Alterations of atrial Ca\(^{2+}\) handling as cause and consequence of atrial fibrillation

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Abstract

Atrial fibrillation (AF) is the most prevalent sustained arrhythmia. As the most important risk factor for embolic stroke, AF is associated with a high morbidity and mortality. Despite decades of research, successful (pharmacological and interventional) ‘ablation’ of the arrhythmia remains challenging. AF is characterized by a diverse aetiology, including heart failure, hypertension, and valvular disease. Based on this understanding, new treatment strategies that are specifically tailored to the underlying pathophysiology of a certain ‘type’ of AF are being developed. One important aspect of AF pathophysiology is altered intracellular Ca\(^{2+}\) handling. While re-entry and multiple wavelets have been demonstrated in AF, the mechanisms underlying the rapid foci in the pulmonary veins, which initiate AF in patients with paroxysmal AF, are presently unknown.

After the onset of AF, atrial effective refractory period (AERP) progressively shortens. This ‘electrical remodelling’ contributes to the perpetuation of the arrhythmia. Atrial contractile function declines (‘contractile remodelling’). Rapid atrial activation after the onset of AF leads to initial intracellular Ca\(^{2+}\) overload, which induces complex changes in intracellular Ca\(^{2+}\) handling. While it is well established that AF-induced changes in Ca\(^{2+}\) handling contribute to contractile remodelling, current research is focused on elucidating the role of altered intracellular Ca\(^{2+}\) handling in cellular pro-arrhythmic mechanisms and atrial arrhythmogenesis. Moreover, recent studies suggest that alterations in Ca\(^{2+}\)-mediated intracellular signalling cascades affect excitation–transcription coupling and contribute to AF pathophysiology.

Here, we review and critique unique features of atrial intracellular Ca\(^{2+}\) handling. While re-entry and multiple wavelets have been demonstrated in AF, the mechanisms underlying the rapid foci in the pulmonary veins, which initiate AF in patients with paroxysmal AF, are presently unknown.

Keywords

Atrial fibrillation • Calcium handling • Contractile remodelling arrhythmogenesis • Excitation–transcription coupling

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1. Introduction

Atrial fibrillation (AF) is the most common sustained arrhythmia. As the most important risk factor for embolic stroke,\(^1\) AF is associated with a high morbidity and mortality\(^2\) and often complicates heart failure.\(^3\)

While re-entry and multiple wavelets have been demonstrated in AF,\(^4,5\) the mechanisms underlying the rapid foci in the pulmonary veins, which initiate AF in patients with paroxysmal AF,\(^6\) are presently unknown.

After the onset of AF, atrial effective refractory period (AERP) progressively shortens.\(^7,8\) This ‘electrical remodelling’ contributes to the perpetuation of the arrhythmia.\(^7\) Additionally, atrial contractile function declines (‘contractile remodelling’). Rapid atrial activation after the onset of AF leads to initial intracellular Ca\(^{2+}\) overload, which induces complex changes in intracellular Ca\(^{2+}\) handling. While it is well established that AF-induced changes in Ca\(^{2+}\) handling contribute to contractile remodelling, current research is focused on elucidating the role of altered intracellular Ca\(^{2+}\) handling in cellular pro-arrhythmic mechanisms and atrial arrhythmogenesis. Moreover, recent studies suggest that alterations in Ca\(^{2+}\)-mediated intracellular signalling cascades affect excitation–transcription coupling and contribute to AF pathophysiology.

Here, we review and critique unique features of atrial intracellular Ca\(^{2+}\) handling. We contrast changes in Ca\(^{2+}\) handling due to AF with those that precede the onset of the arrhythmia in predisposing conditions (e.g. heart failure, atrial dilatation). Finally, the contribution of altered Ca\(^{2+}\) handling to three aspects of AF pathophysiology is discussed: (i) Ca\(^{2+}\)-mediated signalling and transcription; (ii) atrial contractile dysfunction; and (iii) arrhythmogenicity.

2. Normal Ca\(^{2+}\) handling in atrial myocytes

Activation of voltage-gated L-type Ca\(^{2+}\) channels by membrane depolarization leads to the influx of a small amount of Ca\(^{2+}\) into the cardiac myocyte, which activates the release of larger quantities...
of Ca\(^{2+}\) from the nearby Ca\(^{2+}\) stores in the sarcoplasmic reticulum (SR). This amplification of the trigger Ca\(^{2+}\) and its propagation results in a cell-wide transient increase in [Ca\(^{2+}\)], that initiates contraction as free Ca\(^{2+}\) binds to the myofilaments. This Ca\(^{2+}\)-induced Ca\(^{2+}\) release (CICR), which is characteristic for cardiac excitation–contraction (EC) coupling, is fundamentally the same in atrial and ventricular myocytes. Ca\(^{2+}\) homeostasis is restored and relaxation occurs as Ca\(^{2+}\) is released from the myofilaments, pumped back into the SR by the SR Ca\(^{2+}\) ATPase and extruded from the cell by the sarcolemmal Na\(^+/\)Ca\(^{2+}\) exchanger (NCX). Specific differences between atrial and ventricular cells are important and affect contraction of the cells, electrical activity, and vulnerability to arrhythmia. Three features of atrial cells are of central importance to atrial Ca\(^{2+}\) handling: (i) transverse tubule organization; (ii) organization of calcium release units (CRU); and (iii) cellular size.

