Effects of Chronic Atrial Fibrillation on Active and Passive Force Generation in Human Atrial Myofibrils

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Rationale: Chronic atrial fibrillation (cAF) is associated with atrial contractile dysfunction. Sarcomere remodeling may contribute to this contractile disorder.

Objective: Here, we use single atrial myofibrils and fast solution switching techniques to directly investigate the impact of cAF on myofilament mechanical function eliminating changes induced by the arrhythmia in atrial myocytes membranes and extracellular components. Remodeling of sarcomere proteins potentially related to the observed mechanical changes is also investigated.

Methods and Results: Myofibrils were isolated from atrial samples of 15 patients in sinus rhythm and 16 patients with cAF. Active tension changes following fast increase and decrease in [Ca^{2+}] and the sarcomere length–passive tension relation were determined in the 2 groups of myofibrils. Compared to sinus rhythm myofibrils, cAF myofibrils showed (1) a reduction in maximum tension and in the rates of tension activation and relaxation; (2) an increase in myofilament Ca^{2+} sensitivity; (3) a reduction in myofibril passive tension. The slow β-myosin heavy chain isoform and the more compliant titin isoform N2BA were up regulated in cAF myofibrils. Phosphorylation of multiple myofilament proteins was increased in cAF as compared to sinus rhythm atrial myocardium.

Conclusions: Alterations in active and passive tension generation at the sarcomere level, explained by translational and post-translational changes of multiple myofilament proteins, are part of the contractile dysfunction of human cAF and may contribute to the self-perpetuation of the arrhythmia and the development of atrial dilatation.

Key Words: myosin ■ Titin ■ cardiac MyBP-C ■ cardiac troponin ■ atrial light chain-2

Chronic atrial fibrillation (cAF) is characterized by electric, structural, and contractile remodeling that leads to pronounced atrial contractile dysfunction and self-perpetuation of the arrhythmia (reviewed elsewhere[1]). The persistence of atrial contractile disorder after cardioversion to sinus rhythm (SR) in patients with cAF has long been reported[2] and may have dramatic consequences as it favors thromboembolic events.[3] In spite of its clinical significance, the exact mechanism of cAF-induced contractile dysfunction is poorly understood.

Most investigations into the contractile dysfunction of cAF remodeled atria have concentrated on structural alterations[4,5] or disorders of excitation–contraction coupling.[6,7] Recent investigations high-light the role of sarcomere protein modifications in human cAF-associated contractile dysfunction.[8–11] However, the functional impact of myofilament protein changes in cAF is weakly documented because it is difficult to obtain consistent measurements of functionally relevant parameters on human cardiac preparations.

Studies on single myofibrils can significantly document changes in the mechanical performance of human cardiac sarcomeres because these preparations can be obtained in large amounts from very small cardiac samples.[12,13] Single myofibrils are the smallest units of the contractile apparatus that retain the organized myofilament lattice and its entire ensemble of associated proteins. Mechanical measurements of myofibril force combined with rapid perfusion switching techniques have been developed recently to investigate fast

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kinetic events related to cross-bridge action and regulation in human cardiac myofibrils.[12–14]

In this study, sarcomere mechanisms underlying passive and active tension generation and relaxation of atrial myocardium are dissected and compared in myofibrils from surgical samples of cAF and control (SR) patients. In the same samples, we also determined the myosin heavy chain (MHC) and titin isoform expression and the phosphorylation level of several myofilament proteins. The results show that myofilament proteins play a direct role in the altered atrial mechanics associated with cAF suggesting that sarcomere remodeling also contribute to the progressive nature of the arrhythmia.

Non-standard Abbreviations and Acronyms	
AF	atrial fibrillation
cAF	chronic atrial fibrillation
cMyBP-C	cardiac myosin binding protein-C
cTn	cardiac troponin
k_{ACT}	rate constant of tension generation following rapid Ca^{2+} activation
k_{TR}	rate constant of tension generation following rapid mechanical perturbation
k_{REL}	rate constants of the "slow" and "fast" phases of tension decline following rapid Ca^{2+} removal
MHC	myosin heavy chain
n_H	Hill coefficient, slope of the pCa–tension relationship
P_0	maximal Ca^{2+}-activated tension
pCa	$-\log[Ca^{2+}]$
pCa_{50}	pCa at which active tension is 50% of P_0
PK	protein kinase
SR	sinus rhythm
ssTnI	slow skeletal troponin I

Methods

Patients

The investigation conforms with the principles outlined in the Declaration of Helsinki and is approved by the local Ethics Committee (no. 2006/0023797). Samples of atrial appendages were obtained following informed consent from 15 SR patients (67±2 years; 7♀;) and 16 cAF patients (66±2 years; 9♀;) undergoing open heart surgery. In the cAF patients, established atrial fibrillation (AF) was documented for at least 8 months. No significant difference was present in ejection fraction and left atrial diameter between cAF and SR patients. Details for each patient group are given in Online Table I, available in the Online Data Supplement at http://circres.ahajournals.org.

Mechanical Measurements in Myofibrils

Fresh surgical samples were collected in a cold sterile saline solution for myofibril isolation according to previously described methods.[12] Techniques for mechanical measurements in human cardiac myofibrils were as previously described.[12–14] Briefly, myofibrils were transferred to a temperature controlled chamber filled with relaxing solution (pCa8, 15°C). The selected myofibril was horizontally mounted (initial sarcomere length 2.2 to 2.3 μm) between a cantilever force probe and a glass needle mounted on the lever arm of a length control motor. Myofibrils were activated and relaxed by rapid solution switching between 2 continuous streams of solutions. Ionic strength of the experimental solutions was 200 mmol/L and pH 7.0. All solutions contained a MgATP-regenerating system and a cocktail of protease inhibitors.[12,13] To avoid the effects on myofibril force and force kinetics of variable levels of contaminant inorganic phosphate (P_i) in the solutions, [Pi] was reduced to less than 5 μmol/L using a P_i scavenging system.[12,13]

Sarcomeric Protein Analysis

Myosin heavy chain isoforms

Polyacrylamide gel electrophoresis was used to determine MHC isoform composition (MHC-α and MHC-β) after denaturation in sodium dodecyl sulfate (SDS-PAGE) following a procedure described by Talmadge and Roy.[15] Gels were silver stained for isoform recognition or stained with Coomassie Blue for quantitative analysis. Each band (MHC-α or MHC-β) was expressed as percent of the total MHC.

