Altered Excitation-Contraction Coupling in Human Chronic Atrial Fibrillation

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Abstract
This review focuses on the (mal)adaptive processes in atrial excitation-contraction coupling occurring in patients with chronic atrial fibrillation. Cellular remodeling includes shortening of the atrial action potential duration and effective refractory period, depressed intracellular Ca^{2+} transient, and reduced myocyte contractility. Here we summarize the current knowledge of the ionic bases underlying these changes. Understanding the molecular mechanisms of excitation-contraction-coupling remodeling in the fibrillating human atria is important to identify new potential targets for AF therapy.

Introduction

Atrial fibrillation (AF) is the most prevalent cardiac arrhythmia found in the clinical practice, affecting >2 million people in the United States alone (1). AF is often associated with other cardiovascular disorders, such as coronary artery disease, valve dysfunction, congestive heart failure (CHF), and is characterized by significant morbidity. A key determinant of this morbidity is embolic stroke (2), with loss of atrial contractility being one of the major causes of thrombus formation. AF is characterized by a rapid and irregular heartbeat caused when the atria quiver (fibrillate) erratically, sometimes faster than 200 times per minute (2).

Several studies have investigated the molecular and ionic mechanisms involved in the remodeling occurring in the atria of patients with AF, and suggest that structural, electrophysiological, and contractile remodeling are critical factors in the disease progression, i.e., they contribute to the development of a substrate that facilitates the tendency for persistence of AF (3, 4). Structural remodeling involves changes in atrial myocyte and tissue morphology (e.g., cell hypertrophy, fibrosis) (3, 5, 6). Electrical remodeling includes changes in Ca^{2+} and K^+ currents leading to shortening of the action potential (AP) duration (APD) and loss of APD rate-dependent adaptation (6). A growing body of experimental evidence points to perturbations in intracellular Ca^{2+} handling as important players in AF-induced atrial remodeling (7, 8), with intracellular Ca^{2+} transients (CaTs) being reduced. Myofilament protein changes in AF are also likely to contribute to atrial contractile dysfunction (9). However, the mechanisms leading to self-perpetuation of the arrhythmia and depressed cardiac contractility are yet poorly understood. Recently, Llach *et al.* have studied the basis of irregular beat-to-beat response of human atrial myocytes when subjected to elevations of the beating frequency (which often precedes cardiac arrhythmias) and suggested that stability or instability of the response was determined by the sarcoplasmic reticulum (SR) and L-type Ca^{2+} channel activities (10).

In this review, we present the current knowledge about the changes occurring in excitation-contraction (E-C) coupling that characterize the remodeled human atrial myocytes from patients with chronic AF (cAF), and the postulated underlying ionic mechanisms.

Phenotypic consequences of AF on AP, CaT, and contractility

Myocytes from cAF patients are characterized by shorter APs (Fig. 1*A*) and effective refractory period (ERP), and loss of rate adaptation of both atrial repolarization (Fig. 1*A*) and refractoriness (11-15). Typically, the human atrial AP duration at 90% repolarization (APD_{90}) shortens when paced at faster frequencies, but in myocytes isolated from cAF patients this shortening is severely attenuated (Fig. 1*A*).

CaT amplitude is strongly depressed in myocytes from cAF patients compared to those from subjects in sinus rhythm (Figure 1*B*)(16), although the SR Ca^{2+} content is unaltered (16-19). CaTs decay more slowly in cAF compared to sinus rhythm (16, 18). Elevated diastolic $[Ca^{2+}]_i$ has been reported and attributed to enhanced leak of Ca^{2+} from the SR (17). Intracellular $[Ca^{2+}]$ measurements with aequorin light signals in atrial tissue from patients in sinus rhythm display a positive dependency of CaT amplitude on the pacing rate (20). Our recently published mathematical model of the human atrial AP and CaT recapitulated this positive rate-dependence, and importantly showed that this is impaired when simulating cAF conditions (16).

Our simulations indicated that APD rate adaptation in sinus rhythm atrial cells involves accumulation of intracellular Na^+ ($[Na^+]_i$) at high frequencies, which causes outward shifts in Na^+/Ca^{2+} exchange and Na^+/K^+ pump currents. The model also predicted that E-C coupling remodeling in cAF would reduce Na^+ accumulation, thus causing a blunted APD rate-dependent response (16).

Baseline force of contraction of atrial trabeculae is also reduced in human cAF by approximately 70% compared to patients in sinus rhythm (Figure 1*C*)(21-23).

Ionic bases of altered E-C coupling in AF

The molecular bases of AF-induced alterations in E-C coupling are summarized in Table 1 and discussed in detail in the following paragraphs. E-C coupling remodeling can occur at the level of ion channels/transporters expression, or by modification of ion channel/transporter properties (for example, trafficking or phosphorylation). Furthermore, alterations of myofilament proteins may be involved in AF-induced hypocontractility.

Figure 2 depicts simulated APs and CaT for sinus rhythm and cAF myocytes (from (16)) and the major ionic currents that are active during the cardiac cycle, and provides a graphical representation of the main changes occurring in the electrophysiological and Ca^{2+} handling processes in human AF.

Atrial Cell Morphology

Cell capacitance of myocytes from cAF patients is greater than that of myocytes from SR patients, suggesting that AF cells are hypertrophied (24). In fact, cells from AF patients are both longer and wider than those from patients in sinus rhythm (17). Cell hypertrophy may contribute to cAF-induced global atrial dilation, along with changes of the extracellular matrix (with fibrosis and glycogen accumulation). Atrial dilation may itself have important consequences on cellular remodeling and alteration in protein composition and function of the atrial myocytes, as discussed later in this review.

It has recently been shown that atrial myocytes from human tissue sections exhibit extensive t-tubule networks (25). The presence of t-tubules in the human atria (not detected in isolated human atrial myocytes (26)), may play an important role in determining the spatio-temporal properties of the intracellular CaT (25). Notably, one can speculate that t-tubules could be subject to remodeling and contribute to perturbed E-C coupling in cAF, as suggested in sheep (27) and dog (28). However, further investigations will be required to confirm this.