### 2.1 Transverse tubule (TT) organization

Transverse tubules (TTs) of cardiac myocytes are sarcolemmal invaginations of the surface membrane that occur at the Z-line and have transverse and longitudinal dimensions. While TTs are extensively present in mammalian adult ventricular myocytes, occurrence of TTs in atrial myocytes shows large interspecies variability with higher abundance in sheep and dog and fewer TTs in mice, cat, and guinea-pig. Of note, there can be significant variability within a species (mouse), where atrial myocytes with many or few TTs are found next to each other. Also, in rat atrial myocytes, transverse-axial tubule systems (TATS) of variable extent have been reported. However, three rules appear to apply to TTs in atrial myocytes: (i) atrial myocytes generally have relatively few (compared with ventricular myocytes) or no TTs; (ii) the presence or absence of TTs affects many features of Ca\(^{2+}\) handling (see below); (3) thin atrial myocytes tend to have fewer TTs than broad atrial myocytes (e.g. increases in cell width correlate with an increase in TTs in rat atrial myocytes). Also, left atrial rat myocytes were reported to have more TTs than right atrial myocytes.

### 2.2 Calcium release units (CRU)

Ryanodine receptors type 2 (RyR2), the predominant cardiac isoform of the Ca\(^{2+}\) release channel of the SR, are clustered in specialized regions of the SR to form the primary CRU. The CRU RyR2s work as a unit and, when activated, produce a Ca\(^{2+}\) spark, which as the fundamental Ca\(^{2+}\) release event is the elemental unit of EC coupling. CRUs in atrial myocytes are spaced about 1 μm apart along the Z-disk of the sarcomere and thus are organized largely as in ventricular myocytes. If TTs are present (see above), the CRUs would be found close to the TTs. If so, Ca\(^{2+}\) release from a CRU could be triggered by the opening of L-type Ca\(^{2+}\) channels in the TT membrane. Such CRUs would be located in the junctional SR (jSR) and thus be similar to CRUs in jSR in ventricular myocytes. In cells with no TTs, CRUs are found in the SR along the Z-line. These units are called corbular SR or cSR CRUs. In species lacking TTs in atrial myocytes, the majority of CRUs are organized in the cSR with only a minor amount of CRUs found at the jSR-sarcolemmal junctions (Figure 1). These corbular CRUs (cCRUs) are also activated by the elevation of [Ca\(^{2+}\)]; however, in contrast to jCRUs, cCRU activation requires elevated [Ca\(^{2+}\)], to propagate to the cCRU from a neighbouring Ca\(^{2+}\) release site(s). In atrial cells lacking TTs, this results in a centripetally propagating wave of regenerative Ca\(^{2+}\) release (Figure 1B). While many studies found that the central-cellular [Ca\(^{2+}\)], transient was of similar amplitude than the initial subsarcolemmal [Ca\(^{2+}\)], transient studies in rat atrial myocytes reported a reduction in the central-cellular [Ca\(^{2+}\)], compared with the subsarcolemmal [Ca\(^{2+}\)], transient.

### 2.3 Cell size

Atrial cells are thinner and longer than ventricular myocytes. This means that a propagating [Ca\(^{2+}\)], signal from the surface (exterior) sarcolemma to the central part of the atrial myocyte can occur rapidly. Should cells grow enormously in diameter, then there is a longer delay between the action potential (AP) and the [Ca\(^{2+}\)], transient in the centre of the cell. This may lead to the increased likelihood of Ca\(^{2+}\) signalling instability and arrhythmia as discussed in section 3.3. While hypertrophy is a common finding in AF, data on differences in cell size between left and right human atrial myocytes are diverse. While no differences in cell capacitance have been reported between left and right atrial myocytes, atrial myocytes from AF patients were recently found to have increased cell capacitance, while earlier reports showed no differences.

### 3. Changes in intracellular Ca\(^{2+}\) handling preceding the onset of AF

#### 3.1 Genetic alterations

In a familial form of catecholaminergic polymorphic ventricular tachycardia due to genetic defects of RyR2, sinus and AV-nodal dysfunction and AF occur. In rare familial forms of ‘lone’ AF (i.e. AF without structural heart disease), gain-of-function and loss-of-function mutations in K\(^+\) channel genes have been reported. Altered K\(^+\) channel function might indirectly alter intracellular Ca\(^{2+}\) handling by affecting AP and membrane potential. Also, in a recent genome-wide association study in patients with ‘lone’ AF, an association was found with common variants for a gene encoding for Ca\(^{2+}\)-dependent K\(^+\) currents.

In how far these genetic alterations associated with AF might alter intracellular Ca\(^{2+}\) handling is an area of active investigation.

#### 3.2 Cardiac alternans

Cardiac alternans is a beat-to-beat variation in cardiac contractility and repolarization at a constant heart rate. Alternans primarily occurs under conditions of tachycardia and in the presence of structural alterations. Alteration of the central cellular Ca\(^{2+}\) transient has been suggested as the cellular correlate of cardiac alternans. Imaging revealed that in contrast to ventricular cells, Ca\(^{2+}\) alternans in atrial cells resulted in steep [Ca\(^{2+}\)] gradients which caused propagating Ca\(^{2+}\) waves. It has been suggested that an altered Ca\(^{2+}\) sensitivity of RyR2 facilitates the development of Ca\(^{2+}\) alternans in atrial cells. In how far these phenomena contribute to initiation and perpetuation of AF is currently unclear.