Titin Isoforms

Analysis of titin content in SR and cAF samples was adapted from Cazorla et al.[16] Titin content was analyzed with SDS-PAGE (2.5% to 7% acrylamide gradient gels) and stained with 0.1% Coomassie Blue. The integrated optical density of MHC and titin peaks (both N2B and N2BA isoforms) were determined on wet gel images to measure both the total amount of titin relative to MHC and the ratio N2BA:N2B. For each sample a range of loadings was electrophoresed on the same gel. The optical density of titin and MHC peaks were determined and plotted against their loading volume. The linear part of this relation was fitted with a line regression and the slope determined. The slope ratio of titin:MHC was taken as relative amount of titin in the samples and used to determine the N2BA:N2B ratio. Western blotting was performed with the antibodies Z1/Z2 (kindly provided by Dr S. Labeit, University of Mannheim, Germany) to label the N-terminal of titin.

ProQ Phosphostaining

Atrial tissue samples from 8 SR and 8 cAF patients were TCA (trichloroacetic acid)-treated as described previously to fix phosphorylation status of myofilament proteins.[17] Phosphorylation status of myofilament proteins separated on a gradient gel was determined using Pro-Q Diamond phosphostaining.[17] All protein signals were within the linear range and were corrected for protein content determined by SYPRO Ruby staining.

Western Immunoblotting

Gel electrophoresis and Western immunoblotting were performed as described previously[10,18] to analyze content and phosphorylation of cardiac myosin binding protein (cMyBP)-C and cardiac troponin (cTn)I.

Results

Active Tension Generation and Relaxation

Thin bundles of myofibrils obtained from atrial samples of 7 SR and 8 cAF patients were maximally calcium activated at pCa 4.5 and relaxed at pCa 8.0 by rapid solution change. Representative force responses of SR and cAF myofibrils to full activation-relaxation cycles at 15°C are shown in Figure 1A. Average data for maximal isometric tension (P_0) and kinetic parameters of maximal tension generation (k_{ACT}, rate constant of tension generation following Ca^{2+} activation; and k_{TR}, rate constant of tension redevelopment following release–restretch applied to the myofibril under steady-state conditions of force generation) for both SR and cAF myofibrils are in the Table.

P_0 in SR myofibrils was close to that previously reported for control human atrial myofibrils.[12] P_0 was significantly depressed (≈30%) in cAF compared to SR myofibrils. Both k_{TR} and k_{ACT} were markedly slower (≈50%) in cAF than in SR myofibrils (Table). The difference is evident in Figure 1B (left) where tension activation traces of SR and cAF myofibrils are normalized to maximal tension and then superimposed.

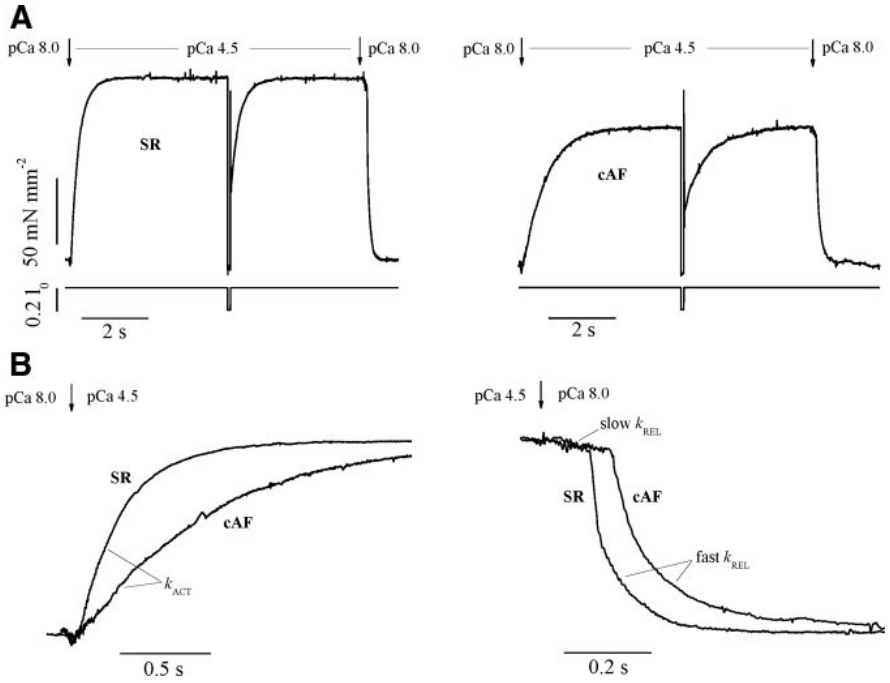

Figure 1. Force activation and relaxation of SR and cAF myofibrils. A, Representative examples of maximum tension activation and full relaxation in response to sudden pCa changes by fast solution switching in SR **(left)** and cAF **(right)** myofibrils. Lower traces show fast release–restretch protocol for k_{TR} registration. **B,** Time courses of tension activation (left, k_{ACT}) and relaxation (**right**, slow and fast k_{REL}) of SR and cAF myofibrils superimposed after normalization to maximal tension (same traces as in **A** on faster time base).