Protein Kinases and Phosphatases

Intracellular CaT is dynamically regulated via phosphorylation by protein kinase A (PKA) and Ca/calmodulin-dependent protein kinase II (CaMKII) of key Ca^{2+} handling and regulatory proteins, such as L-type Ca^{2+} channels, ryanodine receptors (RyRs), and phospholamban (PLN) (29, 30). In addition, sarcomere proteins and various sarcolemmal ion channels are targets of both PKA and CaMKII (29, 31). The phosphorylation state of target proteins is also controlled by serine/threonine protein-phosphatases that are differentially regulated in distinct cardiomyocyte microdomains. Thus, altered protein kinase and phosphatase activity may importantly contribute to E-C coupling remodeling in AF. Indeed, CaMKII has been found to be more expressed and more phosphorylated in human cAF (17, 32). Similar PKA activity was found in cAF *vs.* sinus rhythm in goats (33), but El-Armouche *et al.* detected a higher total activity of type 1 and type 2A phosphatases in human cAF, causing inhomogeneous changes in protein phosphorylation in different cellular compartments (34). This may specifically amplify PKA and CaMKII effects on certain targets without having significant effects on others (e.g., higher phosphatase activity/lower phosphorylation in thick *vs.* thin myofilaments, cell membrane *vs.* SR) (34). Thus there is growing interest in the potential role of CaMKII and protein phosphatase inhibitors in preventing arrhythmogenic remodeling in cAF.

Sarcolemmal Ion Channels

I_{Na}

The Na^+ current (I_{Na}) plays a crucial role in cardiac E-C coupling by initiating the AP, and is also a major determinant of the cardiac AP propagation. Bosch *et al.* reported that I_{Na} density and voltage-dependence of activation were not altered in human AF (15), the steady-state inactivation was shifted to the right (15), and no changes were detected in mRNA levels of the Na^+ channel gene SCN5A (35). In contrast, Sossalla *et al.* provided recent evidence that

expression of Nav1.5 and peak I_{Na} density is decreased (slightly) in the atrial myocardium of patients with cAF (36).

Although it is unclear whether altered fast I_{Na} (Fig. 2*A, 2nd row*) contributes to the electrical remodeling in human AF, it has recently been shown the late Na^+ current component, I_{NaL} (*inset*), is significantly increased in cAF patients (36). Sossalla *et al.* (36) proposed that this increase could be due to the increase in neuronal Na^+ channel isoforms (Nav1.1 expression is increased), or mediated by CaMKII, which is increased in AF (17, 32) and known to regulate I_{NaL} (37), or caused by oxidative stress (38, 39). However, our simulations suggested that an increased I_{NaL} does not contribute significantly to repolarization in cAF, where the overall APD_{90} was still shorter than that in normal healthy cells (16). On the other hand, an increase in I_{NaL} may cause cellular Na^+ and Ca^{2+} overload and lead to contractile dysfunction and electrical instability (via reverse-mode Na^+/Ca^{2+} exchange) (29).

I_{CaL}

The L-type Ca^{2+} current (I_{CaL}) critically regulates E-C coupling by triggering sarcoplasmic reticulum (SR) Ca^{2+} release, and modulating AP shape and duration, i.e., maintaining the AP plateau (29). Reduction in I_{CaL} density (-50% *vs.* sinus rhythm, Fig. 2*A*, *3rd row*) is one of the most consistent electrophysiological features of electrical remodeling in human AF (as seen in (5, 11, 12, 16, 18, 40)). Christ *et al.* (40) demonstrated that decreased I_{CaL} density in cAF is not accompanied by altered expression of the corresponding α_{1c} and β_{2a} channel subunits (although other studies found different results (41)), and proposed that lower basal I_{CaL} is due to decreased channel phosphorylation in AF, which results from an altered ratio of protein kinase/phosphatase activity in favor of increased phosphatase activity. An analogous explanation was proposed for the blunted effect of CaMKII inhibition on I_{CaL} in human cAF (17). It has been shown that blocking I_{CaL} with nifedipine in normal human atrial cells results in an AP characteristic typically seen in AF (11) with respect to morphology, duration and impaired rate-dependent adaptation, i.e., reduction in I_{CaL} seems to be a critical component of the remodeled atrial electrical phenotype. However, Workman *et al.* found that nifedipine did not significantly alter ERP in sinus rhythm myocytes (although APD was shorter), thus supporting the idea that I_{CaL} downregulation may not be sufficient by itself to explain the remodeled atrial electrical phenotype (12).

I_{CaT}

There is no evidence of a T-type Ca^{2+} current (I_{CaT}) in human atrial myocytes (42, 43).

I_f

The hyperpolarization-activated pacemaker current, I_f, has been found to be increased in human AF compared to sinus rhythm, at least at the mRNA level (44), and could contribute to ectopic atrial pacemaker activity. However, functional evidence for I_f involvement is lacking at present.

I_{to} and I_{Kur}

The Ca^{2+}-independent transient outward K^+ current (I_{to}), and the ultra-rapid delayed rectifier K^+ current *(*I_{Kur}) dominate the early AP repolarization phase and confer the atrial AP a characteristic triangular shape. Human cAF is associated with strong reduction of I_{to} (Fig. 2*A*, *4th row*) density (5, 12, 15, 24, 45-47) and downregulation of its channel α subunit Kv4.3 (35, 48). I_{Kur} (Fig. 2*A*, *5th row*) was reduced in cAF (5, 24, 45, 47, 49), paralleled by diminished expression of Kv1.5 (35, 45, 48). However, others have reported no changes in I_{Kur} density (12, 15, 46). Inconsistent results about I_{Kur} function have been commented on previously by Christ *et al.* and attributed to different strategies for identification of I_{Kur} (e.g., pharmacological or with I_{to}-inactivating prepulse), and to a fraction of I_{Kur} that is not accounted for by Kv1.5 (49). The reduction in I_{to} and I_{Kur} explains the slight prolongation in earlier phases of the AP (Figure 2*A*, *1st row*) (16, 50).

