Role of abnormal sarcoplasmic reticulum function in atrial fibrillation

Atrial fibrillation is the most common cardiac arrhythmia and is a cause of significant morbidity and mortality if left untreated. Atrial fibrillation has been associated with profound changes in sarcoplasmic reticulum Ca^{2+} homeostasis, which might contribute to both reduced contractile function and increased arrhythmogenesis in the atria. Studies in human tissue samples and various animal models of atrial fibrillation have revealed changes in both expression levels and post-translational modifications of key Ca^{2+} handling proteins that may contribute to arrhythmogenesis. In this article, we will focus on the molecular basis of alterations in sarcoplasmic reticulum Ca^{2+} handling in atrial fibrillation and their potential therapeutic implications.

KEYWORDS: atrial fibrillation ■ **Ca^{2+}/calmodulin-dependent protein kinase II** ■ **phosphorylation** ■ **remodeling** ■ **ryanodine receptor type 2** ■ **sarcoplasmic reticulum**

Xander HT Wehrens[1†], Sameer Ather[1] & Dobromir Dobrev[2]

[†]Author for correspondence:
[1]Baylor College of Medicine, One Baylor Plaza, BCM 335, Houston, TX 77030, USA
Tel.: +1 713 798 4261
Fax: +1 713 798 3475
wehrens@bcm.edu
[2]Department of Pharmacology & Toxicology, Dresden University of Technology, Germany

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia with a lifetime risk of 20–25% [1]. The major cause of morbidity is thromboembolism leading to stroke, which arises from stasis of blood in the atria due to decreased atrial contractility. Impaired intracellular Ca^{2+} handling is believed to underlie atrial mechanical dysfunction [2]. The smaller amplitude of systolic Ca^{2+} transient is a major determinant of decreased contractility in AF [3]. Nevertheless, the predisposition to spontaneous diastolic sarcoplasmic reticulum (SR) Ca^{2+} release might contribute to arrhythmogenesis in AF by promoting triggered activity [4]. In this article, we will review the cellular and molecular basis of abnormal SR Ca^{2+} handling in AF and discuss how new drug therapies might be developed to reverse these defects.

Excitation–contraction coupling in atrial myocytes

Excitation–contraction coupling is the fundamental process by which an action potential initiates contraction of a cardiomyocyte [5]. Plasmamembrane depolarization leads to the influx of Ca^{2+} via voltage-gated L-type Ca^{2+} channels, which, in turn, triggers a much greater release of Ca^{2+} from the SR via ryanodine receptor type 2 (RyR2) channels (Figure 1). The ensuing systolic Ca^{2+} transient leads to the binding of Ca^{2+} to troponin C, which induces a conformational change in the regulatory complex, eventually leading to actin and myosin filaments sliding past each other and, thereby, shortening the sarcomere. During diastole, Ca^{2+} is sequestered into the SR by sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA2a), reducing cytosolic Ca^{2+} concentrations and facilitating myocyte relaxation [6]. To a smaller extent, Ca^{2+} is also removed from the cytosol through the forward mode of the Na^+/Ca^{2+}-exchanger (NCX). The extent of reloading the SR with Ca^{2+} critically determines the amplitude of the subsequent Ca^{2+} transient and myocyte contractility.

Activation of a physiological stress reaction, such as the fight-or-flight response, leads to enhanced cardiac contractility. At the level of cardiomyocytes, this is mediated by increased SR Ca^{2+} release and reuptake during the excitation–contraction coupling cycle. The amplitude of the L-type Ca^{2+} current ($I_{Ca,L}$) is enhanced by phosphorylation by protein kinase A (PKA), which is activated by the stimulation of β-adrenoceptors on the plasmalemma [7]. PKA also phosphorylates RyR2 and SERCA2a-inhibitor phospholamban (PLN), thereby enhancing the release from and reuptake of Ca^{2+} into the SR, respectively. In addition, Ca^{2+}/calmodulin-dependent kinase II (CaMKII) has been demonstrated to increase RyR2-mediated SR Ca^{2+} release and to relieve the effects of PLN inhibition on SERCA2a activity [8,9]. Thus, several intracellular signaling pathways involving protein phosphorylation of Ca^{2+} channels and transporters dynamically modulate excitation–contraction coupling to maintain homeostasis.

There are important differences in the subcellular architecture of atrial and ventricular cardiomyocyte Ca^{2+} release units that provide the structural framework for intracellular Ca^{2+} release and reuptake associated with excitation–contraction coupling. For example, the Ca^{2+} transient

part of future medicine fsg

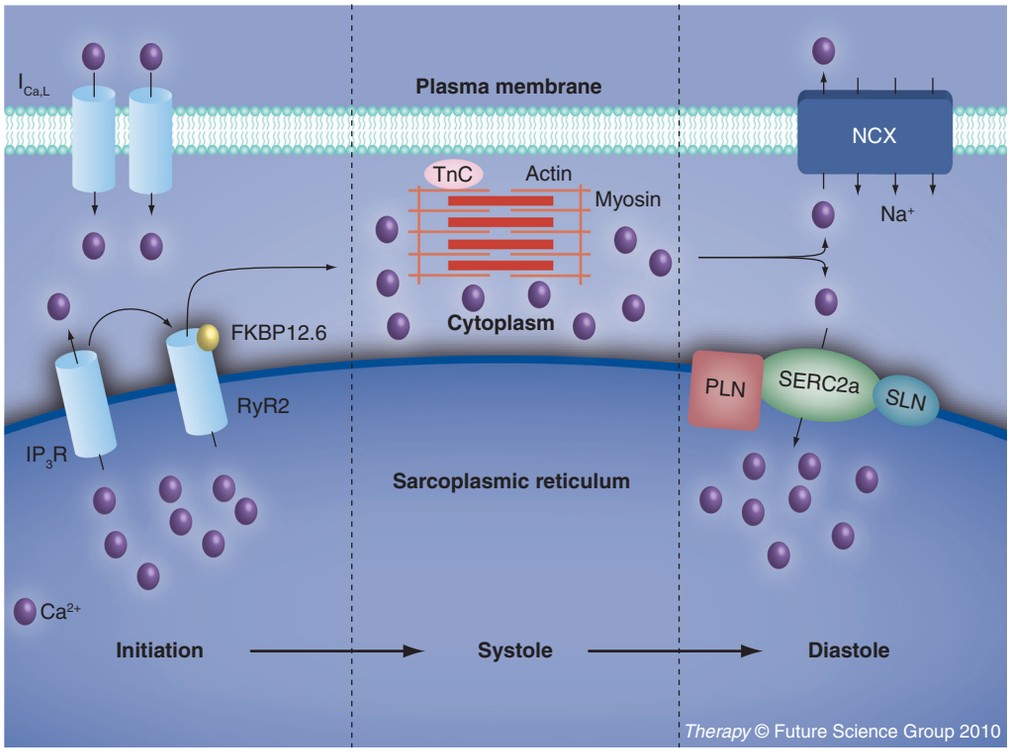

Figure 1. Representation of excitation–contraction coupling in an atrial myocyte. With the advent of an action potential, L-type Ca^{2+} channels are activated resulting in the influx of Ca^{2+}. This influx of Ca^{2+} activates the adjacent ryanodine receptors resulting in outpouring of Ca^{2+} stored in the sarcoplasmic reticulum (SR) into the cytosol. IP_3R are also activated alongside RyR2 and have a role in both inducing independent SR Ca^{2+} release and amplifying SR Ca^{2+} release through RyR2. The release of Ca^{2+} activates the contractile proteins initiating systole. The increased cytosolic Ca^{2+} inactivates L-type Ca^{2+} channels, activates Na^+/Ca^{2+}-exchanger (causing efflux of Ca^{2+}) and activates SERCA2a (causing reuptake of Ca^{2+} into SR). This results in the termination of systole and the onset of diastolic phase.
$I_{Ca,L}$: L-type Ca^{2+} current; IP_3R: Inositol 1,4,5-trisphosphate receptors; NCX: Na^+/Ca^{2+}-exchanger; PLN: Phospholamban; RyR2: Ryanodine receptor type 2; SERCA2a: Sarco/endoplasmic reticulum Ca^{2+}-ATPase; SLN: Sarcolipin; TnC: Troponin C.

is smaller and spatially more dispersed in rat atrial myocytes compared with ventricular myocytes [10]. In rat atrial cells, systolic Ca^{2+} transients arise at the cell periphery and then propagate, as waves, into the cells interior, presumably owing to the relative absence of transverse T-tubules [11]. Reuptake of Ca^{2+} into the SR is more robust in atrial myocytes, and relatively, there is a greater content of SR Ca^{2+} compared with ventricular myocytes. Whereas the enhanced SR Ca^{2+} load might facilitate arrhythmogenesis in pathologically remodeled atria, spontaneous Ca^{2+} release does not occur in healthy atria. Please refer to Dobrev for a detailed review of Ca^{2+} signaling features unique to atrial myocytes [12].