#### 3.3 Heart failure and atrial dilatation

Patients with heart failure frequently develop AF. Progression of atrial dilatation also forms a substrate for AF. While slowing of conduction and interstitial atrial fibrosis contributes to AF development in

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these entities, recent work has also identified alterations in atrial Ca\textsuperscript{2+} handling. \textsuperscript{37,38} In a canine heart failure model and a chronic atrial dilatation model in goats, atrial action potential duration (APD) was prolonged,\textsuperscript{37,38} while L-type Ca\textsuperscript{2+} current (ICa,L) was moderately reduced.\textsuperscript{39} These changes led only to a minor decrease in Ca\textsuperscript{2+} entry per activation compared with the strongly curtailed Ca\textsuperscript{2+} entry in AF-induced atrial remodelling (see below).

3.3.1 Ca\textsuperscript{2+} transient and role of TTs
Due to an extensive t-tubular network, [Ca\textsuperscript{2+}]i transients occurred mostly simultaneously throughout normal sheep atrial myocytes.\textsuperscript{13} However, after the induction of heart failure by rapid ventricular pacing, central-cellular [Ca\textsuperscript{2+}]i transients were decreased while subsarcolemmal [Ca\textsuperscript{2+}]i transients were preserved.\textsuperscript{13} TTs were reduced showing that detubulation significantly alters subcellular Ca\textsuperscript{2+} dynamics in this model. Interestingly, in a similar canine model of rapid ventricular pacing-induced heart failure, whole cell [Ca\textsuperscript{2+}] transients were increased in atrial myocytes,\textsuperscript{38} despite moderately reduced ICa,L. This is likely due to an increase in SR Ca\textsuperscript{2+} load (see below). While canine atrial myocytes possess less TTs\textsuperscript{14} than sheep,\textsuperscript{12,13} it is unclear at present whether TT remodelling contributes to altered Ca\textsuperscript{2+} handling in this model.

3.3.2 Altered SR function and diastolic Ca\textsuperscript{2+} leak
In goats with chronic atrial dilatation, SR Ca\textsuperscript{2+} load was decreased.\textsuperscript{37} Phosphorylation of phospholamban (PLB), the regulatory protein of the SR Ca\textsuperscript{2+} ATPase (Serca2a), increases Ca\textsuperscript{2+} affinity of Serca2a and thus the Ca\textsuperscript{2+} pump rate.\textsuperscript{8} In right atrial myocardium from dilated goat atria, PLB phosphorylation was reduced at a site (Ser-16) specific for protein kinase A (PKA), which is compatible with decreased Serca2a function. Hyperphosphorylation of RyR2 is thought to increase the channels’ open probability and Ca\textsuperscript{2+} sensitivity.\textsuperscript{40} Ca\textsuperscript{2+}/Calmodulin-dependent kinase II (CaMKII) phosphorylation of RyR2 (Ser-2815) was increased in this model, consistent with diastolic Ca\textsuperscript{2+} ‘leak’ from the SR. Thus, in chronic atrial dilatation in goats, reduced SR Ca\textsuperscript{2+} load might be due to increased loss and reduced reuptake of Ca\textsuperscript{2+} into the SR.

In the rapid ventricular pacing-induced canine heart failure model, on the other hand, SR Ca\textsuperscript{2+} load was increased.\textsuperscript{38} CaMKII-mediated phosphorylation of PLB was increased (Thr-17), compatible with increased SR Ca\textsuperscript{2+} reuptake. RyR2 protein expression and PKA phosphorylation were reduced.\textsuperscript{38} Of note, the intra-SR Ca\textsuperscript{2+} buffering protein calsequestrin was also reduced,\textsuperscript{38} which is known to facilitate spontaneous Ca\textsuperscript{2+} release from the SR.\textsuperscript{41} Indeed, spontaneous [Ca\textsuperscript{2+}], transients occurred in left and right atrial myocytes but were prevented when RyR2 were blocked,\textsuperscript{38} thus suggesting

Figure 1 (A) Di-8 ANEPPS membrane staining of a rabbit atrial myocyte reveals the absence of t-tubules. The schematic drawing demonstrates the structural organization of the Ca\textsuperscript{2+} release apparatus in atrial myocytes. See text for further details. IP,R, inositol 1,4,5 trisphosphate receptor; Mito, mitochondrion. (B) Transverse confocal line scan of intracellular Ca\textsuperscript{2+} release in a rabbit atrial myocyte during external field stimulation showing the characteristic spatial and temporal delay between peripheral (ss) and central (cc) Ca\textsuperscript{2+} release (modified from Greiser et al.\textsuperscript{71}).
spontaneous Ca\(^{2+}\) release from the SR. Increased SR Ca\(^{2+}\) load and reduced intra-SR Ca\(^{2+}\) buffering led to arrhythmogenic Ca\(^{2+}\) release in atrial myocytes in this model.\(^3\) This differs from the proposed mechanism for arrhythmogenic Ca\(^{2+}\) release in AF-induced atrial remodelling, which is based on hyperphosphorylated ('leaky') RyR2 (see below).

### 3.4 Changes in \(I_{\text{Ca,L}}\) preceding the onset of AF in patients

In patients at high risk for the development of AF (e.g., mitral valve disease, heart failure), \(I_{\text{Ca,L}}\) in right atrial myocytes was reduced compared with patients at low risk for AF (coronary artery disease).\(^4\) Also, in cells from the high-risk group, the response to phosphodiesterase inhibition, which is known to modulate \(I_{\text{Ca,L}}\), was reduced compared with cells from the low-risk group.\(^5\) Interestingly, in another study, right atrial cells from patients with left ventricular dysfunction had shortened APs but unchanged \(I_{\text{Ca,L}}\).\(^6\)

L-type Ca\(^{2+}\) current data from human atrial myocytes preceding the onset of postoperative AF are inconsistent. Van Wagener et al.\(^7\) described larger \(I_{\text{Ca,L}}\) in right atrial myocytes from patients who developed postoperative AF compared with myocytes from patients who did not, while another study found no differences in APD and \(I_{\text{Ca,L}}\) in right atrial myocytes from patients with or without post-operative AF.\(^8\)

Inconsistent results in human AF likely reflect heterogeneous patient populations and differences in techniques used for detection of post-operative AF.