It has been shown that CaMKII (increased in cAF) positively regulates I_{to} in human atrial

myocytes in acute conditions, as the application of the CaMKII inhibitor KN-93 caused loss of channel function (32). The authors speculated that, by reducing the extent of inactivation of I_{to}, upregulation of CaMKII during atrial fibrillation reduces Ca^{2+} influx and therefore minimizes Ca^{2+} overload. On the other hand, CaMKII overexpression in cAF may impact channel expression, thus contributing to I_{to} downregulation, as recently shown in CaMKII-overexpressing transgenic mice (51).

Experimental evidence suggests that block of I_{Kur} enhances force of contraction of isolated human atrial trabeculae both in patients in sinus rhythm and AF (22, 23, 52). We have recently predicted that block of I_{Kur} results in prolongation and elevation of the AP plateau, which augments the CaT amplitude that would elicit a positive inotropic effect (16). Taken together, these studies suggest that I_{Kur} might be a potentially useful atrial-specific target to potentially counteract hypocontractility associated with cAF. A slight AP prolongation associated to I_{Kur} blockade may also be beneficial.

Caballero *et al.* have recently looked at differences in current density and AF-induced alterations in the right *vs.* left human atrium. They found heterogeneity in the repolarizing currents between the atria in sinus rhythm, and demonstrated that cAF reduced the I_{to} amplitude and density more markedly in the left than in the right atrium, thus creating a right-to-left gradient, whereas I_{Kur} was more markedly reduced in the right than in the left atrium, thus dissipating the left-to-right gradient detected in sinus rhythm (24). However, the data concerning intra-atrial heterogeneities in repolarizing currents in human atrial myocytes are still limited, and it is unclear whether and how these changes may contribute to the perpetuation of arrhythmia (16).

I_{Ks} and I_{Kr}

The delayed rectifier K^+ currents have proven much harder to record and study in isolated human atrial cells (53). Nevertheless, their contribution is likely to be small in cells that lack an appreciable plateau phase (e.g., see current densities in Fig. 2*A*, *6th and 7th rows*) (54). The block of the rapidly activating delayed rectifier K^+ current, I_{Kr}, has been shown to prolong human atrial APD in the late phase of repolarization by a small amount (23), and to date no experimental evidence has suggested its involvement in AF-induced electrical remodeling.

Recently, Caballero *et al.* provided the first demonstration that cAF significantly increased the amplitude of the slow delayed rectifier K^+ current, I_{Ks}, in both atria (24). They suggested that I_{Ks} increase could contribute to cAF-induced shortening of APD and to further promote fibrillatory conduction, especially with current accumulation at high frequencies.

I_{K1} and I_{KACh}

The inwardly rectifying K^+ current (I_{K1}) primarily controls the resting potential of the cardiac cell, and its much lower density in atrial than in ventricular myocytes (55) confers the atrial AP a more depolarized resting potential (16). In cAF, increases in both current density (5, 12, 13, 45, 56) and mRNA levels (5, 13) have been reported (Fig. 2*A*, *8th row*). Increased I_{K1} causes a more negative resting membrane potential in cAF *vs.* sinus rhythm human atrial myocytes (13, 16, 56).

Patients with chronic AF exhibit agonist-independent constitutive $I_{K,ACh}$ activity that contributes to the enhanced basal inward rectifier current and may result from abnormal channel phosphorylation by PKC (13, 56, 57). Constitutively active $I_{K,ACh}$ is considered to support the maintenance of AF, together with increased I_{K1}, by stabilizing reentrant activity sustained by rotors (faster activation, less meander) (58).

Recently, Voigt *et al.* found significant left-to-right gradients in I_{K1} and constitutively active I_{KACh} in patients with paroxysmal AF, which were dissipated in cAF, raising the idea that this may contribute to left-to-right dominant frequency gradients that are often more evident in paroxysmal AF *vs.* cAF (56).

I_{KATP}

The ATP-sensitive K^+ (I_{KATP}) channels generate an inward rectifying current that activates with a decrease in intracellular ATP concentration (59). Gene expression and electrophysiological studies in patients with atrial fibrillation demonstrated reduced mRNA levels of Kir6.2 (48) and current activation (60), but increased current was also reported (61). Interestingly, a KATP channel mutation has been shown to confer risk for adrenergic atrial fibrillation originating from the vein of Marshall (62), and it has been proposed that KATP channel deficit could play a broader role in the pathogenesis of electrical instability (63). It is also conceivable that metabolic and mechanosensitive gating of KATP channels could be altered with structural heart disease and atrial dilation, thus providing a substrate for the more common acquired form of atrial fibrillation (63).

Ca^{2+} and Na^+ handling

I_{NCX}

The Na^+/Ca^{2+} exchanger current (I_{NCX}) is the main Ca^{2+} extrusion and Na^+ influx pathway in cardiac myocytes. It extrudes 1 Ca^+ in exchange for 3 Na^+, thus generating an inward current that influences cardiac repolarization and arrhythmogenesis (29). Increased expression (18, 21, 34) and abnormal function of I_{NCX} protein (16, 18) are implicated in human AF pathophysiology. An increase in I_{NCX} may be an adaptive response to cellular Ca^{2+} loading and contribute to diminish the Ca^{2+} overload induced by rapid atrial pacing (along with I_{Ca} downregulation). Indeed, the decay rate of caffeine-evoked CaT (attributable to Ca^{2+} removal by NCX) is shown to be faster in human cAF *vs.* sinus rhythm myocytes (16-18). Note that simulated I_{NCX} during an AP is smaller in AF than in sinus rhythm (Fig. 2*B*, *2nd row*), due to the reduced CaT (Fig. 2*B*, *1st row*). Na^+ overload-induced Ca^{2+} influx via reverse-mode NCX has been implicated in Ca^{2+} overload and related arrhythmogenesis, whereas increase Ca^{2+} extrusion via forward-mode has been linked to delayed-afterdepolarizations (29, 64). Indeed, Na^+ and Ca^{2+} loading are more favored at increased atrial rates (with AF). However, more studies are needed to assess whether delayed afterdepolarizations (DADs) are important in initiating arrhythmias in AF, and the underlying role of NCX in mediating them, since an increased I_{K1} in cAF will tend to oppose the occurrence of such DADs. These studies will help determine if blocking NCX represents a novel therapeutic strategy in suppressing arrhythmia triggers in cAF.