Regulation of SR Ca^{2+} release in the atria

As in ventricular myocytes, RyR2 are the principal Ca^{2+} release channels responsible for Ca^{2+}-induced Ca^{2+} release in atrial myocytes. The simultaneous opening of a group of RyR2s leads to Ca^{2+} sparks, which are elementary SR Ca^{2+}-release events that can be visualized using confocal microscopy [13]. Many individual sparks coalesce to give a characteristic Ca^{2+} wave that initiates the global Ca^{2+} transient associated with myocyte contractility [14].

A unique property of atrial myocytes is that inositol 1,4,5-trisphosphate receptors (IP_3Rs) are located close to RyR2 in the subsarcolemmal space [15]. It is believed that IP_3Rs can increase local Ca^{2+} concentrations in the vicinity of a RyR2, thereby facilitating Ca^{2+}-induced Ca^{2+} release during excitation–contraction coupling [16]. Expression levels of IP_3R are approximately five- to ten-times higher in rabbit atria compared with ventricular myocytes [17]. Consistent with this observation, direct application of IP_3 to permeabilized rat atrial myocytes evoked a five-times larger global Ca^{2+} transient compared with ventricular cells [15].

The release of Ca^{2+} via RyR2 is also regulated by accessory subunits that bind directly to the RyR2 macromolecular complex [18]. Whereas most initial studies on the composition of the RyR2 protein complex were performed on ventricular myocardium, several recent studies suggest that atrial RyR2 are comprised of the same subunits and binding partners [19–23]. RyR2 are tetramers comprised of four RyR2 subunits, each of which associates with several regulatory subunits. Calmodulin (CaM) is an accessory protein that binds to and regulates RyR2 gating [24]. The FK506-binding protein 12.6 (also known as calstabin2) stabilizes the RyR2 closed conformational state [20,25] and facilitates coupled gating between connected RyR2 channels [26]. Marx *et al.* demonstrated that PKA and protein phosphatases, PP1 and PP2A, bind to RyR2 via specific targeting molecules [18]. These same proteins are present in the atrial RyR2 complex [21]. It has also been demonstrated that atrial RyR2 are a substrate of both PKA and CaMKII-mediated phosphorylation. PKA phosphorylation of an RyR2 at its main phosphorylation site (S2808) increases the RyR2 open probability [25,27]. More recent studies using recombinant RyR2 demonstrated that PKA can also phosphorylate S2030, but the functional implications of this phosphorylation event remain somewhat unclear [28]. The enzyme CaMKII, on the other hand, phosphorylates S2814 [8,19]. CaMKII phosphorylation of S2814 increases the sensitivity to Ca^{2+}-induced activation and increases RyR2 open probability. Taken together, the atrial RyR2/Ca^{2+}-release channel gating properties appear to be similar to those of RyR2 in the ventricle [29]. Additional proteins that bind to the luminal side of RyR2 include junctin and triadin [30]. Finally, RyR2 function is also regulated by the intra-SR Ca^{2+}-buffering protein calsequestrin [31]. The significance of these latter interactions remains to be established in the atria.

Regulation of Ca^{2+} removal mechanisms in the atria

Compared with ventricular myocytes, SR Ca^{2+} reuptake is enhanced in rat atrial myocytes, in part, owing to higher expression levels of SERCA2a [10]. In addition, levels of PLN are lower than in the ventricle [10,32]. Since the SERCA2a/PLN ratio is approximately four-times higher in the atria, SR Ca^{2+} reuptake is enhanced and relaxation of atrial myocardium is facilitated. In the atria, SERCA2a is also regulated by sarcolipin (SLN) [33]. Whereas SLN is predominantly expressed in the atria, PLN expression is more abundant in mouse ventricles [34]. SLN shares a 30% homology with the transmembrane domain of PLN, and its structure and function are very similar to this PLN domain [35]. SLN inhibits SERCA2a activity by decreasing the apparent Ca^{2+} affinity of the pump [36], a process that is dynamically regulated by phosphorylation. Similar to PLN, stimulation of β-adrenoceptors phosphorylates SLN at T5 and enhances contractility by decreasing SERCA2a inhibition [37]. Thus, SLN plays an important regulatory role in the atria.

It has been reported that, in human atria, levels of the NCX are approximately half of those found in the ventricle [38]. Lower NCX levels result in smaller NCX currents compared with ventricular cells [10]. However, atrial myocytes are smaller than ventricular myocytes and when the NCX current amplitude was corrected for cell size, rat atrial cells were found to have a larger NCX current density compared with ventricular cells [10]. Thus, remodeling-related increases in SR Ca^{2+} leak, together with elevated NCX expression levels, may generate large depolarizing Na^{+}/Ca^{2+} exchange currents, which may cause delayed afterdepolarizations and triggered activity, supporting AF maintenance (see later).

Atrial myocytes exhibit markedly increased SR Ca^{2+} content and cellular Ca^{2+} buffering capacity [10], consistent with enhanced SR Ca^{2+} reuptake via SERCA2a. In addition, the Ca^{2+} efflux via sarcolemmal pathways, including NCX, is reduced in atrial cells, and Ca^{2+} entry via L-type Ca^{2+} channels is increased. It has been also suggested that changes in myofilament properties may contribute to the enhanced Ca^{2+} buffering properties of atrial myocytes [39]. As will be discussed, the greater SR Ca^{2+} content in atrial cells may facilitate arrhythmogenic spontaneous SR Ca^{2+} releases in fibrillating atria.

Cellular & molecular mechanisms underlying AF

The two major mechanisms believed to cause AF at the organ level include re-entry and ectopic activity. Sources of these abnormalities are often localized at one of the pulmonary veins or in the posterior wall of the left atrium, near the pulmonary vein junction [40]. Risk factors (i.e., age) and cardiovascular diseases (i.e., heart failure) increase susceptibility to AF by causing specific electrical and structural substrates that promote arrhythmia maintenance once AF has been induced. Cardiovascular diseases can also contribute to the triggers (i.e., acute atrial dilatation) that initiate AF. Thus, the structural

substrate, caused by risk factors and concomitant disease conditions preceding AF, is key for perpetuation of AF [41].

Re-entry requires a susceptible substrate as well as a trigger, usually provided by an ectopic beat. According to the 'leading circle' concept, re-entry results from a balance between tissue refractoriness and conduction speed [42]. Short refractoriness and slow conduction make induction of continuous conduction in a re-entry zone more likely. According to the 'spiral wave' concept [43,44], re-entry is maintained by high-frequency reentrant sources (rotors) with a spiral wave rotating around a central 'core'. The stability of the rotor is determined by the tissue excitability and refractoriness – higher excitability and shorter refractoriness allow the spiral wave to rotate faster, stabilizing the rotor.

Ectopic activity may contribute to AF initiation by acting as a trigger of re-entry. Ectopic activity is caused by abnormal local spontaneous discharges that can be due to afterdepolarizations. Afterdepolarizations are oscillations of the membrane potential of an atrial myocyte that occur during repolarization (early afterdepolarizations [EADs]) or following completion of the action potential (delayed afterdepolarizations [DADs]). Left atrial sources of ectopic activity appear to be of particular importance in a subset of patients with paroxysmal AF [45].

Early afterdepolarizations occur when an abnormal depolarization starts during phase 2 or 3 of the preceding action potential, and are commonly associated with bradycardia or pauses [46]. Pulmonary veins are a preferred site for EADs in canine atria [47]. Excessive action potential prolongations with reactivation of the L-type Ca^{2+} current or late Na^{+} current predispose to the development of EADs in isolated canine right atria [46]. EADs can also occur in the absence of intracellular Ca^{2+}-handling abnormalities. Recently, late phase 3 EADs were suggested to contribute to AF reinitiation [46]. This type of EAD may occur under sympathovagal discharges if acetylcholine-induced action-potential duration abbreviation (parasympathetic effect) is coupled with increased Ca^{2+} transients (sympathetic effect; Ca^{2+}-transient triggering) [48]. It was postulated that this mechanism involves an increase in intracellular Ca^{2+} concentrations during final action potential repolarization, at voltages negative to the equilibrium potential for NCX. These changes are presumed to increase the inward NCX current to generate EADs and trigger activity [47,49].

Delayed afterdepolarizations result from spontaneous diastolic SR Ca^{2+} releases, typically caused by either SR Ca^{2+} overload or dysfunction of the SR Ca^{2+}-release channels. They manifest as an individual or a series of small-amplitude membrane oscillations that could eventually lead to full-blown action potentials. The spontaneous SR Ca^{2+} releases activate a transient inward current that underlies the DAD voltage oscillation [5,50]. The occurrence of DADs have been demonstrated in multicellular preparations from isolated diseased human atrial appendages [51]. Electrical remodeling increases the likelihood of DADs during AF.

