Cellular Mechanisms of Depressed Atrial Contractility in Patients With Chronic Atrial Fibrillation

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Background—After cardioversion of atrial fibrillation (AF), the contractile function of the atria is temporarily impaired. Although this has significant clinical implications, the underlying cellular mechanisms are poorly understood.

Methods and Results—Forty-nine consecutive patients submitted for mitral valve surgery were investigated. Twenty-three were in persistent AF (≥3 months); the others were in sinus rhythm. Before extracorporal circulation, the right atrial appendage was excised. β-Adrenoceptors were quantified by radioligand binding, and G proteins were quantified by Western blot analysis. The isometric contractile response to Ca\(^{2+}\), isoproterenol, Bay K8644, and the postrest potentiation of contractile force were investigated in thin atrial trabeculae, which were also examined histologically. The contractile force of the atrial preparations obtained from AF patients was 75% less than that in preparations from patients in sinus rhythm. Also, the positive inotropic effect of isoproterenol was impaired, and Bay K8644 failed to increase atrial contractile force. In contrast, the response to extracellular Ca\(^{2+}\) was maintained, and the postrest potentiation was preserved. β-Adrenoceptor density and G-protein expression were unchanged. Histological examination revealed 14% more myolysis in the atria of AF patients.

Conclusions—After prolonged AF, atrial contractility was reduced by 75%. The impairment of β-adrenergic modulation of contractile force cannot be explained by downregulation of β-adrenoceptors or changes in G proteins. Dysfunction of the sarcoplasmic reticulum does not occur after prolonged AF. Failure of Bay K8644 to restore contractility suggests that the L-type Ca\(^{2+}\) channel is responsible for the contractile dysfunction. The restoration of contractile force by high extracellular Ca\(^{2+}\) shows that the contractile apparatus itself is nearly completely preserved after prolonged AF.

Key Words: arrhythmia ■ contractility ■ receptors, adrenergic, beta ■ remodeling ■ signal transduction.

Contractile dysfunction of the atria after cardioversion of atrial fibrillation (AF) has been recognized for >30 years.\(^1\) Echo-cardiographic studies showed that the transmitial blood flow during atrial contraction was markedly reduced after restoration of sinus rhythm (SR).\(^2\) The degree of atrial contractile dysfunction and the time required for restoration of normal atrial transport function depend on the duration of AF.\(^2\) This temporary loss of atrial contraction may lead to thromboembolic events even when atrial thrombi are not present at the time of cardioversion.\(^4\)

The cellular mechanisms responsible for AF-induced contractile dysfunction are still poorly understood. First, it has been thought that the electric energy applied during DC cardioversion caused “atrial stunning.”\(^5\) However, later studies have shown that contraction of the atria was also impaired after pharmacological\(^6\) and spontaneous\(^7\) cardioversion. Sustained AF has been shown to cause alterations in atrial cellular ultrastructure. Myolysis and fragmentation of the sarcoplasmic reticulum might explain the contractile dysfunction in remodeled atria.\(^6\) Prolonged rapid atrial rhythms have also been shown to cause a pronounced reduction in the L-type Ca\(^{2+}\) current.\(^9\)–\(^11\) This offers an alternative explanation for the loss of contractile force in the course of prolonged AF. Tachycardia-induced changes of intracellular Ca\(^{2+}\) handling have been studied by Sun et al\(^12\) in canine atrial cardiomyocytes. They found that both contractility and intracellular Ca\(^{2+}\) transients were markedly reduced.

In the present study, we investigated the cellular mechanisms of postfibrillatory atrial contractile dysfunction in humans. In atrial trabeculae isolated from patients with and without AF, the β-adrenergic response, the positive inotropic effect of Ca\(^{2+}\) and Bay K8644, and the postrest potentiation were evaluated. In addition, the most important proteins involved in the β-adrenergic signal transduction pathway were biochemically determined.