As shown in Figure 1A, for both SR and cAF myofibrils, active tension fully relaxed on step reduction of [Ca^{2+}] below the contraction threshold (pCa 8.0). The time course of full tension relaxation is shown in Figure 1B (right) where the normalized relaxation transients are superimposed on a faster time scale. In agreement with previous observations on skeletal and cardiac myofibrils,[19] relaxation of both SR and cAF myofibrils is biphasic starting with a slow linear force decay (rate constant, slow k_{REL}) followed by a fast exponential relaxation phase (rate constant, fast k_{REL}). It has been shown that the slow force decay lasts as long as the sarcomeres remain isometric, whereas the fast exponential phase follows the "give" of one or more sarcomeres and is dominated by intersarcomere dynamics.[19] Average values for the rates of both relaxation phases (Table) indicate that relaxation kinetics were slower or tended to be slower in cAF than in SR myofibrils. Fast k_{REL} was reduced (by 35% $P<0.05$) and slow k_{REL} tended to decrease (by 25% $P<0.1$) in cAF versus SR myofibrils.

Table. Mean Data for Passive Tension and Active Tension Generation and Relaxation in Myofibrils from Seven SR and Eight cAF Patient Samples

	SR	cAF	P Student *t* Test
Resting tension, mN · mm^{-2}	7.98±0.59 (52)	3.26±0.38 (61)	$P<0.01$
Active tension generation			
P_0, mN · mm^{-2}	125±7 (52)	90±7 (61)	$P<0.01$
k_{ACT}, sec^{-1}	3.73±0.18 (54)	2.00±0.10 (72)	$P<0.01$
k_{TR}, sec^{-1}	3.55±0.10 (46)	1.90±0.10 (64)	$P<0.01$
Active tension relaxation			
Slow phase duration, ms	126±6 (47)	139±8 (61)	NS
Slow k_{REL}, sec^{-1}	0.52±0.04 (47)	0.40±0.04 (61)	$P<0.10$
Fast k_{REL}, sec^{-1}	16±1 (47)	10±1 (62)	$P<0.05$

Resting sarcomere length, 2.25±0.01. Data are means±SE (no. of myofibrils).

MHC Isoform Expression

Slower force kinetics at maximal activation in the cAF myofibrils are consistent with previously described[8,9,20] cAF induced changes in the motor protein. Of the 2 MHC isoforms expressed in human cardiac muscle MHC-α is associated with higher actomyosin ATPase activity and faster cross-bridge kinetics than MHC-β. Both SR and cAF myofibrils coexpressed MHC-α and MHC-β isoforms (Figure 2A). As expected from previous characterization of human atrial tissue[21] MHC-α represented the largest fraction (>50%) of the total amount of MHC. The

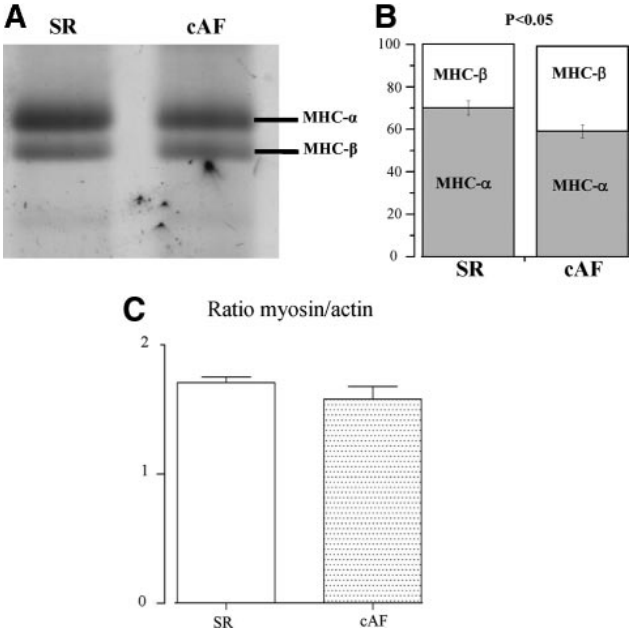

Figure 2. MHC isoform expression in SR and cAF myofibrils. A, Representative SDS-PAGE of the MHC isoform region of SR and cAF samples. **B,** Relative distribution of the MHC isoforms in SR and cAF atrial myofibrils (means±SE, n=8). **C,** Total MHC content expressed relatively to actin.

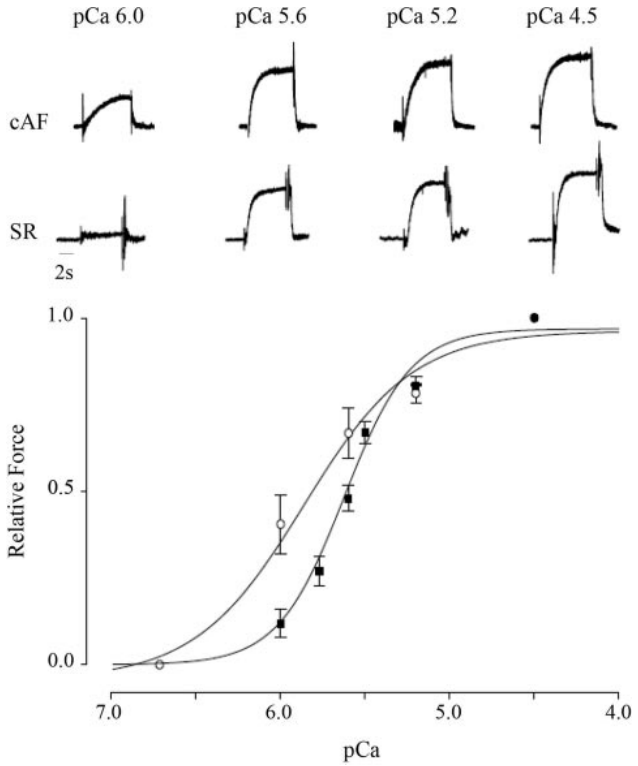

Figure 3. pCa–tension relationships of SR and cAF myofibrils. Top, Representative traces from cAF and SR myofibrils at indicated pCa values. **Bottom,** Mean pCa–tension relationships for cAF (○) and SR myofibrils (■). Tension values are normalized to those measured at pCa 4.5. Data points are means±SE of 6 to 12 myofibrils from 4 SR and 4 cAF patients. Data are fit to a modified Hill equation (continuous lines): $P=P_0/(1+10^{[nH\{pCa-pCa50\}]})$.