I_{NKA}

The Na^+/K^+ pump (NKA) is the main route of Na^+ efflux in cardiac cells thus regulating intracellular [Na^+]. By extruding 3 Na^+ in exchange for 2 K^+, it generates an outward current that is known to influence resting membrane potential and repolarization (29). Workman *et al*. found no difference in NKA pump current in myocytes from cAF patients compared to sinus rhythm, and concluded that I_{NKA} is not involved in AF-induced electrophysiological remodeling in patients (65). Our simulations show different NKA current underlying the AP (Fig. 2*B*, *3rd row*) because of altered Na^+ loading in cAF. Intracellular [Na^+] changes may contribute to the human cAF phenotype, as we postulated in our modeling study (16) but have not yet measured.

Ryanodine Receptors

RyRs directly control SR Ca^{2+} release in cardiac muscles, activating contraction during E-C coupling (29). Spontaneous Ca^{2+}-release events (Ca^{2+} sparks) and Ca^{2+} waves through leaky RyR channels have been reported in myocytes from cAF patients (17, 18, 66, 67) despite unaltered SR Ca^{2+} content. One potential contributor to RyR hyperactivity may be oxidative stress, which is known to play a critical role in AF pathophysiology (38) and increase RyR open probability. Neef *et al.* suggested that the CaMKII-dependent increase in SR Ca^{2+} leak caused by RyR hyperphosphorylation in AF is a potential arrhythmogenic mechanism (17), because elimination of Ca^{2+} via inward I_{NCX} could lead to cell depolarization and cause DADs. Voigt *et al.* measured directly single RyRs isolated from cAF patients and demonstrated a higher channel

open probability in cAF that responded to CaMKII inhibition (68). Thus CaMKII inhibition may reduce the propensity for atrial arrhythmias.

SR Ca^{2+} ATP-ase and PLN

The SR Ca^{2+} ATP-ase (SERCA) is responsible for pumping Ca^{2+} back into the SR after Ca^{2+} release (29). The endogenous inhibitor PLN regulates SERCA and releases its inhibition when phosphorylated by either PKA or CaMKII (29, 30). A decrease in SERCA activity, associated with smaller SERCA protein expression (18, 34), is evident in human cAF and explains the slower CaT decay compared to sinus rhythm (16, 18, 34). On the other hand, reduced inhibition of SERCA by hyperphosphorylated PLN (34) in cAF could help to maintain a normal SR Ca^{2+} load despite increased RyR activity.

Ankyrin-B

Ankyrin-B (encoded by *ANK2*) is an adaptor protein expressed in excitable cells that targets ion channels (e.g., Na^+ and Ca^{2+} channels), transporters (e.g., NKA and NCX), and signaling molecules to specific membrane domains. In the heart, ankyrin-B loss-of-function mutations in humans lead to Long QT syndrome, AF, sinus node dysfunction and stress-induced ventricular arrhythmias (69). Recently, reduced ankyrin-B expression has been demonstrated in atrial samples of patients with paroxysmal AF, and supported an association between ankyrin-B and AF (70). A new potential molecular mechanism underlying ankyrin-associated AF has been proposed involving disrupted $Ca_v1.3$ (atrial L-type Ca^{2+} channels) membrane targeting in atrial myocytes (70). It will be interesting to further explore the role of ankyrin in cAF.

Myofilaments

Altered Ca^{2+} handling (namely, downregulation of the L-type Ca^{2+} channels and increased Ca^{2+} extrusion via NCX) could account for the depressed contractility in remodeled atria, but a reduction of the maximum force generating capacity of the myofilaments and its Ca^{2+}-sensitivity may also be involved. Indeed, recent studies have highlighted the potential role of sarcomeric proteins in the cAF induced hypocontractility (9, 34, 71), although results are somewhat controversial. Compared to sinus rhythm myofibrils, cAF myofibrils exhibited reduced maximum rate of tension generation and maximum active tension, reduced passive tension, and increase in myofilament Ca^{2+} sensitivity (9). An earlier study did not show significant changes in maximum force and passive force, but did report reduced rate of tension redevelopment in cAF (71). One major difference between the two studies is that the former used left atrial samples whereas the latter used right atrial samples.

Altered phosphorylation state of various myofilament proteins was found in cAF *vs.* sinus rhythm. Phosphorylation of the primary sarcomere target of PKA, cTnI, was not altered in cAF atria (9, 34). The expression of the slow β-myosin heavy chain isoform (cMyBP-C) (9, 34, 71) was upregulated in cAF, and its phosphorylation levels were found significantly increased (9) or decreased (34). It has been suggested that discrepancies between these results may be explained by a decrease in cMyBP-C phosphorylation in cAF reflecting atrial dilatation rather than being a component of cAF (9). Another potential reason is the use of samples from the left atrium in the former study and from the right atrium in the latter.

Further studies are needed to resolve these inconsistencies. Furthermore, cell shortening data that are currently missing in human atrial myocytes may help in linking these molecular changes to functional alterations.