Altered SR Ca^{2+} handling in AF

It is well recognized that AF is a progressive disease and that the arrhythmia itself induces electrical remodeling that increases susceptibility and stability of AF [52]. Several factors contribute to electrical remodeling, including shortening of the atrial effective refractory period, which is primarily caused by action-potential shortening [53,54]. At the cellular level, there are profound alterations in mRNA and protein expression levels of various ion channels (for review [55]). Besides reduced $I_{Ca,L}$ [56,57], increased function of inward rectifier K^{+} currents may play a critical role as they abbreviate the effective refractory period and hyperpolarize the resting membrane potential [58,59], thereby, increasing Na^{+} channel availability (and thus excitability) and enhancing rotor frequency [60]. Whereas some of these molecular and cellular alterations are well documented in humans and/or animals with AF, it often remains unknown whether these changes are a cause of AF or a consequence of the chronic atrial arrhythmia. We will review AF-associated changes in proteins involved in intracellular Ca^{2+} release and reuptake.

Structural remodeling of the atria also plays an important role in the susceptibility to atrial arrhythmias. The presence of areas of slow conduction or block may cause spatial dissociation of wavelets and promote re-entry [42]. Atrial dilatation also increases the likelihood of AF due to increased heterogeneity of conduction [61]. The increase in atrial size is often associated with depressed atrial contractility, and together, these changes increase the risk for thromboembolic complications [62].

Multiple studies have shown that abnormal SR Ca^{2+} handling plays a central role in the initiation and/or maintenance of AF in humans [21,55]. Defective Ca^{2+} handling, which may facilitate re-entry and contribute to triggered activity, was

demonstrated to lead to an increase in spontaneous Ca^{2+} release events from the SR in atrial myocytes isolated from patients in chronic AF [4]. Unexpectedly, SR Ca^{2+} load was not increased in atrial myocytes from patients in AF [4], suggesting that the spontaneous SR Ca^{2+} releases most likely occurred owing to alterations in RyR2. By contrast, Liang *et al.* found that the frequency and amplitude of Ca^{2+} sparks were comparable in human atrial myocytes of patients in sinus rhythm and in AF [63]. However, the frequency of small and global Ca^{2+} waves increased in atrial myocytes of AF patients. The authors suggested that the spatiotemporal properties, but not the frequency of Ca^{2+} sparks, were affected in atrial myocytes from patients in AF. These findings and results from animal models point to abnormal SR Ca^{2+} function in AF. Abnormal SR Ca^{2+} release can act as a local trigger generator, initiating a local re-entry circuit or ectopic focal activity in sheep atria [64].

Downregulation of L-type Ca^{2+} channel

Most studies in atrial myocytes isolated from patients in chronic AF have demonstrated a reduction of $I_{Ca,L}$ (FIGURE 2) [56,57]. A reduced $I_{Ca,L}$ current results in the shortening of both the action-potential duration and effective refractory period, which can promote AF maintenance [65]. In addition, reduced $I_{Ca,L}$ contributes to depressed atrial contractility by decreasing Ca^{2+}-induced Ca^{2+} release from the SR [66].

At the molecular level, inconsistent results have been reported regarding mRNA expression of the α_{1C} ($Ca_V1.2$) subunit of voltage-dependent, L-type Ca^{2+} channel, $I_{Ca,L}$, in a variety of animal models and human tissue studies. Some investigations revealed decreased expression of $Ca_V1.2$ [67,68], $\alpha2/\delta1$ [69], β_{1B} [69] and α_{1D} [68], whereas other studies demonstrated unchanged $Ca_V1.2$ [70], $\beta_{1A,C}$ [69] or β_{2A} subunit expression of $I_{Ca,L}$ [57,66]. Several factors might explain these discrepancies, including species differences and time-dependent changes in remodeling in animal models. A recent study in mice lacking the $Ca_V1.3$ subunit of $I_{Ca,L}$ demonstrated an increased vulnerability to AF, suggesting that a reduction of $I_{Ca,L}$ may be causally linked to AF pathogenesis [71].

In addition, it has been demonstrated that abnormal post-translational regulation of $I_{Ca,L}$ might contribute to AF. The $I_{Ca,L}$ subunits are phosphorylated by CaMKII [72,73] and dephosphorylated by PP1 and -2A. The increase of protein phosphatase activity in humans with AF [23,57,70] may overcome the enhanced CaMKII activity in AF, reducing $I_{Ca,L}$ amplitude [57,73].

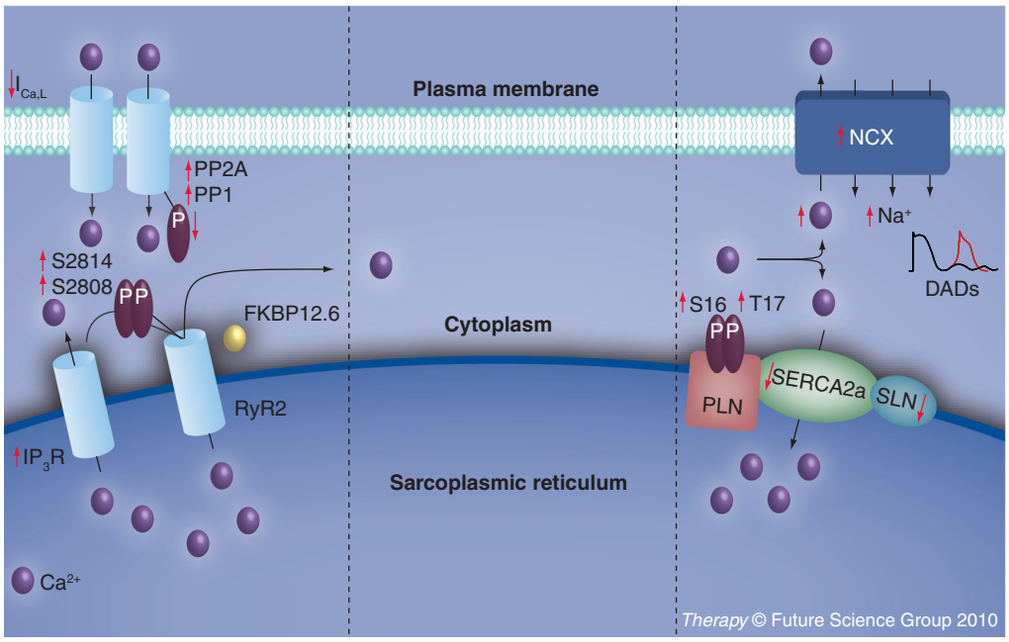

Figure 2. Model of electrical remodeling in atrial fibrillation. Impaired phosphorylation-dependent regulation of Ca^{2+} handling and contractility in human chronic atrial fibrillation. The arrows indicate the direction of change in protein function or phosphorylation.
DAD: Delayed afterdepolarization; $I_{Ca,L}$: L-type Ca^{2+} current; IP_3R: Inositol 1,4,5-trisphosphate receptors; NCX: Na^+/Ca^{2+}-exchanger; PLN: Phospholamban; RyR2: Ryanodine receptor type 2; SERCA2a: Sarco/endoplasmic reticulum Ca^{2+}-ATPase; SLN: Sarcolipin.

These results suggest that imbalances in kinase/phosphatase signaling may contribute to the reduction of $I_{Ca,L}$ in patients with chronic AF. Whereas these findings might explain the reduced contractility in AF, they do not explain the increased incidence of spontaneous Ca^{2+} release events from the SR.

Enhanced RyR2 activity in AF

As mentioned previously, there is ample evidence for defective SR Ca^{2+} handling in AF. Whereas reduced systolic SR Ca^{2+} release can be attributed to downregulation of the $I_{Ca,L}$ current, other mechanisms are likely to be responsible for the paradoxal increase in diastolic nonevoked SR Ca^{2+} release events [4]. Single-channel recordings revealed an increased open probability of RyR2 isolated from dogs in chronic AF [21]. It is likely that enhanced RyR2 activity plays a role in AF pathogenesis, as mice with a gain-of-function mutation in RyR2 exhibit an increased susceptibility to pacing-induced AF [19]. Using these knock-in mice, it was demonstrated that increased SR Ca^{2+} leak in atrial myocytes can promote triggered activity and atrial arrhythmias.

Expression levels of RyR2 were found to be unaltered [74,75] or reduced in dogs and patients with chronic AF [76–78]. However, and perhaps more importantly, the binding levels of accessory subunits and post-translational modifications were altered in chronic AF in humans [21,23]. For example, the level of the RyR2-stabilizing subunit FKBP12.6 (calstabin2) was reduced by approximately 50% in patients with chronic AF, which could explain why RyR2 channels fail to remain fully closed during diastole [21]. Interestingly, mice deficient in FKBP12.6 exhibit an increased vulnerability to pacing-induced AF and enhanced spontaneous SR Ca^{2+} leak [20]. Reduced calsequestrin 2 levels may also contribute to SR dysfunction in AF [78].