Methods

Patients

Right atrial appendages were obtained from 49 patients undergoing mitral valve surgery (Table 1). Twenty-three patients were in chronic AF (≥3 months); the others were in SR. Despite a tendency for a
lower cardiac index and a higher wedge pressure in AF patients, hemodynamics did not differ significantly between the 2 groups. AF patients more often received Ca²⁺ antagonists and digitals for control of their ventricular rate. The patients did not receive drugs at least 12 hours before surgery. All patients gave written informed consent, and the study was approved by an institutional review committee.

**Contractility Studies**

Immediately after surgical excision, right atrial appendages were placed into Tyrode’s solution (pH 7.4, gassed with 5% CO₂/95% O₂). Thin myocardial muscle bundles were prepared in parallel with the muscle fiber direction under stereomicroscopic control. The length of the bundles ranged between 3 and 6 mm, and the diameters were quantified in a nitrocellulose membranes were cut, and single band signals were quantified in a γ-counter. CGP 12177 (1 μmol/L) was used to determine nonspecific binding. Protein content of the membrane preparations was measured according to the method of Bradford.¹⁴

**Western Blot Analysis**

To quantify G proteins, electrophoresis of homogenate aliquots containing 80 μg protein per lane was carried out with the use of 10% polyacrylamide gels. After they were tank-blotted, the nitrocellulose membranes were exposed to primary antibody (antisera AS7 [inhibitory G protein] or RM1 [stimulatory G-protein], NEN Life Sciences). After they were labeled with 125I-protein A, the nitrocellulose membranes were cut, and single band signals were quantified in a γ-counter.

**Statistical Analysis**

Data are expressed as mean±SEM. For EC₅₀ values, 95% CIs are given. Statistical significance was determined with the unpaired Student t test or by 1-way ANOVA for comparison of multiple groups. Significance of differences in medication or sex were calculated by χ² test. A value P<0.05 was considered to be statistically significant.

**Results**

Representative examples of longitudinal and transverse sections of atrial muscle preparations are shown in Figure 1. In SR and AF patients, marked myolytic alterations and pronounced interstitial fibrosis were present. However, the degree of myolysis was more pronounced in chronically fibrillating atria. Also, the cell size was larger in AF patients, with the average transverse cell diameter being 11.2±0.4 μm in SR patients and 17.3±0.6 μm in AF patients (+55%, P<0.01).

In all bundles, the sarcomere content was 14% lower in AF patients compared with SR patients (Figure 1, Table 2). The amount of noncontractile material (mostly glycogen) was nearly 2 times higher in AF patients. The extracellular matrix constituted ≈40% of the muscle bundles in both groups. This relatively high amount of extracellular matrix is consistent with changes described as the result of mitral valve or rheumatic heart disease.¹⁵ In the additional group of patients with coronary heart disease, the amount extracellular
matrix was less (Table 2). However, the differences between coronary artery disease patients in SR compared with those in AF were similar to the observations in patients with mitral valve disease.