It is becoming increasingly clear that studies of remodeling of human atrium by chronic AF are frequently and unavoidably influenced in part by multiple confounding clinical variables such as patient age, sex, disease history, and drug treatments. Furthermore, the changes in ion currents and APs should be considered to be associated with, rather than necessarily caused by, the chronic AF. Nevertheless, the concordance between these human chronic AF data and AF/atrial tachypacing-induced changes in animal models (see Table 1 in (72)) supports the view that

chronic AF causes atrial electrophysiological remodeling in humans.

Consequences of ventricular dysfunction and atrial dilation on human atrial AP

Structural, electrophysiological, mechanical, metabolic and neurohumoral remodeling associated with cardiac disorders, such as coronary artery disease, CHF, and left ventricular systolic dysfunction (LVSD) may increase AF risk (73). In atrial cells isolated from patients with CHF or LVSD, AP duration was either unchanged (Fig. 3*A*) (74, 75) or increased (Fig. 3*B*) (76). In patients in sinus rhythm with reduced left ventricular ejection fraction (< 45%) APD_{90} was shorter than in patients with higher ejection fraction (75), and there was a significant correlation between cellular ERP shortening and decreasing left ventricular ejection fraction (75). Furthermore, multivariate analysis adjusting for 10 relevant clinical covariates confirmed that LVSD was independently associated with atrial cellular ERP-shortening, which may, therefore, be expected to contribute to a predisposition to AF in these patients. The features of ionic remodeling in CHF or LVSD in human atrium are not fully understood. I_{CaL} was either decreased in patients with coronary artery disease, aortic valve disease, or mitral valve disease (77) or unchanged in LVSD or CHF patients (75, 78). Schreieck *et al.* found increased I_{to} in human atrial myocytes of patients with reduced left ventricular function, with no change in its voltage dependence or decay, but with enhanced reactivation (74). However, this I_{to} increase may have been confounded by the lower proportion of patients treated with β-blockers in the reduced LV function group, since such treatment is associated with decreased I_{to} in human atrium (79). In contrast, Workman *et al.* found that LVSD was associated with decreased I_{to}, a positive shift in its activation voltage, and no change in its decay kinetics (75). Koumi *et al.* reported low resting membrane potential in atrial myocytes from CHF patients, possibly due to reduced density of I_{K1} and I_{KACh} (76). Workman *et al.* reported unchanged I_{K1} in LVSD (75), although Ba^{2+}-sensitive I_{K1} or I_{KACh} were not measured. Unchanged atrial I_{Kur} has also been reported in human LVSD (74, 75).

Cardiac dilatation is known to develop frequently during the course of cardiac failure (80). In trabeculae and myocytes taken from dilated atria the AP was shorter and the plateau was markedly depressed (Fig. 3*C*) compared to trabeculae and myocytes from non-dilated atria (80). However, it must be noted that the ventricular dysfunction was not quantified in these patients. AP changes were explained with more severely depressed I_{CaL} compared to the reduction in total outward current (80).

Overall, the ionic bases of altered atrial function in patients with ventricular dysfunction, and how it predisposes to more frequent AF episodes culminating in cAF, remains poorly understood.

Autonomic changes in chronic AF and related myocardial diseases

The autonomic nervous system, and particularly the relative activities of the sympathetic (adrenergic) and parasympathetic (cholinergic) branches, has a major influence on the occurrence of AF. Furthermore, chronic AF, and certain predisposing cardiac pathologies, remodel atrial electrophysiological responses to catecholamines and acetylcholine and thus influence the electrophysiological mechanisms of AF. β-adrenergic stimulation increases human atrial I_{CaL} (11, 40, 42, 81), I_{Kur} (79, 82) and I_f (83, 84), has no effect on I_{K1} (79), I_{KACh} (85) or I_{to} (79), and has markedly different effects on connexin conductance or expression, depending upon their main molecular correlate; i.e. Cx40, Cx43, Cx45 (86). The increased I_{CaL} and I_{Kur}, with lack of effect on other repolarizing currents, results in no net effect of β-stimulation on atrial APD_{90}, as predicted by our model (16), consistent with 5 of 6 reports in human atrial cells or tissues (87). However, the increased I_{CaL} markedly elevates the AP plateau (81) and, coupled with increased $[Ca^{2+}]_i$ from PLN phosphorylation by adrenergic stimulation (88), favors non-reentrant activity such as afterdepolarizations (87). Human atrial studies of α-adrenergic stimulation are sparse: phenylephrine inhibited I_{K1} (85, 89), I_{KACh} (85) and I_{Kur} (82); also potentially promoting afterdepolarizations. Chronic AF consistently potentiates the effect of β-

adrenergic stimulation to increase human atrial I_{CaL} (11, 40, 43, 90). While this could, in theory, increase the propensity for afterdepolarizations in the presence of catecholamines, chronic AF also markedly decreases basal I_{CaL} (6) and attenuates the effects of α-stimulation on I_{K1} and I_{KACh} (85). Chronic AF may also cause increased atrial adrenergic innervation, "neural remodeling", in patients (91). The effects of chronic AF on $[Ca^{2+}]_i$-responses to adrenergic stimulation have yet to be studied in human atrium. Data on effects of myocardial diseases that predispose to AF, on human atrial adrenergic responses, are equivocal: the ability of β-stimulation to increase I_{CaL} was attenuated (92, 93), unchanged (75) or potentiated (77) in association with HF or LVSD. An attenuated I_{CaL}-increase was also reported in cells obtained from dilated atria from explanted hearts (78). Attenuated β-responses may involve reduced β-receptor density or function (87). Post-operative AF was not predicted by any change in the pre-operative atrial I_{CaL} response to β-stimulation (94). Cholinergic elevation and increased levels of acetylcholine activate I_{KACh} and also antagonize effects of catecholamines on I_{CaL}, both shortening APD and ERP, thus promoting reentry (95). Also, combined adrenergic/cholinergic-stimulation may produce "late phase EADs" (96), possibly by concurrently shortening APD and increasing $[Ca^{2+}]_i$ (97). Chronic AF induces a constitutively active I_{KACh} in human atrium (57), likely resulting from a PKC isoform switch (98). However, the acetylcholine-mediated increase in atrial I_{KACh} (13, 57) and shortening in atrial APD (13) were each attenuated in chronic AF, and the cholinergic receptors GIRK1 and GIRK4 were generally downregulated (5). The attenuation of atrial cholinergic responses by chronic AF may be restricted to the right atrium (56). The ability of acetylcholine to increase I_{KACh} and/or shorten atrial APD may be attenuated by HF, as shown in dogs (99). However, corroborative data from human patients with HF or LVSD are sparse and confounded by the presence of chronic AF (76). While much progress has been made, the complex and interacting influences of chronic AF and its predisposing myocardial pathologies on the involvement of the autonomic system in AF are yet to be resolved.