MHC-α fraction was larger in SR samples (70%) compared to cAF (59%) (Figure 2B, n=8 for both groups, $P<0.05$). Total MHC, measured as MHC/actin ratio for each atrial sample, was the same in SR and cAF myofibrils (Figure 2C). The lower content in MHC-α in cAF myofibrils confirms the previously reported shift of MHC isoforms from MHC-α to MHC-β for cAF human atrial myocardium.[8,9,20]

Ca^{2+} Sensitivity of Active Tension

Ca^{2+} sensitivity of tension was investigated by activating myofibrils with various pCa solutions then assembling the pCa–tension relationship. Each preparation was exposed to up to 4 different pCa solutions. pCa 4.5 determined maximum Ca-activated tension and pCa 8 fully relaxed the preparation between each activation. Values of force at any given pCa were normalized to maximal force at pCa 4.5. pCa–tension points were fit to a Hill equation and the pCa at which tension was half maximum (pCa_{50}) and the Hill coefficient (n_H; slope of the pCa–tension relationship) were determined for each group.

pCa–tension relationships for SR and cAF myofibrils are given in Figure 3, with representative experimental traces at indicated pCa values. The pCa–tension relationship for cAF was clearly shifted to the left compared to that of SR myofibrils. Average pCa_{50} was 5.83±0.10 in cAF, which was significantly different ($P<0.05$) from pCa_{50} of SR myofibrils (5.61±0.03). The average n_H was 2.16±0.30 in SR and 1.44±0.43 in cAF myofibrils ($P<0.05$).

Sarcomeric Protein Phosphorylation

To investigate if the higher Ca^{2+} sensitivity of force in cAF can be ascribed to differences in myofilament protein phosphorylation between SR and cAF sarcomeres, myofibril proteins were separated on 1D gradient gels and stained with SYPRO Ruby and Pro-Q Diamond (Figure 4). On average, no significant difference was found in cTnI phosphorylation between SR and cAF, whereas phosphorylation of cMyBP-C, desmin, cTnT, and atrial light chain-2 was significantly higher in cAF compared to SR samples.

The increase in cMyBP-C phosphorylation found in human cAF using the Pro-Q Diamond phosphostaining (Figure 4B) is in sharp contrast with previously reported decrease in cMyBP-C phosphorylation measured with specific phospho-cMyBP-C antibody[10] in human cAF atria. To determine whether the opposite result was attributable to the different measurement methods we analyzed the content and phosphorylation of cMyBP-C and cTnI of our cAF and SR samples using Western immunoblotting. The analysis revealed no changes in cMyBP-C (Figure 5A) and cTnI (Figure 5B) protein content in cAF samples compared to SR. In accordance with ProQ analysis of protein phosphorylation, the specific antibodies directed against phosphorylated protein kinase (PK)A sites in cMyBP-C (Ser282) and cTnI (Ser23/24) showed higher cMyBP-C phosphorylation in cAF compared to SR ($P=0.08$, Figure 5A) and confirmed no difference in cTnI phosphorylation at Ser23/24 (Figure 5B).

The previously reported decrease in cMyBP-C phosphorylation in cAF[10] may reflect atrial dilatation rather than being a component of cAF because, at variance with the present study (see Online Table I), in the previous study[10] the atrial size of SR patients was much less than that of cAF patients (see the table in the article by El-Armouche et al[10]). Consistent with this explanation, a recent study in goat models of atrial dilatation and atrial fibrillation[22] has reported that reduction in PKA phosphorylation of cMyBP-C is a distinctive feature of atrial dilatation.

Passive Tension

Passive tension at optimum overlap was lower in cAF compared to SR myofibrils (Table), suggesting that passive stiffness differs in the 2 myofibril types. To better investigate passive properties, the force responses of relaxed SR and cAF myofibrils to various ramp elongations were recorded and the steady-state sarcomere length–resting tension relationship determined in the 2 myofibril groups (Figure 6). Sarcomere length and resting tension were measured 20 seconds after each length change was completed, ie, when most of the stress relaxation was over. Details of the length elongation protocol applied to myofibrils are shown in the inset of Figure 6 together with tension traces for a representative SR myofibril. The average sarcomere length–passive tension relationships of SR and cAF myofibrils (Figure 6) are evidence that passive stiffness is significantly reduced in cAF myofibrils.

Titin Isoform Expression

Mammalian cardiac muscle coexpresses 2 titin isoforms, a short N2B and a long, more compliant, N2BA titin.[16–23] Considering that titin is the only significant source of resting