Changes in the phosphorylation level of RyR2 have been reported consistently in chronic AF. Vest *et al.* demonstrated increased PKA phosphorylation of S2808 on RyR2 in dogs with chronic pacing-induced AF and patients with chronic AF [21]. Although total protein phosphatase levels and activity of PP1 and -2A are increased in the atria of patients in chronic AF, it is still unclear what happens to PP1 and -2A levels within the RyR2 macromolecular complex [23]. PP1 is regulated by inhibitor 1 and 2 [79]. El-Armouche *et al.* demonstrated that the levels of inhibitor 1 and 2 were not altered in patients with AF, but that inhibitor 1 phosphorylation at T35 was increased [23], which should lead to a strong inhibition of PP1 within the SR compartment, possibly contributing to enhanced RyR2 phosphorylation [80].

Ryanodine receptor type 2 is further phosphorylated by CaMKII at S2814 in human atrial samples from patients in chronic AF [19,81]. It was demonstrated that enhanced CaMKII activity and CaMKII phosphorylation of RyR2 led to an increased propensity to diastolic SR Ca^{2+} leak and atrial arrhythmias. Goats with sustained AF also demonstrated enhanced autophosphorylation and, thus, activity of CaMKII along with increased CaMKII-dependent RyR2 phosphorylation [22], clearly suggesting that the high atrial rate is sufficient to cause these alterations. Notably, goats with atrial dilatation, but absence of sustained AF, also exhibit increased CaMKII activity and CaMKII-dependent RyR2 phosphorylation [22], pointing to the possibility that structural atrial diseases may predispose to AF by producing changes in cellular Ca^{2+} signaling. Indeed, genetic and pharmacological inhibition of CaMKII phosphorylation of RyR2 reduced the inducibility of AF in mice, suggesting that hyperphosphorylation of the RyR2 at S2814 might play a central role in AF pathogenesis [19]. The absence of carbachol-induced AF in RyR2-S2814A knock-in mice, in which RyR2 phosphorylation by CaMKII was genetically inhibited, confirms the importance of this single phosphorylation event in the pathogenesis of atrial arrhythmias [19].

Diastolic Ca^{2+} leak persistence is promoted only if normal or enhanced SR Ca^{2+} load can be maintained [82]. In AF patients, PLN is hyperphosphorylated at both Ser-16 (PKA site) and Thr-17 (CaMKII site), respectively, and this may prevent SR Ca^{2+} depletion in AF, explaining the preserved SR Ca^{2+} content [4,23,81,83]. Dogs with experimental heart failure show increased SR Ca^{2+} load, along with enhanced CaMKII phosphorylation of PLN and increased frequency of DAD events in atrial cells [78]. Together, these data and the results discussed earlier suggest that part of the changes detected in patients with chronic AF might result from structural changes of the atria, heart failure and other risk factors and clinical conditions. Further extensive work in suitable patient populations and experimental models is needed to dissect the specific contributions of the preceding cardiac pathologies to the promotion of AF.

Enhanced IP_3R activity in AF

Yamda *et al.* demonstrated an upregulated expression of IP_3R in atrial tissue samples from patients with chronic AF [84]. Other studies

have confirmed this in human atrial tissue [85] and a canine model of rapid atrial pacing [77]. Since activation of IP_3R increases the frequency of spontaneous Ca^{2+} sparks [86], Ca^{2+} waves [87] and arrhythmogenic contractions [88], it is likely that the additional presence of IP_3R contributes to spontaneous Ca^{2+} release events from the SR [89,90]. Activation of IP_3R facilitates the generation of EADs and DADs in atrial myocytes, and these effects can be counteracted by an IP_3R blocker, such as aminoethoxydiphenyl borate 2 [91,92]. In addition, a causal link between IP_3R and arrhythmias were demonstrated in IP_3R2-deficient mice, as endothelin failed to induce arrhythmogenic spontaneous Ca^{2+} release events in the knockout mice [93]. Thus, upregulation of IP_3R may play a role in enhancing SR Ca^{2+}-release defects in AF. Further studies are clearly required to elucidate the complex interplay of IP_3R and RyR2 in the pathogenesis of AF.

Reduced SR Ca^{2+} reuptake

The levels of SERCA2a are downregulated by approximately 25% in patients with chronic AF [23]. Nevertheless, several reports have demonstrated that mRNA and protein expression levels of PLN are unaltered in patients with chronic AF [23,94–96]. However, there is evidence that both PKA- and CaMKII-mediated phosphorylation of PLN are increased in patients with chronic AF [23]. Interestingly, elevated PLN phosphorylation occurs despite globally increased activity levels of PP1 and -2A [23], which highlights the importance of local differences in protein phosphatase activity and/or targeting within different microdomains in atrial myocytes.

In addition, it has been demonstrated that the expression of SLN is decreased in chronic AF in humans [96]. Together with the changes in PLN regulation, reduced SLN binding to SERCA2a could, theoretically, be expected to enhance SR Ca^{2+} reuptake, which may offset the Ca^{2+} loss due to increased SR Ca^{2+} leak, potentially explaining the preserved SR Ca^{2+} content in patients with chronic AF [4]. A recent genetic-association study has suggested that some single-nucleotide polymorphisms in the *SLN* gene might be associated with AF [97].

Upregulation of NCX function in AF

Expression levels of NCX are upregulated in patients and goats with chronic AF [23,94]. Despite potentially increased reverse-mode activity of NCX, contractile function is typically decreased in chronic AF, which could possibly be attributed to reduced $I_{Ca,L}$ in combination with increased myofibrillar Ca^{2+} sensitivity and impaired atrial relaxation [23,94]. Nevertheless, if sufficient SR Ca^{2+} is released into the cytosol during diastole, the greater abundance of NCX protein may result in a larger NCX inward current (forward-mode action) for a given Ca^{2+} release, possibly promoting the occurrence of DADs and triggered activity that may contribute to AF maintenance.

Taken together, there is emerging evidence that altered cellular Ca^{2+} signaling in the atria may contribute to both re-entry and triggered activity, thereby promoting AF maintenance. However, there are important gaps in our knowledge regarding impaired atrial Ca^{2+} handling. For instance, persistence of diastolic Ca^{2+} leak requires a maintained SR Ca^{2+} load [82]. Although preliminary results in AF patients point to preserved global SR Ca^{2+} load and enhanced functional NCX [83], it remains to be determined whether increased SR Ca^{2+} leak and sufficient SR Ca^{2+} load occur at the subsarcolemmal SR compartment. The current evidence of increased SR Ca^{2+} leak in AF patients is indirect [19,21,81]. Precise quantification of SR Ca^{2+} leak in atrial myocytes from AF patients in combination with single-channel recordings of RyR2 properties are clearly required. The quantitative relation between SR Ca^{2+} leak, probability of occurrence of diastolic Ca^{2+} waves and amplitude of NCX is currently unknown and it remains to be determined whether the size of the NCX current associated with SR Ca^{2+} leak is sufficient to depolarize the membrane to the threshold needed to trigger an action potential. In addition, future work should also address the time course of reversibility of SR Ca^{2+} abnormalities. Finally, although Ca^{2+}-dependent focal sources are suggested to contribute to the maintenance of clinical AF [98], direct experimental evidence of the causal relationship between Ca^{2+}-related cellular proarrhythmic events and focal sources in fibrillating human atria is still lacking.

Novel therapeutic approaches targeting abnormal SR function in AF

Traditionally, AF has been treated pharmacologically using drugs that block voltage-gated ion channels. Most of these agents, however, are also characterized by a profound proarrhythmogenic potential, which strongly limits their clinical applicability. New therapeutic strategies have emerged during the past 5 years as a result of a better understanding of the molecular pathways involved in atrial arrhythmogenesis (reviewed in [99]). It was suggested that the commonly

used AF drug, amiodarone, might prevent atrial arrhythmias, at least in part, by preventing the AF-related downregulation of $I_{Ca,L}$ and thus electrical remodeling [100].

New therapeutic modalities may include compounds that inhibit spontaneous SR Ca^{2+} leak by normalizing the function of the RyR2 macromolecular complex (reviewed in [5]). The JTV519 (K201) has been demonstrated to prevent AF in a canine model of sterile pericarditis [101]. JTV519 was also demonstrated to reduce RyR2-mediated SR Ca^{2+} leak in mice by reversing disease-associated loss of FKBP12.6 binding to RyR2 [21,102,103]. Lehnart *et al.* demonstrated that, in mouse ventricular myocytes, JTV519 could block spontaneous SR Ca^{2+} releases and DADs that arise as a consequence of the SR Ca^{2+} leak [50]. In another study, JTV519 reduced firing rates in rabbit pulmonary vein cardiomyocytes, decreased amplitude of DADs, prolonged action potential duration and reduced incidence of provoked AF [104].