Oxidative stress and inflammation-related changes in human AF

Patients undergoing cardiac surgery often experience post-operative AF. It has been shown that these patients did not exhibit the electrophysiological remodeling seen in patients with cAF as far as Ca^{2+} and K^+ currents and AP characteristics are concerned (94), whereas altered atrial Ca^{2+} handling in post-operative AF patients has not yet been studied. However, Van Wagoner *et al.* showed that patients with the highest I_{CaL} density pre-surgery, were associated with post-operative AF, thus indirectly suggesting a role for Ca^{2+} overload, mediated via oxidative/inflammatory stress, as a possible trigger (11). This is because increased levels of inflammatory markers are often recorded after cardiac surgery, and recent evidence suggests oxidative stress may play an important role in the pathogenesis and perpetuation of post-operative AF (100).

Several studies have shown increased myocardial oxidative stress associated with AF (38, 101). In addition, inflammatory markers such as interleukin-6 and C-reactive protein have been found elevated in AF patients (101, 102). Evidences suggest that in several pathophysiological conditions inflammation and oxidative stress are highly interrelated, whereby inflammation augments oxidative stress and viceversa, and may be involved in AF pathogenesis. Importantly, oxidant and inflammatory mechanisms may contribute to the described structural, electrophysiological, and contractile remodeling that favors maintenance of AF (103), and thus could be considered as targets for AF-treatment, as discussed in more comprehensive reviews on the topic (101, 104). Inflammatory processes may contribute to atrial injury resulting in myocyte hypertrophy and fibrosis. Furthermore, several Ca^{2+} channels and transporters are the subject of redox modulation (105). For example, oxidative stress may play an important role in I_{Ca} changes, as it has been shown that S-nitrosylation of the L-type Ca^{2+} channel α subunit is increased in AF, and exogenously applied glutathione partially restores the AF-related I_{Ca} reduction (106). We also discussed above the potential contribution of oxidation

in AF-associated RyR hyperactivity. Several K^+ channels (e.g., I_{to} (107) and I_{KATP}) are also sensitive to redox state. Kv1.5 currents are inhibited by oxidation by S-nitrosylation (108), which may contribute to I_{Kur} suppression in AF. Redox-dependent modulation of Na^+ channel activity has also been reported (109). Additionally, oxidative stress may affect myofilament protein function (38) and influence the activity of protein kinases (e.g., CaMKII (110)) and phosphatases that alter E-C coupling via phosphorylation of target proteins and are also redox sensitive. Suppressing E-C coupling remodeling with anti-inflammatory and antioxidant drugs (such as glucocorticoids and statins) has proven clinically useful in some cases in preventing AF recurrence (104). In sum, we are still limited in our understanding regarding how oxidative/inflammatory stress influences E-C coupling, particularly in the context of chronic AF, and further studies are warranted.

Conclusions

Chronic AF is associated with altered expression and activity of numerous sarcolemmal ion channels, transporters, Ca^{2+} handling and myofilament proteins. Understanding the ionic mechanisms underlying E-C coupling remodeling in fibrillating human atria, and distinguishing compensatory responses from maladaptive mechanisms, may allow for identification of new therapeutic targets to improve electrical and contractile function in cAF patients.

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Table 1– Molecular bases of altered E-C Coupling in human AF (changes *vs.* sinus rhythm) [Modified from (16)].

Cell Size/Structure	
Size	Increased length and width (17)
C_m	Increased (24)
Sarcolemmal Ion Channels	
I_{Na}	No changes (15, 35) Steady-state inactivation shifted right (15) Slightly reduced current density (36) Late current increased (36)
I_{CaL}	Reduced current density by ~50% (5, 11, 12, 18, 40) No changes in voltage dependence of activation and inactivation (11)
I_f	Increased mRNA levels (44)
I_{to}	Reduced density -80% in the RA -45% in the LA (5, 12, 15, 24, 45-47)
I_{Kur}	Reduced density -55% in the RA -45% in the LA (5, 24, 45, 47, 49) Unchanged (12, 15, 46)
I_{Ks}	Increased 2-fold (24)
I_{K1}	Upregulated +100% (5, 12, 13, 15, 45)
I_{KACh}	Increased basal current by receptor-independent, constitutively active component; increased (15) or reduced carbachol-activated current (13, 56, 57)
I_{KATP}	Decreased (60) Increased (61)
Ca and Na handling	
I_{NCX}	Upregulated (17, 18, 21, 34)
SERCA	Reduced maximal pump rate (18) and protein expression (34)
PLN	Enhanced PKA and CaMKII phosphorylation (34) Unaltered CaMKII-dependent phosphorylation (17)
RyR	Increased phosphorylation at PKA and CaMKII sites (17, 18, 67) resulting in increased channel open probability (68) and SR Ca^{2+} leak (17, 111)
I_{NKA}	Unchanged function (65)
Ankyrin-B	Downregulated (70)
Protein kinases and phosphatases	
CaMKII	Increased expression (32) and phosphorylation (17)
PKA	Similar activity in cAF *vs.* sinus rhythm (34)
PP1, PP2A	Higher activity (34)
Myofilaments	Reduced maximum rate of tension generation and maximum active tension, reduced passive tension, and increase in myofilament Ca sensitivity (9) No changes in maximum force and passive force, reduced rate of tension redevelopment (71) Increased phosphorylation of cMyBP-C (9) Decreased phosphorylation of cMyBP-C (34) No changes in cTnI phosphorylation (9, 34)

Figure Legends

Figure 1 – Altered E-C coupling in human AF. A) APs recorded at different pacing rates in a control human atrial myocyte (*left*) and in a cell from a cAF patient (*right*) (11). B) Intracellular Ca^{2+} transients measured in human atrial cells from sinus rhythm (*left*) and cAF (*right*) patients at physiological temperature. C) Twitch force measurements (22) in myocytes from sinus rhythm and cAF patients are shown at various doses of AVE0118 (I_{Kur} blocker).