### 4. Changes in intracellular Ca\(^{2+}\) handling resulting from AF

After the onset of AF, the AERP progressively shortens\(^7\) due to a concomitant shortening of APD.\(^9\) Increase in inwardly rectifying K\(^+\) currents (mainly a constitutively active acetylcholine-activated K\(^+\) current \([I_{\text{K,ac}}])\),\(^9\) a decrease in the transient outward K\(^+\) current \([I_{\text{to}}])\) and a pronounced reduction of the L-type Ca\(^{2+}\) current \([I_{\text{Ca,L}}])\) by \(70\%)\) underlie APD shortening and also render the shape of the AP more triangular (for a review of ion currents in human AF see Workman et al.).\(^10\) This atrial 'electrical remodelling' contributes to the perpetuation of the arrhythmia\(^7\) and has profound effects on intracellular Ca\(^{2+}\) handling.\(^11\)

#### 4.1 Mechanisms underlying \(I_{\text{Ca,L}}\) reduction in AF

\(I_{\text{Ca,L}}\) reduction is a hallmark of AF-induced electrical remodelling.\(^1\) Reduced protein expression of the pore-forming \(\alpha_{1C}\) subunit,\(^2\) which is inconsistent in AF,\(^3\)\(^4\) and altered channel regulation have been suggested as underlying mechanisms. \(I_{\text{Ca,L}}\) is regulated by a variety of kinases (e.g., PKA, protein kinase C, CaMKII).\(^5\)\(^6\) which increase the current. While PKA signal transduction is unchanged in AF,\(^5\) increased CaMKII activity is a consistent finding.\(^7\)\(^8\) Interestingly, increased CaMKII activity in AF seems to be offset by an increased phosphatase (PP) activity resulting in a net reduction of \(I_{\text{Ca,L}}\).\(^9\)\(^10\) Indeed, the PP inhibitor okadaic acid increased \(I_{\text{Ca,L}}\) to almost normal levels in human atrial myocytes from AF patients.\(^11\)

While the CaMKII inhibitor KN-93 reduced \(I_{\text{Ca,L}}\) in control cells, it did not affect \(I_{\text{Ca,L}}\) in AF cells, thus further supporting the hypothesis that increased CaMKII activity is offset by a higher increase in PP activity in AF myocytes.\(^12\)

The effect of the non-receptor tyrosine kinase src on \(I_{\text{Ca,L}}\) in human atrial myocytes is controversial. Two studies found an inhibitory effect on \(I_{\text{Ca,L}}\) in control cells,\(^13\)\(^14\) which was lost in AF.\(^15\) Another study reported no effect of src kinases on \(I_{\text{Ca,L}}\) in control cells but found an increase in src-kinase protein expression in AF tissue.\(^16\) Further studies are needed to elucidate the role of tyrosine kinases on \(I_{\text{Ca,L}}\) regulation in control and during AF.

Surprisingly, single channel recordings in human AF cells revealed an increased open probability of L-type Ca\(^{2+}\) channels,\(^17\) which at present cannot be reconciled with the strongly reduced whole cell L-type Ca\(^{2+}\) current. While kinase/phosphatase imbalances clearly affect \(I_{\text{Ca,L}}\) regulation in AF, further insight could be gained from directly measuring channel (subunit) phosphorylation at kinase-specific phosphorylation sites. Also, subunit trafficking and channel assembly have not yet been evaluated in AF.

Further evidence for altered channel regulation comes from a sheep model of AF, where \(I_{\text{Ca,L}}\) was only slightly reduced (\(\sim 24\%))\)\(^18\) However, when measured with Ca\(^{2+}\) chelating agents in the pipette, \(I_{\text{Ca,L}}\) increased in control cells only, while the current did not change in AF cells. This is compatible with less Ca\(^{2+}\)-dependent inactivation of \(I_{\text{Ca,L}}\), which might be due to reduced subsarcolemmal Ca\(^{2+}\) transients or increased intracellular Ca\(^{2+}\) buffering in AF.

#### 4.2 Reduced whole cell \([\text{Ca}^{2+}]_i\) transients are not solely caused by \(I_{\text{Ca,L}}\) reduction

In AF, due to reduced \(I_{\text{Ca,L}}\), significantly less Ca\(^{2+}\) enters the cell per activation, thus reducing the amount of trigger Ca\(^{2+}\) for Ca\(^{2+}\) release from the jSR and the subsequent activation of the centripetal Ca\(^{2+}\) wave (see above). Indeed, \(I_{\text{Ca,L}}\) and whole cell \([\text{Ca}^{2+}]_i\) transients were reduced in various AF models,\(^19\)\(^20\) suggesting that reduced \(I_{\text{Ca,L}}\) might underlie the reduced \([\text{Ca}^{2+}]_i\). However, recent subcellular analysis revealed that \([\text{Ca}^{2+}]_i\) transients in the subsarcolemmal region were not reduced in atrial tachycardia-induced remodelling in sheep and rabbit models.\(^21\)\(^22\) Interestingly, pharmacological reduction of \(I_{\text{Ca,L}}\) in normal left atrial rabbit myocytes significantly reduced subsarcolemmal \([\text{Ca}^{2+}]_i\) transients, suggesting that additional factors, apart from \(I_{\text{Ca,L}}\) reduction, contribute to altered subsarcolemmal Ca\(^{2+}\) release and reduced \([\text{Ca}^{2+}]_i\). In this model,\(^23\) A potential explanation for the preserved subsarcolemmal \([\text{Ca}^{2+}]_i\), might be an increased Ca\(^{2+}\) sensitivity of SR Ca\(^{2+}\) release (e.g. increased RyR2 phosphorylation, see below).