**Contractility**

Single contractions at baseline conditions (37°C, 1 Hz, and 2.5 mmol/L Ca\(^{2+}\)) of all muscle preparations are superimposed in Figure 2. In trabeculae isolated from patients in SR, the strength (FC) was 11.5 mN/mm\(^2\) (n=55, range 4.1 to 21.9 mN/mm\(^2\)). In contrast, in muscle bundles of AF patients, FC was only 3.1 mN/mm\(^2\) (n=51, range 0.9 to 7.4 mN/mm\(^2\), \(P<0.01\)). Thus, in the atrial myocardium of AF patients, baseline contractility was reduced by \(\approx75\%\). Increasing the extracellular Ca\(^{2+}\) concentration increased FC in SR and AF patients (Figure 3). However, the relative increase was more pronounced in the AF group. As a result, at Ca\(^{2+}\) concentrations \(\geq7.4\) mmol/L, FC was no longer significantly lower than in the SR group (but still slightly below the force in the SR group). Thus, high extracellular Ca\(^{2+}\) concentrations nearly overcame the contractile dysfunction in muscle preparations of AF patients. In 31 bundles of 19 SR patients and 30 bundles of 15 AF patients, maximal FC (F\(_{\text{max}}\)) was directly compared with the differences in sarcomere content. In AF patients, the reduction in sarcomere content (\(-14\%\)) was similar to the slight decrease in F\(_{\text{max}}\) (\(-15\%\)), although the
The β-adrenoceptor agonist isoproterenol exerted a pronounced positive inotropic effect in the SR and AF groups (Figure 4A). However, in AF patients, the concentration-response curve was shifted downward and to the right. FC at the maximal isoproterenol concentration (10^−6 mol/L) was lower in the AF group (18.3±1.8 versus 30.0±1.3 mN/mm², P<0.01). In the SR group, the half-maximal positive inotropic effect of isoproterenol was reached at 2.6 (1.8 to 4.4) nmol/L (EC50). A nearly 10-fold higher isoproterenol concentration was needed in the AF group to elicit the half-maximal response (EC50 22 [14 to 41] nmol/L, P<0.01). Thus, the positive inotropic potency of isoproterenol was markedly reduced in atrial muscle preparations from AF patients. Neither in the SR patients nor in the AF patients did β-blocker therapy alter the maximal response to isoproterenol (for SR patients, 32.2±4.3 mN/mm² [n=4, with β-blocker] versus 29.2±1.9 mN/mm² [n=12, no β-blocker], P=NS; for AF patients, 20.1±2.1 mN/mm² [n=4, with β-blocker] versus 17.4±1.8 mN/mm² [n=9, no β-blocker], P=NS). Similarly, the potency of isoproterenol (EC50) was not changed.

β-Adrenergic Signaling

[^3H]Iodoscyanopindolol binding experiments on atrial membrane preparations showed saturation characteristics in SR and in AF patients (Figure 4B). The β-adrenoceptor density was 58±5 fmol/mg in the SR group (n=11) and 54±5 fmol/mg in the AF group (n=10) (P=NS). Thus, compared with the right atrial myocardium of 6 healthy donor hearts (81±7 fmol/mg), right atrial β-adrenoceptor density was reduced to a similar extent in mitral valve disease patients in SR or AF.

Figure 4C shows representative immunoblots of the stimulatory G protein. The antiserum AS/7 bound specifically to a single band at 40 kDa, which represents the Gα5 subunit. RM/1 was bound to a 45-kDa and a 52-kDa protein representing 2 splicing variants of the stimulatory G-protein α subunit. Neither the level of the stimulatory G protein (12 719±838 cpm/mg in 14 SR patients versus 14 676±1243 cpm/mg in 12 AF patients, P=NS) nor the level of the inhibitory G protein (3951±300 cpm/mg in 14 SR patients versus 3476±309 cpm/mg in 12 AF patients, P=NS) was affected by AF.

Loss of Effect of Bay K8644

The positive inotropic effect of Bay K8644 is shown in Figure 5. In the SR group, the Ca^2+ channel agonist exerted a pronounced positive inotropic effect. At 10−5 mol/L Bay K8644, FC was comparable to FC at 10.8 mmol/L extracellular Ca^2+. In contrast, the response to Bay K8644 was abolished in the AF group, although these muscle preparations did respond to an increase in extracellular Ca^2+ concentration. At the highest concentration of Bay K8644 (10−3 mol/L), FC was 4.5±1.1 mN/mm² compared with 28.0±2.6 mN/mm² at 10.8 mmol/L Ca^2+ (P<0.01). Treatment with Ca^2+ antagonists did not change the response to Bay K8644 in the AF patients. In the treated patients, FC at 10−5 mol/L Bay K8644 was 4.7±1.3 mN/mm² (n=7) versus 4.2±1.3 mN/mm² (n=5) in the patients who were not treated with Ca^2+ antagonists (P=NS). Digitalis did also not affect the response to Bay K8644.