Figure 2 – Ionic bases of altered E-C coupling in AF. A) Simulated time courses of human atrial APs and contributing ionic currents are shown for sinus rhythm and cAF. Currents are listed on the left (with changes in cAF *vs.* sinus rhythm), and the genes encoding the channels are shown on the right. B) Simulated human atrial Ca^{2+} transients for sinus rhythm and cAF are shown with NCX and NKA currents (with changes in cAF *vs.* sinus rhythm on the left). C) Schematic representation of a human atrial myocyte illustrating the cAF-induced changes in Ca^{2+} handling proteins.

Figure 3 – Altered atrial APs in human ventricular dysfunction. A) Atrial APs from patients with moderate or severe LVSD (*bottom*) and from patients without LVSD (*top*) (75). B) Representative AP and response to ACh in isolated atrial myocytes from HF (*top*) and donor (*bottom*) hearts at a stimulation frequency of 1 Hz (76). C) Representative APs from a non-dilated (normal, *left*) and a dilated (*right*) atrium recorded at a pacing frequency of 1 Hz (80).

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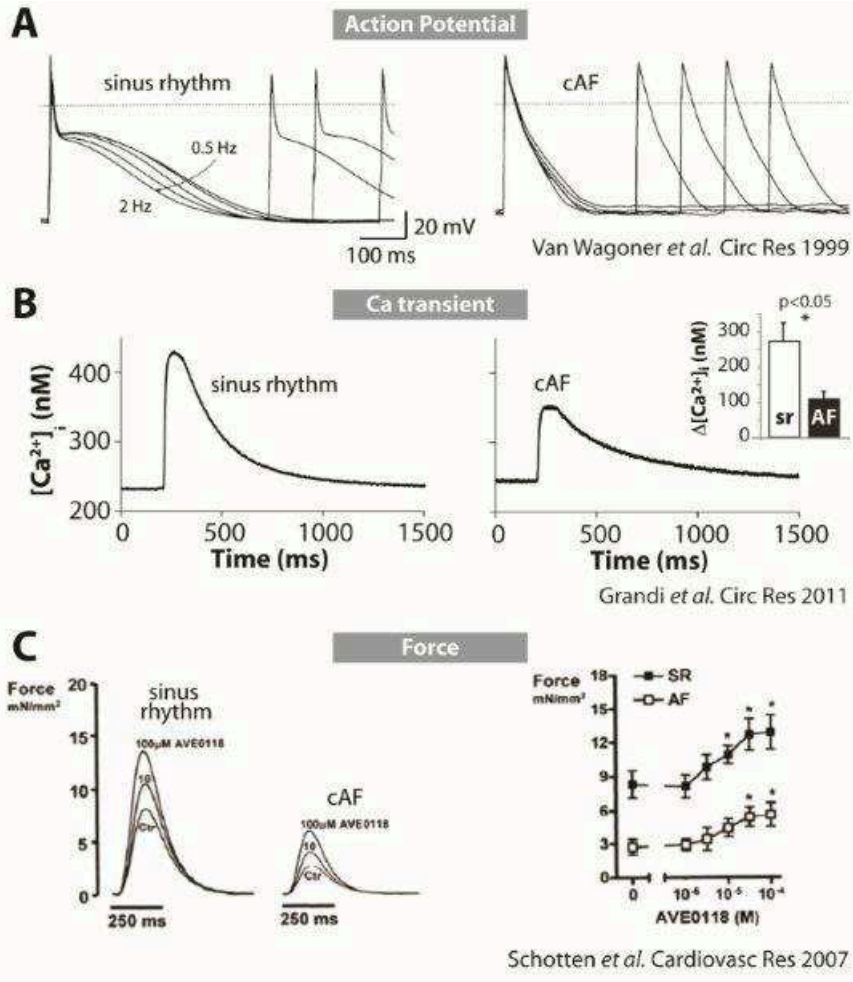
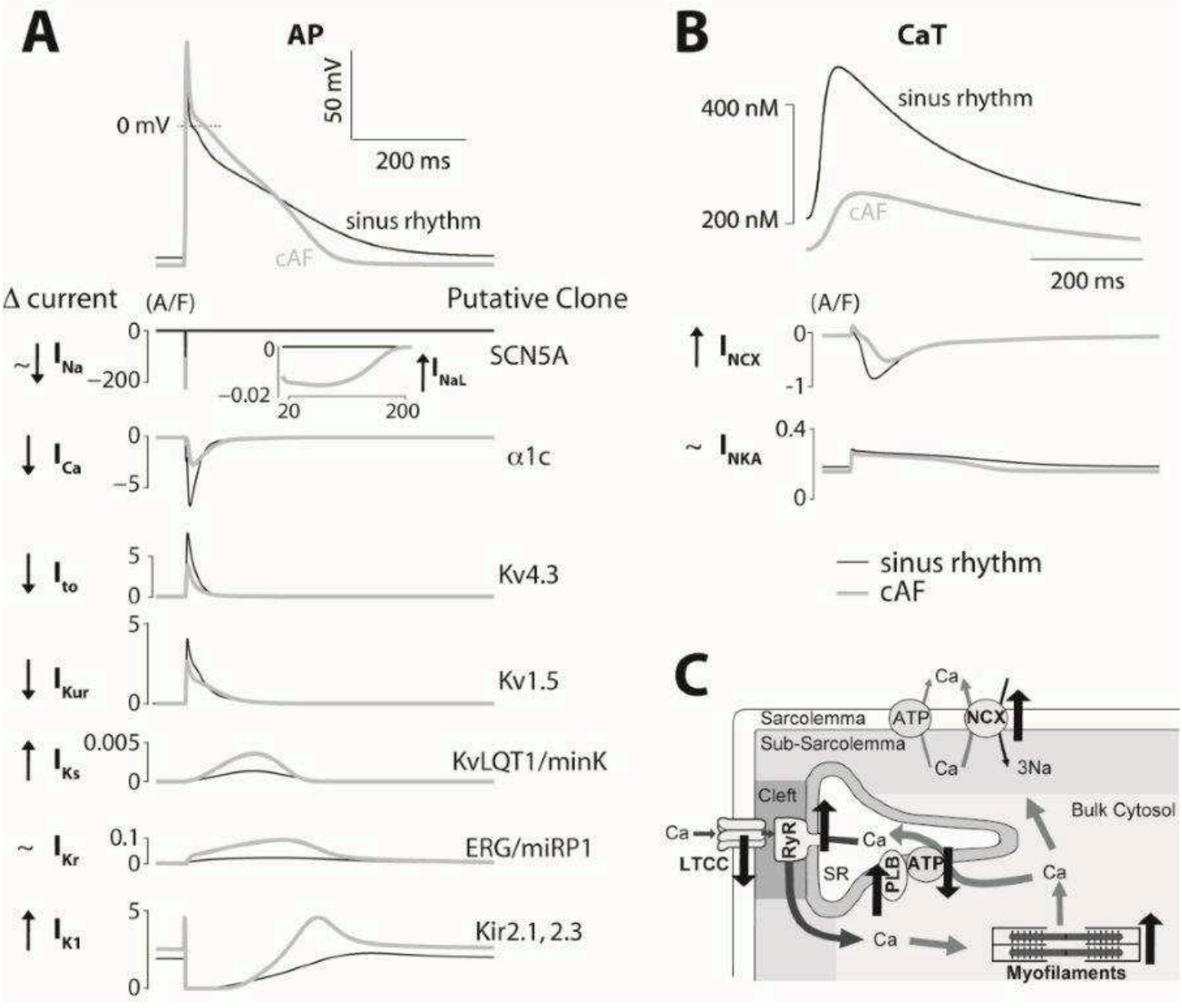