Other drugs may have similar effects on RyR2. For example, tetracaine was able to completely suppress Ca^{2+} sparks in atrial myocytes from patients in chronic AF [63]. The type 1C antiarrhythmic drug flecainide, better known for its Na^+ channel-blocking effects, was demonstrated to effectively inhibit arrhythmias induced by SR Ca^{2+} leak in mouse myocytes [105]. Hilliard *et al.* demonstrated that flecainide inhibits SR Ca^{2+} leak by blocking RyR2 in the open state [106]. Flecainide was also demonstrated to inhibit intracellular Ca^{2+} waves, probably owing to a combined effect on RyR2 gating and voltage-gated Na^+ channels [106,107].

Dantrolene, a drug generally used to treat malignant hyperthermia, effectively prevents abnormal Ca^{2+} release via type 1 RyR2 in skeletal muscle. Kobayashi *et al.* recently demonstrated that dantrolene may also inhibit spontaneous SR Ca^{2+} leak in the heart [108]. Therefore, dantrolene or its derivatives may also be promising therapeutic agents for the treatment of cardiac arrhythmias, including AF. In addition to direct inhibition of RyR2, SR Ca^{2+} leak may also be reduced by suppressing the activity of CaMKII in the atrium. Chelu *et al.* demonstrated that pharmacological inhibition of CaMKII could inhibit the induction of AF in mice by reducing SR Ca^{2+} leak [19].

Inhibition of Ca^{2+} influx via reverse-mode NCX (Ca^{2+} influx) might decrease Ca^{2+} overload and halt the progression of the remodeling process. The NCX inhibitor KB-R7943 prevents short-term electrical remodeling-induced action potential duration shortening in dogs [109] and prevents DADs and triggered activity in rabbit pulmonary veins [110]. A blockade of forward-mode NCX (Ca^{2+} efflux) should suppress DADs and trigger activity, but is expected to worsen Ca^{2+} overload. Although NCX inhibitors, such as KB-R7943, are suggested to block NCX more efficiently in the reverse (Ca^{2+} influx) mode than forward (Ca^{2+} efflux) mode [111], KB-R7943 is not selective and blocks L-type Ca^{2+} channels [112] and transient receptor potential channels [113]. Compounds specifically reducing forward-mode NCX without interference with other key ion channels and transporters, may prove efficient for AF treatment.

Executive summary

Fundamental mechanisms of atrial fibrillation include re-entry & triggered activity/automaticity

- Atrial fibrillation (AF), the most common sustained cardiac arrhythmia, is induced and maintained by a combination of re-entry and triggered activity/automaticity mechanisms.

Electrical remodeling promotes AF

- AF induces electrical remodeling of atrial myocytes resulting in altered expression levels and post-translational modifications of various ion channels and transporters.
- Reduced L-type Ca^{2+} current and enhanced inward rectifier K^+ currents underlie the shortening of the atrial effective refractory period, which promotes the formation and maintenance of re-entry circuits.

Impaired Ca^{2+}-induced Ca^{2+} release leads to contractile dysfunction in AF

- Reduced Ca^{2+} influx via L-type Ca^{2+} channels as a trigger for Ca^{2+}-induced Ca^{2+} release decreases systolic Ca^{2+} release from the sarcoplasmic reticulum (SR), which may be associated with atrial contractile failure.

Spontaneous diastolic Ca^{2+} releases from the SR are proarrhythmic

- Changes in subunit composition and post-translational regulation of intracellular Ca^{2+} release channels/ryanodine receptors increases the likelihood of spontaneous SR Ca^{2+} release events despite preserved SR Ca^{2+} content. This can generate depolarizing Na^+/Ca^{2+}-exchanger current, which may induce delayed afterdepolarizations and triggered activity.
- Increased activity of Ca^{2+}/calmodulin-dependent protein kinase II in AF can potentiate SR Ca^{2+} leak and may promote AF inducibility.

Conclusion

- Defective SR Ca^{2+} handling may contribute to both the induction and perpetuation of AF.
- Pharmacological inhibition of aberrant SR Ca^{2+} release might be a promising new strategy for the treatment of AF.

Conclusion & future perspective

There is emerging evidence from animal models and human studies that abnormal Ca^{2+} handling by the SR plays an important role in AF. Reduced $I_{Ca,L}$ current may be key to contractile dysfunction in AF. However, an increased incidence of spontaneous diastolic SR Ca^{2+} release events might contribute to both the induction and maintenance of AF. Increased PKA and CaMKII phosphorylation of RyR2, and reduced binding of the FKBP12.6 subunit to RyR2, might evoke triggered activity [21,114]. Relatively increased SERCA2a function, due to alterations in PLN phosphorylation and SLN expression, may enhance SR Ca^{2+} reuptake, compensating for the RyR2-mediated diastolic SR Ca^{2+} leak. Recent work in genetically-modified mice has provided important insights into the causal relationships between molecular alterations of single molecules in the context of AF susceptibility. However, it remains to be established whether cellular Ca^{2+}-related proarrhythmic events are the underlying mechanism of atrial arrhythmogenic foci in patients *in vivo*. Nevertheless, new pharmacological agents are being developed to inhibit potentially arrhythmogenic diastolic SR Ca^{2+} leak. These drugs might offer unique therapeutic advantages as they could improve atrial contractility and may reduce atrial arrhythmogenesis by normalizing SR Ca^{2+} handling.

Financial & competing interests disclosure

XHT Wehrens is a WM Keck Foundation Distinguished Young Scholar in Medical Research, and is also supported by NIH/NHLBI grants R01 HL089598 and R01 HL091947. D Dobrev is supported by the German Federal Ministry of Education and Research through the Atrial Fibrillation Competence Network (grant 01Gi0204; projects C3-C5) and the Deutsche Forschungsgemeinschaft (Do 769/1-3). This work was also supported by grants from the Fondation Leducq (07CVD03, European North American Atrial Fibrillation Research Alliance, to D Dobrev; 08CVD01, Alliance for Calmodulin Kinase Signaling in Heart Disease, to XHT Wehrens). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Bibliography

Papers of special note have been highlighted as:
- ▪ of interest
- ▪▪ of considerable interest

1 Lloyd-Jones DM, Wang TJ, Leip EP *et al.*: Lifetime risk for development of atrial fibrillation: the framingham heart study. *Circulation* 110(9), 1042–1046 (2004).

2 Lenaerts I, Bito V, Heinzel FR *et al.*: Ultrastructural and functional remodeling of the coupling between Ca^{2+} influx and sarcoplasmic reticulum Ca^{2+} release in right atrial myocytes from experimental persistent atrial fibrillation. *Circ. Res.* 105(9), 876–885 (2009).

3 Sun H, Gaspo R, Leblanc N, Nattel S: Cellular mechanisms of atrial contractile dysfunction caused by sustained atrial tachycardia. *Circulation* 98(7), 719–727 (1998).

4 Hove-Madsen L, Llach A, Bayes-Genis A *et al.*: Atrial fibrillation is associated with increased spontaneous calcium release from the sarcoplasmic reticulum in human atrial myocytes. *Circulation* 110(11), 1358–1363 (2004).

▪ **Original report of spontaneous sarcoplasmic reticulum calcium release in atrial myocytes from patients with chronic atrial fibrillation.**

5 Santonastasi M, Wehrens XH: Ryanodine receptors as pharmacological targets for heart disease. *Acta Pharmacol. Sin.* 28(7), 937–944 (2007).

6 Bers DM: Cardiac excitation–contraction coupling. *Nature* 415(6868), 198–205 (2002).

7 Wehrens XH, Lehnart SE, Marks AR: Intracellular calcium release and cardiac disease. *Annu. Rev. Physiol.* 67, 69–98 (2005).

8 Wehrens XH, Lehnart SE, Reiken SR, Marks AR: Ca^{2+}/calmodulin-dependent protein kinase II phosphorylation regulates the cardiac ryanodine receptor. *Circ. Res.* 94(6), E61–E70 (2004).

9 Mattiazzi A, Mundina-Weilenmann C, Guoxiang C, Vittone L, Kranias E: Role of phospholamban phosphorylation on Thr17 in cardiac physiological and pathological conditions. *Cardiovasc. Res.* 68(3), 366–375 (2005).

10 Walden AP, Dibb KM, Trafford AW: Differences in intracellular calcium homeostasis between atrial and ventricular myocytes. *J. Mol. Cell Cardiol.* 46(4), 463–473 (2009).

11 Mackenzie L, Bootman MD, Berridge MJ, Lipp P: Predetermined recruitment of calcium release sites underlies excitation–contraction coupling in rat atrial myocytes. *J. Physiol.* 530(Pt 3), 417–429 (2001).