#### 4.3 Alterations in SR function: increased CICR efficacy, preserved SR Ca\(^{2+}\) load, and increased RyR2 phosphorylation

At a constant SR Ca\(^{2+}\) load, subsarcolemmal SR Ca\(^{2+}\) release in atrial myocytes is finely graded by the amplitude of \(I_{\text{Ca,L}}\).\(^24\) As indicated above, the preserved subsarcolemmal \([\text{Ca}^{2+}]_i\) transient in tachycardia-induced remodelling in a rabbit model suggests that less \(I_{\text{Ca,L}}\) elicits a \([\text{Ca}^{2+}]_i\) transient that is similar to control cells. This is indicative of more efficient CICR. Hyperphosphorylation of RyR2s has been suggested to increase the channels’ Ca\(^{2+}\) sensitivity and open probability and thus to facilitate SR Ca\(^{2+}\) release.\(^25\) Although this issue is controversial,\(^26\) Indeed, increased RyR2 phosphorylation at the PKA (Ser-2809) and CaMKII (Ser-2815) sites was found in human AF (Figure 2).\(^27\)\(^28\) despite an increase in phosphatases
Interestingly, increased PP1 activity seems to be locally offset by an increased activation of (PP1)-inhibitor 1 and a general increase in CaMKII activity (Figure 2). Increased RyR2 phosphorylation and preserved SR Ca2+ load were found in human AF together with an increased Ca2+ spark frequency. Also, lipid bilayer studies of RyR2 isolated from AF patients revealed an increased open probability of the channel. This is compatible with an increased Ca2+ ‘leak’ from the SR in AF. However, without a higher compensatory Ca2+ reuptake, an increased Ca2+ leak would reduce SR Ca2+ load. Estimation of Serca2a function in AF has been inconclusive with reports of no changes, reduced, and increased function (based on protein expression and phosphorylation levels). Recent work in rats has shown that the PLB/Serca2a ratio is lower in atria compared with ventricles compatible with increased Serca2a function in atria. However, Serca2a function in atrial myocardium is also potently regulated by another accessory protein, sarcolipin (SLN), which is atrial specific. SLN but not PLB mRNA was reduced in right atrial tissue from AF patients, suggesting that SNL expression and function might be regulated in AF.

Also, upregulation of the NCX, which is a consistent finding in AF (see below), would further ‘unload’ the SR by increasing Ca2+ extrusion. Therefore, it is at present unclear whether (1) increased RyR2 phosphorylation and open probability lead to a significant loss of
Ca$^{2+}$ from the SR and (2) whether there is compensatory increase in Ca$^{2+}$ reuptake into the SR in AF. Quantification of SR Ca$^{2+}$ leak and reuptake would help clarify these issues.

4.4 Altered subcellular Ca$^{2+}$ release

In sheep atrial myocytes, which have functionally relevant TTs, AF induced a significant reduction in TTs. This was associated with dys-synchrony of SR Ca$^{2+}$ release, which contributed to the reduced whole-cell Ca$^{2+}$ transient in this model. In rabbit atrial myocytes, which do not have t-tubules, tachycardia-induced atrial remodelling led to the failure of the centripetally propagating intracellular Ca$^{2+}$ wave resulting in the blunted Ca$^{2+}$ release in the central region of the myocyte (Figure 3).71

Thus, if TTs are present, AF-induced detubulation is an important mechanism contributing to the altered subcellular Ca$^{2+}$ release. In atrial cells without TTs, AF also significantly alters subcellular Ca$^{2+}$ handling.

4.5 Na$^+$/Ca$^{2+}$ exchanger upregulation

While upregulation of the NCX is consistently found in AF (Figure 3B),12,57,72 its functional implications for subcellular Ca$^{2+}$ handling during AF require further investigation. A canine study showed that KB-R7943, a blocker of the Ca$^{2+}$ entry mode (‘reverse mode’) of the NCX, prevented initial electrical remodelling after the onset of rapid atrial activation.73 This suggested that Ca$^{2+}$ entry via reverse mode of NCX, which is negligible under physiological conditions, played a role during the early phase of atrial remodelling. However, KB-R7943 is not selective for the NCX and also inhibits Na$^+$, K$^+$, and L-type Ca$^{2+}$ currents.74 Thus, the described effect of KB-R7943 could also be due to its effect on I_{Ca,L}. The NCX is mainly activated by local [Ca$^{2+}$], and [Na$^+$], where the NCX, was reduced in a canine model of AF.75 Reduced [Na$^+$], would favour increased Ca$^{2+}$ removal from the subsarcolemmal region by the NCX (‘forward mode’). In AF sheep and rabbit models, abbreviated subsarcolemmal Ca$^{2+}$ transients were found, which might indicate increased Ca$^{2+}$ extrusion by NCX.12,63 The contribution of NCX upregulation to cellular pro-arrhythmic mechanisms in AF is discussed in section 5.3.

