontaneous SR Ca^{2+} release in patients with AF. For ethical reasons, we only had access to right atrial tissue, and we are therefore unable to determine whether spontaneous SR Ca^{2+} release is also elevated in the left atrium of patients with AF. In favor of our data suggesting that afterdepolarizations taking place in the right atrium may induce AF, a recent study in the arterially perfused canine right atrium show that afterdepolarizations reinitiate AF.¹⁴ Finally, catecholaminergic polymorphic ventricular tachycardia has also been ascribed to mutations that increase the open probability of the SR Ca^{2+} release channel,³⁰ corroborating the notion that an increased spontaneous SR Ca^{2+} release may promote arrhythmias.

Considerations of the Model

In the present study, confocal microscopy and patch-clamp recordings were done in two separate experimental series. This allowed measurements of Ca^{2+} spark and Ca^{2+} wave frequencies in nonclamped human atrial myocytes bathed in a physiological-like solution. It also allowed us to confirm that the two techniques give comparable spontaneous Ca^{2+} wave frequencies.

Our results afford novel evidence that the frequency of spontaneous sarcoplasmic Ca^{2+} release is increased in myocytes from patients with AF and that this is likely to be due to an upregulation of the SR Ca^{2+} release channel activity. This channel therefore appears to be a potentially important target for pharmacological control of AF, either by directly manipulating its open probability or by modulating cellular mechanisms that regulate spontaneous SR Ca^{2+} release.

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