12 Dobrev D, Teos LY, Lederer WJ: Unique atrial myocyte Ca^{2+} signaling. *J. Mol. Cell Cardiol.* 46(4), 448–451 (2009).

13 Cheng H, Lederer WJ, Cannell MB: Calcium sparks: elementary events underlying excitation–contraction coupling in heart muscle. *Science* 262(5134), 740–744 (1993).

▪▪ **First description of elementary calcium release events (sparks) in cardiac myocytes.**

14 Cannell MB, Soeller C: Sparks of interest in cardiac excitation–contraction coupling. *Trends Pharmacol. Sci.* 19(1), 16–20 (1998).

15 Lipp P, Laine M, Tovey SC *et al.*: Functional InsP3 receptors that may modulate excitation–contraction coupling in the heart. *Curr. Biol.* 10(15), 939–942 (2000).

16 Zima AV, Blatter LA: Inositol-1,4,5-trisphosphate-dependent Ca^{2+} signalling in cat atrial excitation–contraction coupling and arrhythmias. *J. Physiol.* 555(Pt 3), 607–615 (2004).

17 Domeier TL, Zima AV, Maxwell JT, Huke S, Mignery GA, Blatter LA: IP3 receptor-dependent Ca^{2+} release modulates excitation–contraction coupling in rabbit ventricular myocytes. *Am. J. Physiol. Heart Circ. Physiol.* 294(2), H596–H604 (2008).

18 Marx SO, Reiken S, Hisamatsu Y *et al.*: Phosphorylation-dependent regulation of ryanodine receptors. A novel role for leucine/isoleucine zippers. *J. Cell. Biol.* 153(4), 699–708 (2001).

19 Chelu MG, Sarma S, Sood S *et al.*: Calmodulin kinase II-mediated sarcoplasmic reticulum Ca^{2+} leak promotes atrial fibrillation in mice. *J. Clin. Invest.* 119(7), 1940–1951 (2009).

▪ **Demonstration of synergistic role of calmodulin kinase II and intracellular calcium leak in atrial fibrillation.**

20 Sood S, Chelu MG, van Oort RJ *et al.*: Intracellular calcium leak due to FKBP12.6 deficiency in mice facilitates the inducibility of atrial fibrillation. *Heart Rhythm* 5(7), 1047–1054 (2008).

21 Vest JA, Wehrens XH, Reiken SR *et al.*: Defective cardiac ryanodine receptor regulation during atrial fibrillation. *Circulation* 111(16), 2025–2032 (2005).

22 Greiser M, Neuberger HR, Harks E *et al.*: Distinct contractile and molecular differences between two goat models of atrial dysfunction: AV block-induced atrial dilatation and atrial fibrillation. *J. Mol. Cell Cardiol.* 46(3), 385–394 (2009).

23 El-Armouche A, Boknik P, Eschenhagen T *et al.*: Molecular determinants of altered Ca^{2+} handling in human chronic atrial fibrillation. *Circulation* 114(7), 670–680 (2006).

24 Fruen BR, Black DJ, Bloomquist RA *et al.*: Regulation of the RYR1 and RYR2 Ca^{2+} release channel isoforms by Ca^{2+}-insensitive mutants of calmodulin. *Biochemistry* 42(9), 2740–2747 (2003).

25 Marx SO, Reiken S, Hisamatsu Y *et al.*: PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. *Cell* 101, 365–376 (2000).

▪▪ **First demonstration of ryanodine receptor remodeling in heart failure.**

26 Ondrias K, Mojzisova A: Coupled gating between individual cardiac ryanodine calcium release channels. *Gen. Physiol. Biophys.* 21, 73–84 (2001).

27 Wehrens XH, Lehnart SE, Reiken S, Vest JA, Wronska A, Marks AR: Ryanodine receptor/calcium release channel PKA phosphorylation: a critical mediator of heart failure progression. *Proc. Natl Acad. Sci. USA* 103(3), 511–518 (2006).

28 Xiao B, Zhong G, Obayashi M *et al.*: Ser-2030, but not Ser-2808, is the major phosphorylation site in cardiac ryanodine receptors responding to protein kinase a activation upon β-adrenergic stimulation in normal and failing hearts. *Biochem. J.* 396(1), 7–16 (2006).

29 Cote K, Proteau S, Teijeira J, Rousseau E: Characterization of the sarcoplasmic reticulum K^+ and Ca^{2+}-release channel-ryanodine receptor-in human atrial cells. *J. Mol. Cell Cardiol.* 32(11), 2051–2063 (2000).

30 Carl SL, Felix K, Caswell AH *et al.*: Immunolocalization of sarcolemmal dihydropyridine receptor and sarcoplasmic reticular triadin and ryanodine receptor in rabbit ventricle and atrium. *J. Cell Biol.* 129(3), 672–682 (1995).

31 Chopra N, Knollmann BC: Cardiac calsequestrin: the new kid on the block in arrhythmias. *J. Cardiovasc. Electrophysiol.* 20(10), 1179–1185 (2009).

32 Koss KL, Grupp IL, Kranias EG: The relative phospholamban and SERCA2 ratio: a critical determinant of myocardial contractility. *Basic Res. Cardiol.* 92(Suppl. 1), 17–24 (1997).

33 Asahi M, Sugita Y, Kurzydlowski K *et al.*: Sarcolipin regulates sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) by binding to transmembrane helices alone or in association with phospholamban. *Proc. Natl Acad. Sci. USA* 100(9), 5040–5045 (2003).

34 Minamisawa S, Wang Y, Chen J, Ishikawa Y, Chien KR, Matsuoka R: Atrial chamber-specific expression of sarcolipin is regulated during development and hypertrophic remodeling. *J. Biol. Chem.* 278(11), 9570–9575 (2003).

35 Maclennan DH, Kimura Y, Toyofuku T: Sites of regulatory interaction between calcium ATPases and phospholamban. *Ann. NY Acad. Sci.* 853, 31–42 (1998).

36 Asahi M, Kurzydlowski K, Tada M, Maclennan DH: Sarcolipin inhibits polymerization of phospholamban to induce superinhibition of sarco(endo)plasmic reticulum Ca^{2+}-ATPases (SERCAS). *J. Biol. Chem.* 277(30), 26725–26728 (2002).

37 Bhupathy P, Babu GJ, Ito M, Periasamy M: Threonine-5 at the N-terminus can modulate sarcolipin function in cardiac myocytes. *J. Mol. Cell Cardiol.* 47(5), 723–729 (2009).

38 Wang J, Schwinger RH, Frank K *et al.*: Regional expression of sodium pump subunits isoforms and Na^+–Ca^{2+} exchanger in the human heart. *J. Clin. Invest.* 98(7), 1650–1658 (1996).

39 Forbes MS, Van Niel EE, Purdy-Ramos SI: The atrial myocardial cells of mouse heart: a structural and stereological study. *J. Struct. Biol.* 103(3), 266–279 (1990).

40 Nattel S: Basic electrophysiology of the pulmonary veins and their role in atrial fibrillation: precipitators, perpetuators, and perplexers. *J. Cardiovasc. Electrophysiol.* 14(12), 1372–1375 (2003).

41 Nattel S, Burstein B, Dobrev D: Atrial remodeling and atrial fibrillation: mechanisms and implications. *Circ. Arrhythm. Electrophysiol.* 1(1), 62–73 (2008).

42 Allessie MA, Bonke FI, Schopman FJ: Circus movement in rabbit atrial muscle as a mechanism of tachycardia. III. The 'leading circle' concept: a new model of circus movement in cardiac tissue without the involvement of an anatomical obstacle. *Circ. Res.* 41(1), 9–18 (1977).

43 Davidenko JM, Pertsov AV, Salomonsz R, Baxter W, Jalife J: Stationary and drifting spiral waves of excitation in isolated cardiac muscle. *Nature* 355(6358), 349–351 (1992).

▪ **Identification of spiral waves as an arrhythmogenic mechanism.**

44 Pertsov AM, Davidenko JM, Salomonsz R, Baxter WT, Jalife J: Spiral waves of excitation underlie reentrant activity in isolated cardiac muscle. *Circ. Res.* 72(3), 631–650 (1993).

45 Haissaguerre M, Jais P, Shah DC *et al.*: Spontaneous initiation of atrial fibrillation by ectopic beats originating in the pulmonary veins. *N. Engl. J. Med.* 339(10), 659–666 (1998).

▪▪ **Demonstration of ectopic foci in pulmonary veins as initiator of atrial fibrillation.**

46 Burashnikov A, Antzelevitch C: Late-phase 3 EAD. A unique mechanism contributing to initiation of atrial fibrillation. *Pacing Clin. Electrophysiol.* 29(3), 290–295 (2006).

47 Patterson E, Lazzara R, Szabo B *et al.*: Sodium-calcium exchange initiated by the Ca^{2+} transient: an arrhythmia trigger within pulmonary veins. *J. Am. Coll. Cardiol.* 47(6), 1196–1206 (2006).