4.6 Effect of altered neurohormonal regulation on intracellular Ca$^{2+}$ handling

5-Hydroxytryptamine (5-HT), via 5-HT$_4$ receptors and subsequent PKA-mediated increases in L-type Ca$^{2+}$ current,76 prolongs the plateau potential of the AP and induces afterdepolarizations in human right atrial myocytes.77 Interestingly, in right atrial myocytes from AF patients, 5-HT failed to induce afterdepolarizations, although mRNA of 5-HT$_4$ was increased.78 In short-term AF in pigs, infusion of a 5-HT$_4$ antagonist reduced initial ERP and AP shortening.79 Thus, while apparently regulated in AF, the role of altered 5-HT$_4$ signalling in Ca$^{2+}$ handling in AF is unclear at present.

Due to the activation of the renin–angiotensin system (RAS) in AF patients80 and the fact that angiotensin II (AT II) increased Ca$^{2+}$

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**Figure 3** (A) Whole-cell Ca$^{2+}$ transient amplitude in rabbit atrial myocytes is significantly reduced after 5 days of atrial tachycardia induced by rapid atrial pacing (RAP). (B) Plot of the Na$^+$/Ca$^{2+}$ exchange current ($i_{\text{NCX}}$) vs. [Ca$^{2+}$]. ($\Delta F_0/F_0$) is a measure for the activity of the Na$^+$/Ca$^{2+}$ exchanger (NCX). In remodelled cells (after RAP, shown in red), this relation is significantly steeper than in control (shown in black) showing more $i_{\text{NCX}}$ for a given [Ca$^{2+}$], which denotes a higher NCX activity. (C) Transverse confocal line scans show a significantly blunted central-cellular Ca$^{2+}$ wave in remodelled atria (modified from Greiser et al.71).
5. Pathophysiological consequences of altered Ca\textsuperscript{2+} handling

Adaptation to the high atrial rate in AF is mediated by complex changes in intracellular signalling cascades, many of which are regulated by [Ca\textsuperscript{2+}]. While intracellular Ca\textsuperscript{2+} loading in atrial myocytes transiently increases upon the onset of AF, it gradually declines again to normal levels due to the subsequent remodelling process (Figure 4).\textsuperscript{75,83} After cessation or cardioversion of AF, the Ca\textsuperscript{2+} load will rapidly decrease due to the restoration of a normal (slower) atrial rate.\textsuperscript{84} These conditions are equivalent to the slow stimulation rate at which Ca\textsuperscript{2+} handling is usually studied in \textit{in vitro} experiments. Thus, most \textit{in vitro} studies of Ca\textsuperscript{2+} handling in AF reflect the consequences of adaptation to the high rate and high Ca\textsuperscript{2+} load (remodelling). At the same time, however, Ca\textsuperscript{2+} loading in the cells is actually low as a consequence of the low-rate.

5.1 Ca\textsuperscript{2+}-dependent signal transduction pathways and excitation–transcription coupling

Several studies demonstrated activation of the Ca\textsuperscript{2+}-dependent proteases calpain I and calpain II in patients with AF.\textsuperscript{85,86} Calpain I-dependent degradation of troponin T is thought to contribute to the contractile dysfunction in AF.\textsuperscript{86} After 24 h of rapid pacing, an \textit{in vitro} study in HL-1 atrial myocytes reported a significant reduction in the plasmalemmal protein expression levels of the L-type Ca\textsuperscript{2+} channel \(\alpha_{1C}\) subunit, myolysis, and nuclear condensation. This was paralleled by a 14-fold increase in calpain activity.\textsuperscript{87} Interestingly, inhibition of calpain, but not treatment with the calcium antagonist verapamil, prevented these ultrastructural changes.\textsuperscript{87}

Elevated [Ca\textsuperscript{2+}], leads to calmodulin saturation and the subsequent activation of the phosphatase calcineurin (Cn).\textsuperscript{88} Activated Cn dephosphorylates nuclear factor of activated T cells (NFAT), allowing translocation of NFAT into the nucleus where this transcription factor induces activation of hypertrophic pathways (Figure 5). Cellular hypertrophy is a consistent finding in AF.\textsuperscript{89,90} Increased Cn activity and nuclear translocation of NFAT-c3 and NFAT-c4 were shown in pigs with AF.\textsuperscript{91} Interestingly, FK506, a Cn inhibitor, abolished the hypertrophic response induced by electrical pacing of atrial tissue slices.\textsuperscript{92} It appears, however, that the calcineurin NFAT pathway is not only involved in hypertrophic responses. A recent \textit{in vitro} study demonstrated a transient increase in Cn activity in canine right atrial myocytes after 8 h of rapid pacing, which returned to baseline at 24 h.\textsuperscript{93} Cn and NFAT inhibition fully prevented \(I_{Ca,L}\) downregulation in these cells suggesting that the Cn NFAT pathway plays a pivotal role in frequency-dependent regulation of \(I_{Ca,L}\) in this setting.\textsuperscript{93}

Another consequence of increased [Ca\textsuperscript{2+}]\textsuperscript{2+} during AF is the activation of CaMKII, which is discussed in sections 3.3, 4.1, and 4.3.

The acute ‘Ca\textsuperscript{2+} unloading’ right after the cessation of AF together with the adaptive processes developed during AF will decrease [Ca\textsuperscript{2+}]\textsuperscript{2+} overload and calmodulin saturation. This will reduce calpain activation, thereby contributing to reverse remodelling of the atria. Indeed, changes in ion currents, action potential duration, and atrial contractility were reversible within days to weeks.\textsuperscript{94} whereas structural alterations might only reverse very slowly if at all.