Preserved Postrest Potentiation of FC

Figure 6 shows representative experiments on the postrest potentiation of FC (rest interval 10 seconds). FC of the first postrest twitch was markedly increased in the SR and the AF patients. Compared with the force amplitude at steady-state conditions, the postrest FC was equally enhanced in both patient groups (4.1±1.0 mN/mm² [SR, n=16] versus 3.9±1.1 mN/mm² [AF, n=16], P=NS).

**TABLE 2. Relative Tissue Composition of Right Atrial Muscle Bundles**

<table>
<thead>
<tr>
<th>Muscle preparations/patients, n/h</th>
<th>Mitral Valve Disease</th>
<th>Coronary Artery Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SR</td>
<td>AF</td>
</tr>
<tr>
<td>Cardiomyocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sarcomeres</td>
<td>50.4±2.0</td>
<td>43.5±2.0*</td>
</tr>
<tr>
<td>Intracellular noncontractile material</td>
<td>6.8±0.3</td>
<td>11.7±0.3*</td>
</tr>
<tr>
<td>Nuclei</td>
<td>1.2±0.1</td>
<td>1.1±0.1</td>
</tr>
<tr>
<td>Extracellular space</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extracellular matrix including fibroblasts</td>
<td>38.7±2.0</td>
<td>41.1±1.9</td>
</tr>
<tr>
<td>Vessels including perivascular cells</td>
<td>2.9±0.2</td>
<td>2.6±0.2</td>
</tr>
</tbody>
</table>

Values are mean±SEM (percentage of total tissue volume).
*P<0.05 for AF vs SR; †P<0.05 for coronary artery disease vs mitral valve disease.

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Figure 2. Superimposed force recordings of atrial muscle preparations from 25 SR patients (55 preparations) and from 22 AF patients (51 preparations) recorded under isometric conditions (37°C, 1 Hz).
The present study provides the first insight into the cellular mechanisms of the postfibrillatory atrial contractile dysfunction in humans. It demonstrates that the contractility of atrial muscle bundles isolated from AF patients is reduced by ~75%. The data confirm previous observations in a dog model of tachycardia-induced atrial contractile dysfunction in which the systolic shortening of atrial cardiomyocytes was found to be reduced to a similar extent. Downregulation and/or altered function of the L-type Ca\(^{2+}\) channels seems to underlie AF-induced atrial dysfunction.

**Discussion**

The present study provides the first insight into the cellular mechanisms of the postfibrillatory atrial contractile dysfunction in humans. It demonstrates that the contractility of atrial muscle bundles isolated from AF patients is reduced by ~75%. The data confirm previous observations in a dog model of tachycardia-induced atrial contractile dysfunction in which the systolic shortening of atrial cardiomyocytes was found to be reduced to a similar extent. Downregulation and/or altered function of the L-type Ca\(^{2+}\) channels seems to underlie AF-induced atrial dysfunction.
Myolysis and AF-Induced Loss of Atrial Contractility

In patients with chronic AF, recovery of atrial contractile function after cardioversion may require several weeks or months. Restoration of the cellular (ultra)structure might explain such a long recovery of contractile function. Indeed, the present study shows that myolysis and replacement of sarcomeres by glycogen was more pronounced in the trabeculae of AF patients. The same alterations have previously been reported in the goat model of AF, and it has been concluded that the loss of sarcomeres might contribute to the delayed recovery of atrial function after conversion to SR. However, in the latter study, a direct relation between structural changes and the contractile function of the atrial myocardium was not investigated. In the present study, the loss of sarcomeres was compared with the maximal achievable FC in the same muscle preparations to evaluate the contribution of structural remodeling to the AF-induced atrial contractile dysfunction. At high extracellular Ca²⁺ concentrations, atrial FC was only slightly reduced in the AF group (∼15%). This moderate reduction in maximal FC corresponded to a similar reduction in sarcomere content (∼14%). There was a strong correlation between the maximal FC and the sarcomere content of the individual muscle preparations. Therefore, AF-induced myolysis can only partly explain the pronounced reduction of contractility in remodeled atria. The main mechanism of postcardioversion atrial contractile dysfunction is a disturbed activation rather than a loss of atrial myofilaments.