48 Burashnikov A, Antzelevitch C: Reinduction of atrial fibrillation immediately after termination of the arrhythmia is mediated by late phase 3 early after depolarization-induced triggered activity. *Circulation* 107(18), 2355–2360 (2003).

49 Patterson E, Po SS, Scherlag BJ, Lazzara R: Triggered firing in pulmonary veins initiated by *in vitro* autonomic nerve stimulation. *Heart Rhythm* 2(6), 624–631 (2005).

50 Lehnart SE, Terrenoire C, Reiken S *et al.*: Stabilization of cardiac ryanodine receptor prevents intracellular calcium leak and arrhythmias. *Proc. Natl Acad. Sci. USA* 103(20), 7906–7910 (2006).

51 Benardeau A, Hatem SN, Rucker-Martin C *et al.*: Primary culture of human atrial myocytes is associated with the appearance of structural and functional characteristics of immature myocardium. *J. Mol. Cell Cardiol.* 29(5), 1307–1320 (1997).

52 Wijffels MC, Kirchhof CJ, Dorland R, Allessie MA: Atrial fibrillation begets atrial fibrillation. A study in awake chronically instrumented goats. *Circulation* 92(7), 1954–1968 (1995).

▪▪ **First demonstration that atrial fibrillation itself changes electrophysiological properties of atria making them more susceptible to sustain atrial fibrillation.**

53 Yue L, Feng J, Gaspo R, Li Gr, Wang Z, Nattel S: Ionic remodeling underlying action potential changes in a canine model of atrial fibrillation. *Circ. Res.* 81(4), 512–525 (1997).

54 Rostock T, Steven D, Lutomsky B *et al.*: Atrial fibrillation begets atrial fibrillation in the pulmonary veins on the impact of atrial fibrillation on the electrophysiological properties of the pulmonary veins in humans. *J. Am. Coll. Cardiol.* 51(22), 2153–2160 (2008).

55 Dobrev D: Electrical remodeling in atrial fibrillation. *Herz* 31(2), 108–112 (2006).

56 van Wagoner DR, Pond AL, Lamorgese M, Rossie SS, McCarthy PM, Nerbonne JM: Atrial L-type Ca^{2+} currents and human atrial fibrillation. *Circ. Res.* 85(5), 428–436 (1999).

57 Christ T, Boknik P, Wohrl S *et al.*: L-type Ca^{2+} current downregulation in chronic human atrial fibrillation is associated with increased activity of protein phosphatases. *Circulation* 110 (17), 2651–2657 (2004).

58 Dobrev D, Friedrich A, Voigt N *et al.*: The G protein-gated potassium current $I_{K,ACh}$ is constitutively active in patients with chronic atrial fibrillation. *Circulation* 112(24), 3697–3706 (2005).

59 Dobrev D, Graf E, Wettwer E *et al.*: Molecular basis of downregulation of G-protein-coupled inward rectifying K^+ current $I_{K,ACh}$ in chronic human atrial fibrillation: decrease in GIRK4 mRNA correlates with reduced $I_{K,ACh}$ and muscarinic receptor-mediated shortening of action potentials. *Circulation* 104(21), 2551–2557 (2001).

60 Pandit SV, Berenfeld O, Anumonwo JM *et al.*: Ionic determinants of functional re-entry in a 2-D model of human atrial cells during simulated chronic atrial fibrillation. *Biophys. J.* 88(6), 3806–3821 (2005).

61 Verheule S, Sato T, Everett TT *et al.*: Increased vulnerability to atrial fibrillation in transgenic mice with selective atrial fibrosis caused by overexpression of TGF-β1. *Circ. Res.* 94(11), 1458–1465 (2004).

62 Di Tullio MR, Sacco RL, Sciacca RR, Homma S: Left atrial size and the risk of ischemic stroke in an ethnically mixed population. *Stroke* 30(10), 2019–2024 (1999).

63 Liang X, Xie H, Zhu PH *et al.*: Ryanodine receptor-mediated Ca^{2+} events in atrial myocytes of patients with atrial fibrillation. *Cardiology* 111(2), 102–110 (2008).

64 Mandapati R, Skanes A, Chen J, Berenfeld O, Jalife J: Stable microreentrant sources as a mechanism of atrial fibrillation in the isolated sheep heart. *Circulation* 101(2), 194–199 (2000).

65 van der Velden HMW, van der Zee L, Wijffels MC *et al.*: Atrial fibrillation in the goat induces changes in monophasic action potential and mRNA expression of ion channels involved in repolarization. *J. Cardiovasc. Electrophysiol.* 11(11), 1262–1269 (2000).

66 Schotten U, Haase H, Frechen D *et al.*: The L-type Ca^{2+}-channel subunits α_{1C} and β2 are not downregulated in atrial myocardium of patients with chronic atrial fibrillation. *J. Mol. Cell Cardiol.* 35(5), 437–443 (2003).

67 Bosch RF, Scherer CR, Rub N *et al.*: Molecular mechanisms of early electrical remodeling: transcriptional downregulation of ion channel subunits reduces $I_{Ca,L}$ and I_{to} in rapid atrial pacing in rabbits. *J. Am. Coll. Cardiol.* 41(5), 858–869 (2003).

68 Gaborit N, Steenman M, Lamirault G *et al.*: Human atrial ion channel and transporter subunit gene-expression remodeling associated with valvular heart disease and atrial fibrillation. *Circulation* 112(4), 471–481 (2005).

69 Grammer JB, Zeng X, Bosch RF, Kuhlkamp V: Atrial L-type Ca^{2+}-channel, β-adrenorecptor, and 5-hydroxytryptamine type 4 receptor mRNAs in human atrial fibrillation. *Basic Res. Cardiol.* 96(1), 82–90 (2001).

70 Greiser M, Halaszovich CR, Frechen D *et al.*: Pharmacological evidence for altered Src kinase regulation of $I_{Ca,L}$ in patients with chronic atrial fibrillation. *Naunyn Schmiedebergs Arch. Pharmacol.* 375(6), 383–392 (2007).

71 Mancarella S, Yue Y, Karnabi E, Qu Y, El-Sherif N, Boutjdir M: Impaired Ca^{2+} homeostasis is associated with atrial fibrillation in the α_{1D} L-type Ca^{2+} channel KO mouse. *Am. J. Physiol. Heart Circ. Physiol.* 295(5), H2017–H2024 (2008).

72 Ono K, Fozzard HA: Phosphorylation restores activity of L-type calcium channels after rundown in inside-out patches from rabbit cardiac cells. *J. Physiol.* 454, 673–688 (1992).

73 van der Heyden MA, Wijnhoven TJ, Opthof T: Molecular aspects of adrenergic modulation of cardiac L-type Ca^{2+} channels. *Cardiovasc. Res.* 65(1), 28–39 (2005).

74 Brundel BJ, van Gelder IC, Henning RH *et al.*: Gene expression of proteins influencing the calcium homeostasis in patients with persistent and paroxysmal atrial fibrillation. *Cardiovasc. Res.* 42(2), 443–454 (1999).

75 Lai LP, Su MJ, Lin JL *et al.*: Down-regulation of L-type calcium channel and sarcoplasmic reticular Ca^{2+}-ATPase mRNA in human atrial fibrillation without significant change in the mRNA of ryanodine receptor, calsequestrin and phospholamban: an insight into the mechanism of atrial electrical remodeling. *J. Am. Coll. Cardiol.* 33(5), 1231–1237 (1999).

76 Ohkusa T, Ueyama T, Yamada J *et al.*: Alterations in cardiac sarcoplasmic reticulum Ca^{2+} regulatory proteins in the atrial tissue of patients with chronic atrial fibrillation. *J. Am. Coll. Cardiol.* 34(1), 255–263 (1999).

77 Zhao ZH, Zhang HC, Xu Y *et al.*: Inositol-1,4,5-trisphosphate and ryanodine-dependent Ca^{2+} signaling in a chronic dog model of atrial fibrillation. *Cardiology* 107(4), 269–276 (2007).

78 Yeh YH, Wakili R, Qi XY *et al.*: Calcium-handling abnormalities underlying atrial arrhythmogenesis and contractile dysfunction in dogs with congestive heart failure. *Circ. Arrhythm Electrophysiol.* 1(2), 93–102 (2008).

79 Herzig S, Neumann J: Effects of serine/threonine protein phosphatases on ion channels in excitable membranes. *Physiol. Rev.* 80(1), 173–210 (2000).

80 El-Armouche A, Wittkopper K, Degenhardt F *et al.*: Phosphatase inhibitor-1-deficient mice are protected from catecholamine-induced arrhythmias and myocardial hypertrophy. *Cardiovasc. Res.* 80(3), 396–406 (2008).