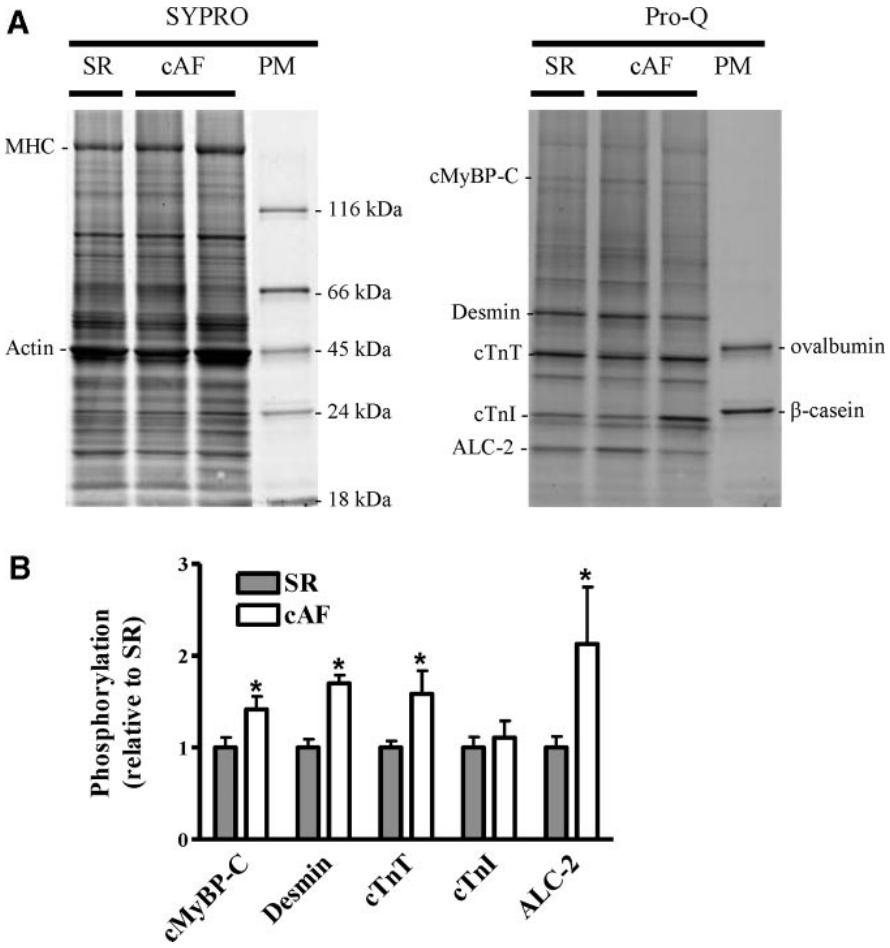

Figure 4. Myofilament protein phosphorylation. A, Tissue samples (20 μg/lane) were separated on a 4% to 15% gradient gel stained with SYPRO and Pro-Q Diamond. ProQ Diamond–stained signals (cMyBP-C; desmin; cTnT; cTnI; and atrial light chain 2 [ALC-2]) were divided by SYPRO-stained protein signals to correct for differences in protein loading. PM indicates molecular weight marker (PeppermintStick phosphoprotein marker in which ovalbumin and β-casein are phosphorylated). **B,** Student's *t* test analysis revealed significantly higher phosphorylation of cMyBP-C, desmin, cTnT, and atrial light chain-2 in cAF (n=8) compared to SR (n=8). Values are given relative to SR group, which was set to 1. *$P<0.05$, cAF vs SR.

tension in single myofibrils, we investigated whether the lower passive stiffness found in cAF myofibrils correlates with changes in titin isoform expression.

In Figure 7A, SDS-PAGE reveals that human atrium coexpresses N2B and N2BA titin isoforms; this is confirmed by Western blotting. Titin degradation product T2 was hardly detectable and T3 undetectable in our human atrial samples indicating a good titin preservation. As shown in Figure 7B, SR atrial tissue (n=6) expressed more N2BA (61%) than N2B (39%) and cAF (n=6 patients) expressed even more N2BA than SR (78%, $P<0.01$). The titin/MHC ratio was the same in SR and cAF patients (Figure 7C). Therefore, the total content of titin was not different in SR and cAF atrial tissue but the mean expression of N2BA in cAF was increased at the expense of N2B.

Discussion

Passive and active mechanics of human atrial myofibrils are significantly altered in cAF indicating that myofilament changes contribute to the atrial contractile dysfunction that persists after cardioversion. The decrease in passive stiffness in the cAF myofibrils can be entirely explained by the shift in titin isoform expression. Besides contributing to the altered mechanics of cAF atria, changes in sarcomere diastolic properties likely participate in the progressive atrial dilatation that often accompanies cAF. The contractile alterations found in cAF myofibrils can be only partly explained by the shift in the isoform expression of the cardiac motor protein. Altered phosphorylation of multiple myofilament proteins are likely related to most contractile alterations. Changes associated with increased myofilament Ca^{2+} sensitivity may play a role in the self-perpetuation of cAF.

The observed mechanical changes seem to be a component of cAF rather than atrial dilatation because the atrial size of cAF patients, although relatively large, is not significantly different from that of SR patients (see Online Table I).

Changes in Titin and Diastolic Stiffness

To our knowledge titin isoform expression has never been studied in human atrial myocardium. We find that the N2BA:N2B expression ratio in the atrial myocardium of SR patients is 1.64±0.27, much higher than that reported for ventricular myocardium of normal human hearts (0.56±0.06).[24] Interestingly, in humans as in other big mammals,[16] the expression of N2BA dominates in atrial myocardium. It is an important finding that the N2BA isoform is significantly upregulated at the expense of the N2B isoform in cAF (N2BA:N2B 3.80±0.50). As expression shifts toward the N2BA isoform, because of the different compliance of the 2 titin isoforms, passive myofibril stiffness is expected to decrease. Consistent with this notion, passive stiffness of cAF myofibrils is significantly reduced (on average 2.5-fold) compared to SR myofibrils.