Nuclear Ca\textsuperscript{2+} transients are thought to be due to diffusion of cytosolic Ca\textsuperscript{2+} through the nuclear pores into the nucleoplasm resulting in Ca\textsuperscript{2+} transients that follow cytosolic Ca\textsuperscript{2+} transients in time. However, the presence of a Ca\textsuperscript{2+} release machinery including IP\textsubscript{3}-receptors, RyR2, angiotensin receptors, and \(\beta\)-adrenoceptors in the nuclear envelope has been shown in numerous studies.\textsuperscript{95} Indeed,
the intrinsic Ca\textsuperscript{2+} release in the perinuclear region and from the nuclear envelope has been documented (Figure 5). IP\textsubscript{3}-receptor activation appears to play an important role in this process.\textsuperscript{96} It was recently shown that after 5 days of rapid atrial pacing, the cytosolic and nuclear Ca\textsuperscript{2+} transients were reduced in rabbit atrial myocytes.\textsuperscript{97} Whether this is caused by reduced diffusion of Ca\textsuperscript{2+} from the cytosol or altered intrinsic Ca\textsuperscript{2+} release is currently unknown. IP\textsubscript{3} receptors are upregulated in right atrial tissue of AF patients, which might increase nuclear IP\textsubscript{3} signalling and perinuclear Ca\textsuperscript{2+} release.\textsuperscript{98}

Activation of nuclear CaMKII leads to phosphorylation of proteins involved in the epigenetic regulation of cardiac myocytes. CaMKII-dependent phosphorylation of histone-deacetylases (HDAC) results in their nuclear export. Pharmacological inhibition or knock-out of HDAC in ventricular myocytes prevented reactivation of a hypertrophic gene program suggesting a role of HDAC export in activation of hypertrophic genes (Figure 5).\textsuperscript{99} Recently, the pathophysiological relevance of this pathway was also demonstrated in the atria. In transgenic mice, constitutive activation of HDAC resulted in cardiac hypertrophy with increased left atrial dimensions and a higher inducibility of atrial arrhythmias, including AF.\textsuperscript{100} Pharmacological inhibition of this pathway prevented the development of an AF substrate and reduced the inducibility of atrial arrhythmias.\textsuperscript{100} This study demonstrates the importance of Ca\textsuperscript{2+}-dependent gene regulation in atrial myocytes and also suggests a potential role for HDAC inhibition as a new therapeutic approach in AF.

5.2 Altered Ca\textsuperscript{2+} handling in AF and its implications for atrial contractile dysfunction

After AF initiation, atrial hypococontractility develops in parallel with electrical remodelling.\textsuperscript{101} Upon cardioversion after 5 days of AF in a goat model, shortened AERPs and hypococontractility returned to baseline in a parallel time course,\textsuperscript{101} thus emphasizing the importance of APD, which determines ERP, for atrial contractility. Additionally, atrial cell shortening also critically depends on AP morphology, specifically the plateau potential.\textsuperscript{102} APD prolongation is one important mechanism of antiarrhythmic therapy or cardioversion of AF.\textsuperscript{103} Interestingly, APD prolongation and alteration of AP morphology also restored atrial contractile force in AF.\textsuperscript{102} AVE0118, a blocker of I\textsubscript{to} and the atrial-specific ultra rapid inward rectifier potassium current (IKur), prolonged early repolarization (APD\textsubscript{30}) and increased contractility in atrial muscle bundles from patients with AF\textsuperscript{102} and restored atrial contractility in the AF goat model in vivo.\textsuperscript{104} The prolongation of APD\textsubscript{30} increases Ca\textsuperscript{2+} entry via ‘reverse’ mode of the NCX, which is the underlying mechanism of AVE0118-mediated inotropy.\textsuperscript{102} However, changes in AP shape alone cannot fully explain atrial contractile remodelling in AF.\textsuperscript{14}

Atrial tachycardia/AF-induced remodelling,\textsuperscript{12,49,63} heart failure,\textsuperscript{13} and chronic atrial dilatation\textsuperscript{37} all lead to a significant reduction of atrial contractile force. In all of these models, except the canine heart failure model,\textsuperscript{38} steady-state [Ca\textsuperscript{2+}], transients were reduced, thereby decreasing atrial contractile force. The increased [Ca\textsuperscript{2+}], transients

Figure 5 Alterations in Ca\textsuperscript{2+}-dependent intracellular signalling pathways and excitation–transcription coupling during AF. Activation of G protein-coupled receptors (GPCR), e.g. angiotensin 1 and 2 receptors, endothelin receptors (ETA/ETB), alpha-adrenoceptors, and growth factor receptors (GFR) increase cytosolic inositol 1,4,5-trisphosphate (IP\textsubscript{3}) concentration via activation of phospholipase C (PLC). The intracellular target of IP\textsubscript{3} is IP\textsubscript{3} receptors (IP\textsubscript{3}R), which are predominantly located in the subsarcolemmal region and in the perinuclear envelope in atrial myocytes. See text for further details. NFAT, nuclear factor of activated T cells; P, phosphate group; Cn, calcineurin; HDAC, histone deacetylases; CaMKII, Ca\textsuperscript{2+}/Calmodulin-dependent kinase II; TnI, troponin I.
found in the canine heart failure model suggest that atrial contractile dysfunction in this model is due to alterations in the contractile apparatus.

Indeed, PKA phosphorylation of myosin-binding protein-C (MyBP-C) was reduced.

PKA phosphorylation of MyBP-C is important for maximum force development and stretch-dependent augmentation of force generation. 

MyBP-C phosphorylation was increased in human right atria from AF patients together with reduced myofibrillar force generation, relaxation, and Ca2+ sensitivity (Figure 2).