81 Neef S, Dybkova N, Sossalla S *et al.*: CaMKII-dependent diastolic SR Ca^{2+} leak and elevated diastolic Ca^{2+} levels in right atrial myocardium of patients with atrial fibrillation. *Circ. Res.* (2010) (Epub ahead of print).

82 Venetucci LA, Trafford AW, O'Neill SC, Eisner DA: The sarcoplasmic reticulum and arrhythmogenic calcium release. *Cardiovasc. Res.* 77(2), 285–292 (2008).

83 Voigt N, Trafford AW, Ravens U, Dobrev D: Cellular and molecular determinants of altered atrial Ca^{2+} signaling in patients with chronic atrial fibrillation. *Circulation* 120(Suppl. 1), S667–S668 (2009).

84 Yamda J, Ohkusa T, Nao T *et al.*: Up-regulation of inositol 1,4,5 trisphosphate receptor expression in atrial tissue in patients with chronic atrial fibrillation. *J. Am. Coll. Cardiol.* 37(4), 1111–1119 (2001).

▪ **Demonstration of upregulation of IP_3R in atrial tissue of patients with atrial fibrillation.**

85 Yamada J, Ohkusa T, Nao T *et al.*: Up-regulation of inositol 1, 4, 5-trisphosphate receptor expression in atrial tissue in patients with chronic atrial fibrillation. *J. Cardiol.* 39(1), 57–58 (2002).

86 Gassanov N, Brandt MC, Michels G, Lindner M, Er F, Hoppe UC: Angiotensin II-induced changes of calcium sparks and ionic currents in human atrial myocytes: potential role for early remodeling in atrial fibrillation. *Cell Calcium* 39(2), 175–186 (2006).

87 Liang X, Xie H, Zhu PH *et al.*: Enhanced activity of inositol-1,4,5-trisphosphate receptors in atrial myocytes of atrial fibrillation patients. *Cardiology* 114(3), 180–191 (2009).

88 Burrell KM, Molenaar P, Dawson PJ, Kaumann AJ: Contractile and arrhythmic effects of endothelin receptor agonists in human heart *in vitro*: blockade with SB 209670. *J. Pharmacol. Exp. Ther.* 292(1), 449–459 (2000).

89 Kockskamper J, Zima AV, Roderick HL, Pieske B, Blatter LA, Bootman MD: Emerging roles of inositol 1,4,5-trisphosphate signaling in cardiac myocytes. *J. Mol. Cell Cardiol.* 45(2), 128–147 (2008).

90 Bootman MD, Harzheim D, Smyrnias I, Conway SJ, Roderick HL: Temporal changes in atrial EC-coupling during prolonged stimulation with endothelin-1. *Cell Calcium* 42(4–5), 489–501 (2007).

91 Mackenzie L, Bootman MD, Laine M *et al.*: The role of inositol 1,4,5-trisphosphate receptors in Ca^{2+} signalling and the generation of arrhythmias in rat atrial myocytes. *J. Physiol.* 541(Pt 2), 395–409 (2002).

92 Mackenzie L, Roderick HL, Berridge MJ, Conway SJ, Bootman MD: The spatial pattern of atrial cardiomyocyte calcium signalling modulates contraction. *J. Cell Sci.* 117(Pt 26), 6327–6337 (2004).

93 Li X, Zima AV, Sheikh F, Blatter LA, Chen J: Endothelin-1-induced arrhythmogenic Ca^{2+} signaling is abolished in atrial myocytes of ino sitol-1,4,5-trisphosphate(IP_3)-receptor type 2-deficient mice. *Circ. Res.* 96(12), 1274–1281 (2005).

94 Schotten U, Greiser M, Benke D *et al.*: Atrial fibrillation-induced atrial contractile dysfunction: a tachycardiomyopathy of a different sort. *Cardiovasc. Res.* 53(1), 192–201 (2002).

95 Li M, Reddy LG, Bennett R, Silva ND Jr, Jones LR, Thomas DD: A fluorescence energy transfer method for analyzing protein oligomeric structure: application to phospholamban. *Biophys. J.* 76(5), 2587–2599 (1999).

96 Uemura N, Ohkusa T, Hamano K *et al.*: Down-regulation of sarcolipin mRNA expression in chronic atrial fibrillation. *Eur J. Clin. Invest.* 34(11), 723–730 (2004).

97 Tsai CT, Lai LP, Hwang JJ, Lin JL, Chiang FT: Molecular genetics of atrial fibrillation. *J. Am. Coll. Cardiol.* 52(4), 241–250 (2008).

▪ **Overview of genetic forms of atrial fibrillation.**

98 Patterson E, Jackman WM, Beckman KJ *et al.*: Spontaneous pulmonary vein firing in man: relationship to tachycardia-pause early afterdepolarizations and triggered arrhythmia in canine pulmonary veins *in vitro*. *J. Cardiovasc. Electrophysiol.* 18(10), 1067–1075 (2007).

99 Dobrev D: Atrial Ca^{2+} signaling in atrial fibrillation as an antiarrhythmic drug target. *Naunyn Schmiedebergs Arch. Pharmacol.* doi: 10.1007/s00210-009-0457-1(2009) (Epub ahead of print).

100 Shinagawa K, Shiroshita-Takeshita A, Schram G, Nattel S: Effects of antiarrhythmic drugs on fibrillation in the remodeled atrium: insights into the mechanism of the superior efficacy of amiodarone. *Circulation* 107(10), 1440–1446 (2003).

101 Kumagai K, Nakashima H, Gondo N, Saku K: Antiarrhythmic effects of JTV-519, a novel cardioprotective drug, on atrial fibrillation/flutter in a canine sterile pericarditis model. *J. Cardiovasc. Electrophysiol.* 14(8), 880–884 (2003).

102 Wehrens XH, Marks AR: Novel therapeutic approaches for heart failure by normalizing calcium cycling. *Nat. Rev. Drug Discov.* 3(7), 565–573 (2004).

103 Wehrens XH, Lehnart SE, Reiken SR *et al.*: Protection from cardiac arrhythmia through ryanodine receptor-stabilizing protein calstabin2. *Science* 304(5668), 292–296 (2004).

▪▪ **Demonstration of anti-arrhythmic effects of ryanodine receptor-stabilizing compound.**

104 Chen YJ, Chen YC, Wongcharoen W, Lin CI, Chen SA: Effect of K201, a novel antiarrhythmic drug on calcium handling and arrhythmogenic activity of pulmonary vein cardiomyocytes. *Br. J. Pharmacol.* 153(5), 915–925 (2008).

105 Watanabe H, Chopra N, Laver D *et al.*: Flecainide prevents catecholaminergic polymorphic ventricular tachycardia in mice and humans. *Nat. Med.* 15(4), 380–383 (2009).

106 Hilliard FA, Steele DS, Laver D *et al.*: Flecainide inhibits arrhythmogenic Ca^{2+} waves by open state block of ryanodine receptor Ca^{2+} release channels and reduction of Ca^{2+} spark mass. *J. Mol. Cell Cardiol.* 48(2), 293–301 (2010).

107 Lehnart SE, Lederer WJ: An antidote for calcium leak: targeting molecular arrhythmia mechanisms. *J. Mol. Cell Cardiol.* 48(2), 279–282 (2009).

108 Kobayashi S, Yano M, Suetomi T *et al.*: Dantrolene, a therapeutic agent for malignant hyperthermia, markedly improves the function of failing cardiomyocytes by stabilizing interdomain interactions within the ryanodine receptor. *J. Am. Coll. Cardiol.* 53(21), 1993–2005 (2009).

109 Miyata A, Zipes DP, Hall S, Rubart M: KB-R7943 prevents acute, atrial fibrillation-induced shortening of atrial refractoriness in anesthetized dogs. *Circulation* 106(11), 1410–1419 (2002).

110 Wongcharoen W, Chen YC, Chen YJ *et al.*: Effects of a Na^+/Ca exchanger inhibitor on pulmonary vein electrical activity and ouabain-induced arrhythmogenicity. *Cardiovasc. Res.* 70(3), 497–508 (2006).

111 Iwamoto T: Na^+/Ca^{2+} exchange as a drug target – insights from molecular pharmacology and genetic engineering. *Ann. NY Acad. Sci.* 1099, 516–528 (2007).

112 Ouardouz M, Zamponi GW, Barr W, Kiedrowski L, Stys PK: Protection of ischemic rat spinal cord white matter: dual action of KB-R7943 on Na^+/Ca^{2+} exchange and L-type Ca^{2+} channels. *Neuropharmacology* 48(4), 566–575 (2005).

113 Kraft R: The Na^+/Ca^{2+} exchange inhibitor KB-R7943 potently blocks TRPC channels. *Biochem. Biophys. Res. Commun.* 361(1), 230–236 (2007).

114 Durham WJ, Wehrens XH, Sood S, Hamilton SL: Diseases associated with altered ryanodine receptor activity. *Subcell Biochem.* 45, 273–321 (2007).