It has been reported that the length dependence of activation of cardiac muscle (that is the mechanism underlying the

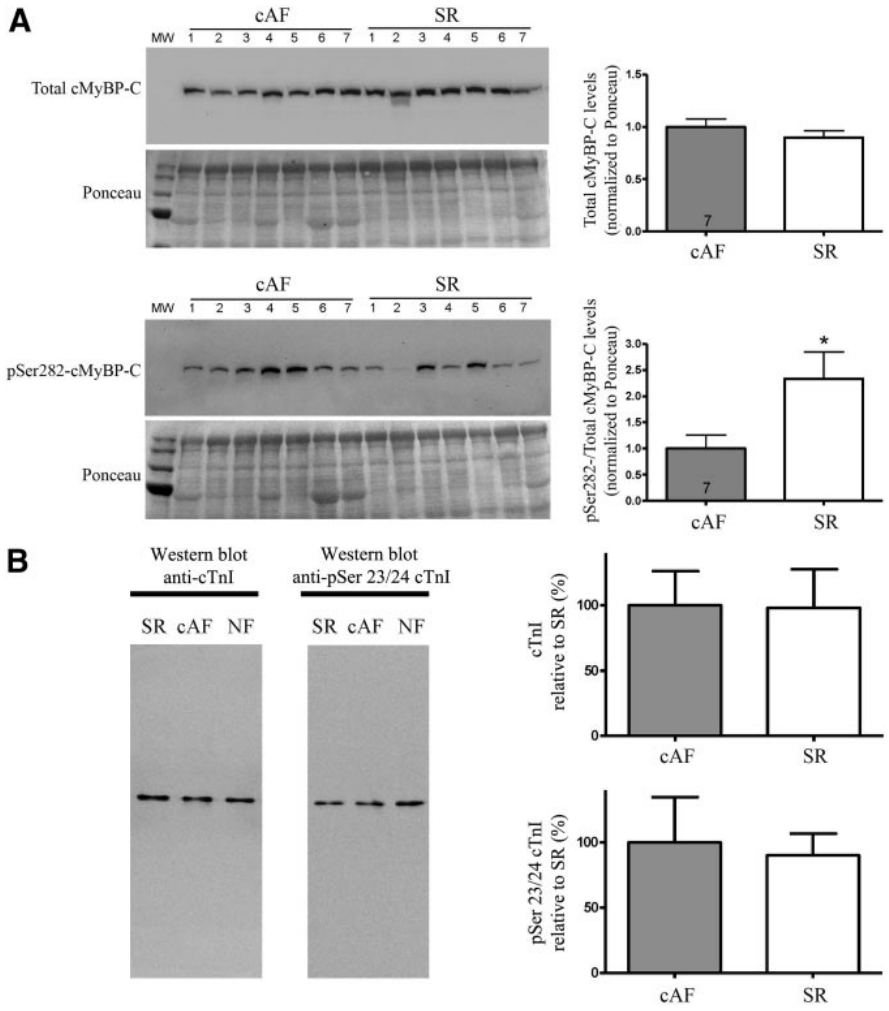

Figure 5. cMyBP-C and cTnI protein content and phosphorylation at PKA sites. Western blot analysis did not reveal reduced content or degradation of cMyBP-C **(A)** and cTnI **(B)**. In accordance with ProQ analysis of protein phosphorylation, cMyBP-C phosphorylation at Ser282 was higher in cAF than in SR **(A)**, whereas phosphorylation at Ser23/24 in cTnI did not differ between SR and cAF. NF indicates nonfailing ventricular donor sample included as positive control **(B)**. Values are given relative to SR, which was set to 100%.

Starling's law of the heart) is titin-based and is reduced in preparations that express high levels of N2BA titin.[25] One can speculate, therefore, that length dependence of activation is reduced in cAF myofibrils because of the increase in N2BA titin. A reduction in the length dependence of activation will contribute to cAF induced atrial contractile dysfunction and possibly to progressive dilation. Progressive atrial dilation in cAF[26] may contribute to self-perpetuation of the arrhythmia.[27] The mechanism behind cAF related increase in atrial size is unclear but increase in N2BA expression and reduction in myofibril resistance to elongation may contribute to it.

Alterations in Contractile Function

The major changes in contractile function of cAF compared to SR myofibrils are (1) a marked reduction in the maximum rate of tension generation, (2) a significant decrease in maximum active tension, and (3) a significant increase in myofilament Ca^{2+} sensitivity.

In both SR and cAF myofibrils, k_{ACT} was the same as k_{TR}. This similarity suggests that k_{ACT} is not limited by the rate with which thin filaments are switched on by Ca^{2+}; rather it predominantly reflects the rate with which crossbridges enter their force generating states. Both k_{ACT} and k_{TR} were markedly reduced (~50%) in cAF compared to SR myofibrils indicating a slower cross-bridge turnover rate. Consistent with the present results, a significant reduction in k_{TR} was previously reported in permeabilized human atrial myocytes from cAF patients.[9] Relaxation kinetics, determined by sudden Ca^{2+} removal from myofibrils, predominantly reflects the apparent rate with which attached crossbridges leave force-generating states.[19] Relaxation rates were slower (25% to 35%) in the cAF myofibrils than in the SR myofibrils implying that both cross-bridge attachment and detachment rates contribute to the slower overall cross-bridge turnover in cAF myofibrils.

Increase in the relative amount of the slow MHC-β isoform expressed in cAF versus SR myofibrils (41% versus 30%), though smaller than in previous reports (63% versus 25%[20]; 41% versus 23%[9]), directly accounts for the reduction in activation and relaxation kinetics of cAF compared to SR myofibrils. The negative impact of the MHC isoform change on the power output and velocity of atrial contraction may contribute to atrial contractile dysfunction in cAF.