A
Action Potential
sinus rhythm
0.5 Hz
2 Hz
20 mV
100 ms
cAF
Van Wagoner et al. Circ Res 1999
B
Ca transient
[Ca2+]i (nM)
400
300
200
sinus rhythm
0
500
1000
1500
Time (ms)
cAF
p<0.05
Δ[Ca2+]i (nM)
300
200
100
0
sr
AF
Grandi et al. Circ Res 2011
C
Force
Force
sinus rhythm
100µM AVE0118
10
-Ca2+
250 ms
cAF
100µM AVE0118
10
-Ca2+
250 ms
SR
AF
AVE0118 (M)
Schotten et al. Cardiovasc Res 2007


A
AP
0 mV
50 mV
200 ms
sinus rhythm
cAF
Δ current
(A/F)
Putative Clone
~↓ I_Na
0
−200
0
−0.02
20
200
I_NaL
SCN5A
↓ I_Ca
0
−5
α1c
↓ I_to
5
0
Kv4.3
↓ I_Kur
5
0
Kv1.5
↑ I_Ks
0.005
0
KvLQT1/minK
~ I_Kr
0.1
0
ERG/miRP1
↑ I_K1
5
0
Kir2.1, 2.3
B
CaT
400 nM
200 nM
sinus rhythm
cAF
200 ms
(A/F)
↑ I_NCX
0
-1
~ I_NKA
0.4
0
sinus rhythm
cAF
C
Ca
Sarcolemma
ATP
NCX
Sub-Sarcolemma
Ca
3Na
Cleft
Bulk Cytosol
Ca
RyR
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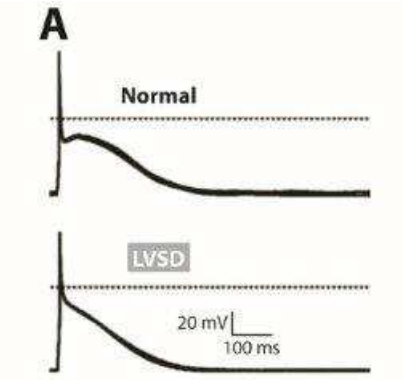
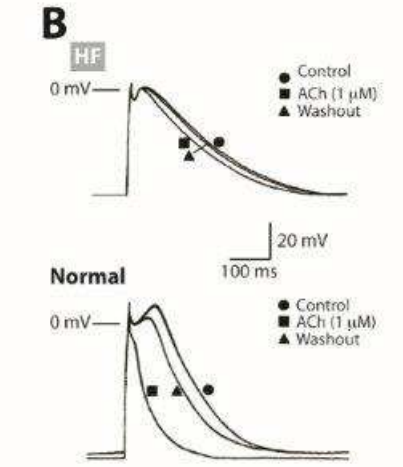
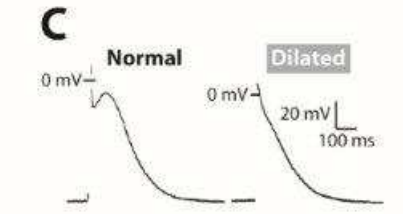
LTCC
SR
PLB
ATP
Ca
Ca
Myofilaments



Workman *et al.* Heart rhythm 2009



Koumi *et al.* Circulation 1994



LeGrand *et al.* Cardiovasc Res 1994