This is in contrast to previous human studies where MyBP-C phosphorylation was reduced and myofibrillar force generation was not altered.

Interestingly, oxidation markers were increased in myofibrillar proteins from AF patients, suggesting that oxidative injury might contribute to atrial contractile dysfunction.

Thus, atrial contractile dysfunction either due to or preceding AF, except in a canine heart failure model, is caused by a combination of decreased [Ca2+]i, transients and altered myofibrillar function.

5.3 Cellular proarrhythmic mechanisms, arrhythmogenic Ca2+ waves, and ectopic focal discharges

If atrial remodelling leads to cellular Ca2+ instability, there are three cellular-based factors that contribute to cellular proarrhythmic phenomena: (i) Ca2+ waves, (ii) Ca2+-activated inward current, (iii) electrical membrane resistance. A discussion of these mechanisms (below) is viewed as a brief introduction rather than a comprehensive treatment.

5.3.1 Ca2+ waves

Under physiological conditions in atrial cells, propagating Ca2+ signals occur (see above). This is part of the normal EC coupling process in atrial cells that do not have TTs. Importantly, the cells are thin and thus Ca2+ waves propagate over short distances and the direction of propagation tends to be perpendicular to the long axis of the cell.

For all of these reasons, the normal Ca2+ wave propagation, while representing instability in ventricular myocardies, is not necessarily arrhythmogenic in atrial myocardies.

When Ca2+ ‘overload’ (i.e. increased [Ca2+]i, see above) occurs, the sensitivity of RyR2s to be triggered by cytosolic [Ca2+]i increases. This increase in [Ca2+]i, sensitivity means that there is a greater probability of spontaneous Ca2+ sparks and spontaneous Ca2+ waves. Under these conditions, in addition to the AP-triggered Ca2+ release that underlies EC coupling, there may be Ca2+ release events (including spontaneous Ca2+ sparks and waves) that arise between the normal AP-triggered events. Such spontaneous events may propagate in any direction, including along the long axis of the cell. Whenever they occur, the [Ca2+]i elevation associated with these abnormal Ca2+ waves may activate membrane current during abnormal periods (e.g. during repolarization and diastole).

5.3.2 Ca2+-activated inward current

The primary Ca2+-activated inward current is the NCX current (I\(_{\text{NCX}}\)). This sarcocendymal protein is the primary transport system that pumps Ca2+ out of the cell. When there is an elevation of [Ca2+]i, the NCX responds rapidly to produce inward current as Ca2+ is extruded. I\(_{\text{NCX}}\) has been described as the arrhythmogenic ‘transient inward current or I\(_{\text{T}}\)’, that is responsible for delayed afterdepolarizations (DADs).

DADs occur in diastole and, when sufficiently large, can trigger an extrasystole. Work has shown that I\(_{\text{T}}\) is observed in a single cardiac myocyte when a wave of elevated Ca2+ propagates within a single myocyte.

Additionally, Ca2+-activated I\(_{\text{I1}}\) can occur during AP repolarization and then contribute to early afterdepolarizations (EADs), which may also trigger extrasystoles. Also, the combination of short APs and the presence of a high SR Ca2+ load has been reported to provoke NCX-mediated late phase 3 EADs.

While most studies on Ca2+-dependent arrhythmias have been carried out in ventricular myocytes and Purkinje fibres, this mechanism has also been proposed for atrial cells.

In AF, protein expression of NCX is upregulated. This means that for any given elevation of [Ca2+]i, the I\(_{\text{NCX}}\) will be larger. Thus, a propagating wave of Ca2+ would be associated with a higher peak I\(_{\text{NCX}}\).

However, these cellular proarrhythmic mechanisms have so far not been shown to underlie the initiation of sustained AF in vivo. To date, all studies addressing Ca2+-mediated cellular proarrhythmic mechanisms in human AF used cells from the right atrial appendage. Although there are alterations in Ca2+ signalling in these cells (e.g. increased frequency of Ca2+ sparks and waves) that are compatible with the generation of cellular proarrhythmic mechanisms, the right atrial appendage is less arrhythmogenic than other areas in the right and left atrium. Thus, to determine the importance of these potentially pro-arrhythmic mechanisms for atrial arrhythmogenesis in vivo, it will be important to determine whether the frequency of Ca2+ sparks and waves is also increased in atrial myocytes from more arrhythmogenic regions of human atria.

5.3.3 Electrical membrane resistance

The efficacy of a given level of inward current to trigger an extrasystole in a single cell depends on the total membrane resistance of the cell. For example, in human AF, inward rectifying K+ currents are increased. Under these conditions, a larger arrhythmogenic inward current is required to produce an extrasystole than under the conditions when the inward rectifying K+ current is normal (smaller). Thus, DADs and EADs would be less likely in these cells, a result that is opposite to that in ventricular myocytes in heart failure.

Thus, this is an area of active investigation.

6. Conclusions

Here, we have reviewed Ca2+ handling alterations associated with AF. An important observation is that changes in Ca2+ handling in atrial cells might contribute to initiation and perpetuation of AF but also develop as a result of AF. While it is clear that AF is a multifactorial disease with alterations in atrial tissue, cellular, and molecular features, Ca2+ instability has been demonstrated to be important to the manifestation of AF. Current work seeks to further unravel the contribution of altered intracellular Ca2+ handling to specific subtypes of AF. Further understanding of the genetic and molecular causes of the disease forms the basis for improved treatment by antiarrhythmic agents and ablation therapies but might also lead to the development of more specific preventive concepts targeted at patients at risk for AF.

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