Maximal Ca^{2+} activated tension was reduced by ≈30% in cAF compared to SR myofibrils. Similar effect of cAF on maximal active tension (33% reduction) was recently reported for permeabilized human atrial myocytes,[9] though in that study the difference between cAF and SR maximal tension was not significant. A 75% reduction in twitch tension has been reported in intact atrial trabeculae from cAF patients compared to SR patients.[6] In that study, however, following positive inotropic

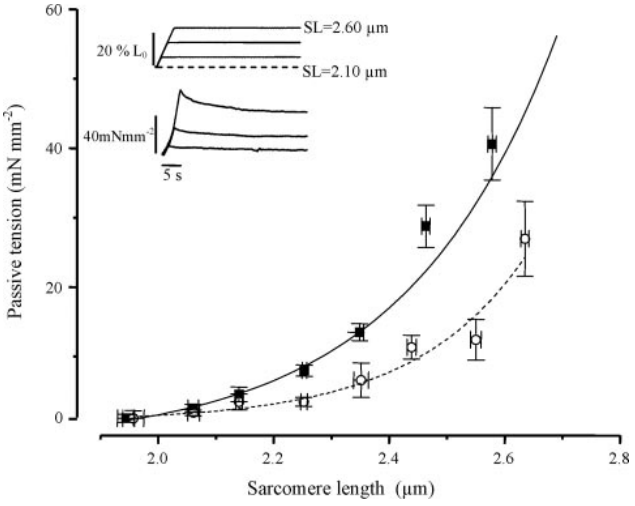

Figure 6. Passive tension in SR and cAF myofibrils. Average sarcomere length–passive tension relationships for SR (■, continuous line) and cAF (○, dashed line) myofibrils. Data are means±SE of 9 to 15 myofibrils from 6 SR and 6 cAF patients. In the **inset**, representative passive tension responses (lower traces) to ramp elongations (upper traces) of SR myofibril in relaxing conditions (pCa 8.0).

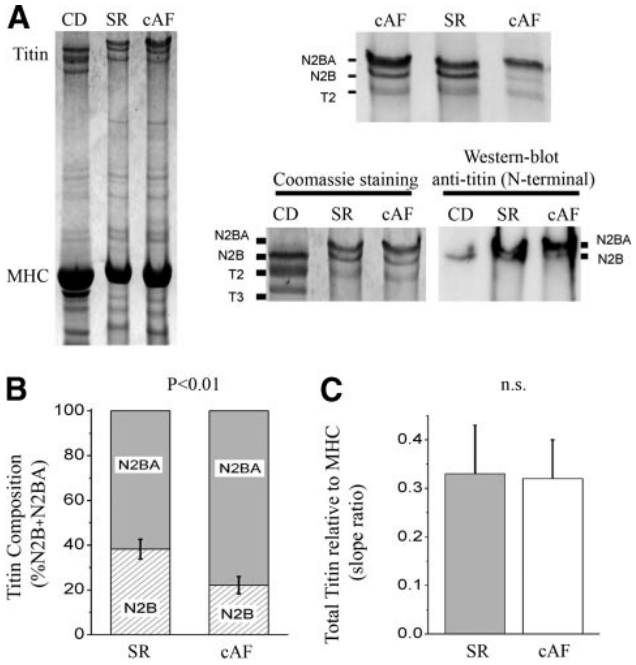

Figure 7. Titin isoform expression in SR and cAF atrial myocardium. A, SDS-PAGE of SR and cAF myocardium. Rat cardiac degraded control (CD) was coelectrophoresed for isoform reference. **Top right,** Expanded titin region with evidence that degradation product T2 is hardly detectable and T3 undetectable in the human atrial samples. **Bottom right,** Western blot using the antibody Z1/Z2 for isoform band confirmation. **B,** Titin isoform percent distribution in SR and cAF (means±SE, n=6, $P<0.01$). **C,** Total titin content, expressed relatively to MHC in SR and cAF atrial tissue (means±SE, n=6, $P>0.9$).

interventions (high extracellular [Ca^{2+}], postrest potentiation) the difference in twitch tension decreased to ≈15% consistent with the AF-induced reduction in myofibril density per unit cross sectional area of the trabeculae (-14%). Those results suggested that most of the atrial contractile dysfunction of cAF is associated with impaired Ca^{2+} handling whereas the loss of atrial myofibrils plays a minor role. Here we directly determined the maximal tension of isolated myofibrils and eliminate contamination by either AF-induced excitation–contraction coupling dysfunction or myofibrillar loss. Thus, in cAF myofibrils reduction in maximal active tension must be attributable to defects in the myofilament themselves.

The shift in MHC expression toward the slow β isoform in the myofibrils of AF patients is an unlikely explanation for reduction in maximal tension of cAF myofibrils; maximal tension does not differ in human atrial and ventricular myofibrils that express quite different patterns of MHC isoforms.[12] Though maximal active tension loss of cAF myofibrils may come from multiple sources, present results support a role for altered phosphorylation of myofilament proteins (see below).

We found evidence that regulation of contraction at the myofilament level is also altered in cAF. pCa_{50} of active tension was significantly increased, whereas the Hill coefficient (n_H) was decreased in cAF myofibrils compared to SR myofibrils. No significant change in Ca^{2+} sensitivity of tension development has been previously reported for permeabilized human cAF preparations,[8,9] though a significant increase in the Ca^{2+} sensitivity of ATP consumption under isometric conditions has been found in the same cAF preparations.[8] This latter observation seems consistent with the present results, although a discrepancy between the Ca^{2+} sensitivity of force and isometric ATPase is difficult to explain.

Several mechanisms, mostly leading to changes in regulatory proteins associated with thin and thick filaments, can impact the pCa–tension relationship of atrial myofibrils. cAF is accompanied by re-expression of fetal genes in the atria.[3,4] The predominant TnI isoform expressed by the fetal human heart is the slow skeletal (ssTnI) isoform[28,29]; this imparts higher Ca^{2+} sensitivity to cardiac sarcomere.[30] Although re-expression of ssTnI has been described in the early stages of AF in human atria and in a goat model of the disease, ssTnI was undetectable in atrial samples from patients with cAF.[31] A relation has been found in human atria between cAF and the activity of calpain-1,[32] a protease involved in degradation of contractile proteins,[33] including cTn.[34] Degradation of cTn by calpain has been reported to occur in both human cAF and a cell model for tachypacing-induced remodeling.[11] The predominant cTnI degradation product ($cTnI_{1\ to\ 192}$) has been shown to significantly increase Ca^{2+} sensitivity of human cardiac myofibrils.[14] However, in the present and previous studies[9,31] no changes in troponin content were observed and cTnI degradation products were undetectable in atrial samples from cAF patients.

The most likely explanation for the increased Ca^{2+} sensitivity and reduced maximal force resides in the complex changes in myofilament protein phosphorylation found in cAF versus SR samples. As previously reported,[9,10] phosphorylation of cTnI—the primary sarcomere target of PKA—was the same in cAF and SR atria. Our analysis revealed, instead, significantly higher phosphorylation of other myofilament proteins in cAF. CaMKII (Ca^{2+}/calmodulin-dependent protein kinase II) is overexpressed in human cAF[35] and this may explain why cMyBP-C phosphorylation is increased because Ser282 is both a PKA and CamKII site. Increased phosphorylation of cMyBP-C in the absence of

increased PKA phosphorylation of cTnI may increase myofibril Ca^{2+} sensitivity,[36] though the specific effect of cMyBP-C phosphorylation on Ca^{2+} sensitivity of tension is at present unresolved.[37] Increased atrial light chain-2 phosphorylation by myosin light chain kinase or PKC has been associated with increased myofilament Ca^{2+} sensitivity,[38,39] whereas increased cTnT phosphorylation by PKC has been implicated in reduced maximal force generating capacity.[40] As conclusion, present results suggest that the increased Ca^{2+}-responsiveness and the lower maximal tension of cAF myofibrils cannot be explained by contractile protein degradation or isoform shifts but result from the complex interplay between changes in the phosphorylation status of multiple myofilament proteins.

The fact that phosphorylation of multiple myofilament proteins is increased favors a role for altered phosphatase rather than kinase activity, which would result in more specific protein phosphorylation changes. A role for altered phosphatase activity in cAF has been suggested in a previous study[10] and warrants further investigation of protein phosphatases and their cellular compartmentalization.

In intact cAF myocardium, increased myofilament Ca^{2+} sensitivity may partly counteract the decrease in active force generation and impaired excitation–contraction coupling but may have detrimental effects on relaxation. Moreover, increase in Ca^{2+} sensitivity of cAF myofibrils may contribute to electric remodeling and self-perpetuation of atrial arrhythmia. Because Ca^{2+} binding to the troponin complex represents the largest component of dynamic Ca^{2+} buffering during the cardiac cycle, intracellular Ca^{2+} transients may significantly change with altered myofilament Ca^{2+} sensitivity.[41,42] This, in turn, may lead to action potential remodeling and altered intracellular Ca^{2+} handling to create both an arrhythmogenic substrate and a trigger in atrial myocardium. In isolated mouse heart myofilament Ca^{2+}-sensitization by drugs or troponin mutations associated with familial hypertrophic cardiomyopathy are reported to induce arrhythmias by (1) shortening the effective refractory period and the action potential duration, (2) slowing conduction velocity, and (3) predisposing to early after-depolarizations and triggered activity.[43]

Clinical Implications

This study provides new insights into the alterations of myofilament function in cAF that may help us understand persistent atrial contractile dysfunction, a major contributor to atrial thrombogenesis. In cAF remodeled sarcomeres, we identified altered titin isoform expression and increased myofilament Ca^{2+} sensitivity as contributors to progressive and self-perpetuating arrhythmia. If mechanoelectric feedback between increased myofilament Ca^{2+} sensitivity and increased propensity for arrhythmias will be definitely established, restoration of sarcomere Ca^{2+} sensitivity (likely via restoration of myofilament protein phosphorylation levels) may become a novel therapeutic option for AF treatment.

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Disclosures

None.

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Novelty and Significance

What Is Known?

- Chronic atrial fibrillation (cAF) is associated with persistent atrial contractile dysfunction, a major contributor to atrial thrombogenesis.
- Mechanisms responsible for impaired contractility are poorly defined and available therapies do not address this dysfunction.
- Most studies focus on alterations in atrial myocyte Ca^{2+} handling, but we and others emphasize the role of myofilament protein remodeling.

What New Information Does This Article Contribute?

- We demonstrate that diastolic and systolic sarcomere mechanics and myofilament Ca^{2+} sensitivity are altered in atrial myofibrils from cAF patients.
- These mechanical changes are explained by shifts in protein isoforms and by increased phosphorylation of multiple myofilament proteins.
- Myofilament remodeling is part of atrial contractile dysfunction in human cAF and probably contributes to the progressive and self-perpetuating nature of the arrhythmia.

It is hypothesized that maladaptive remodeling of the myofilaments is responsible, at least in part, for human cAF-associated atrial contractile dysfunction. To document the functional impact of myofilament protein changes in human cAF we dissected the sarcomere diastolic and systolic properties of single atrial myofibrils from surgical samples of cAF and control patients. cAF myofibrils show (1) a reduction in diastolic stiffness, (2) a reduction in maximum active tension and in the rates of contraction and relaxation, and (3) an increase in myofilament Ca^{2+} sensitivity. These mechanical changes are associated with changes in myofilament proteins. In cAF remodeled sarcomeres we identify altered titin and myosin isoform expression and increased levels of phosphorylation of multiple proteins. The latter finding suggests that altered phosphatase activity leads to increased Ca^{2+} sensitivity that, in turn, may contribute to the self-perpetuation of the atrial arrhythmia. These results show that translational and post-translational changes in myofilament proteins play a direct role in the altered atrial mechanics associated with cAF and contribute to the progression of the arrhythmia. Restoration of sarcomere Ca^{2+} sensitivity (likely via restoration of myofilament protein phosphorylation levels) may become a novel therapeutic option for AF treatment.