β-Adrenergic Signaling

An important mechanism controlling myocardial force in atrial and ventricular myocardium is the β-adrenergic signal transduction pathway. Our data demonstrate that in atrial myocardium of AF patients, the positive inotropic effect of isoproterenol was markedly reduced. Although the impaired β-adrenergic response cannot explain the reduced atrial contractility in vitro, it might contribute to the atrial contractile dysfunction in vivo by blunting the positive inotropic response to the physiological variations in sympathetic tone.

In human failing ventricular myocardium and in a canine model of pacing-induced heart failure, desensitization toward catecholamines has been shown to be due to a reduced density of the β-adrenoceptors and an upregulation of the inhibitory G protein. In contrast, in the present study, atrial β-adrenoceptor density and G-protein levels were not altered in AF patients. This indicates that the atrial contractile dysfunction after the cessation of AF is due to mechanisms different from the ventricular cardiomyopathy in heart failure or experimental tachycardia-induced cardiomyopathy on the ventricular level.

Role of the L-Type Ca²⁺ Channel

Because the positive inotropic effect of catecholamines is mainly due to activation of the L-type Ca²⁺ channel, we tested the hypothesis that alterations of the Ca²⁺ channels were responsible for the diminished isoproterenol effect. A reduction in Ca²⁺ current has been shown to be the most important ionic mechanism of AF-induced electrical remodeling. Several studies have demonstrated a downregulation of L-type Ca²⁺ channel subunits in AF. In our present study, the L-type Ca²⁺ channel agonist Bay K8644 failed to increase atrial FC in AF patients. Potential explanations include changes in the number and/or function of the L-type Ca²⁺ channels. Downregulation of channel subunits is expected to reduce the positive inotropic effect of the Ca²⁺ channel agonist and might have caused the lack of response to Bay K8644. Altered function of the channel due to changes of the phosphorylation state is a second possibility to explain the loss of effect of Bay K8644. In failing ventricular myocardium, the spatial relationship between the L-type Ca²⁺ channel and the Ca²⁺-release channel of the sarcoplasmic reticulum is disturbed. This would provide a third explanation for the absence of the Bay K8644 effect. The latter possibility would also explain why the positive inotropic effect of Bay K8644 is abolished in atrial myocardium of AF patients, whereas Bay K8644 could partly overcome the decrease of the Ca²⁺ inward current in the atrial cells of dogs undergoing rapid atrial pacing.
The blunted response to Bay K8644 not only emphasizes that a change in the function and/or number of the L-type Ca\(^{2+}\) channels is the key to understanding post-AF atrial contractile dysfunction, it also explains why the positive inotropic effect of isoproterenol was reduced in AF patients, although \(\beta\)-adrenoceptor density and G-protein levels were unaltered. Isoproterenol increases FC by increasing the Ca\(^{2+}\) inward current and by enhancing the Ca\(^{2+}\) reuptake by the Ca\(^{2+}\)-ATPase of the sarcoplasmic reticulum (SERCA). As a result of both effects, the Ca\(^{2+}\) load of the sarcoplasmic reticulum is increased. In atrial myocardium of AF patients, the inotropic effect of isoproterenol was reduced, which can be explained by the above-mentioned changes of the L-type Ca\(^{2+}\) channel. Interestingly, isoproterenol still elicits some positive inotropic effect in the atrial myocardium of AF patients. This remaining stimulatory effect is most likely due to stimulation of the Ca\(^{2+}\) reuptake into the sarcoplasmic reticulum by SERCA, which would be consistent with our hypothesis that the function of the sarcoplasmic reticulum is preserved in the atrial myocardium of AF patients (see below). In contrast, Bay K8644, acting purely on the L-type Ca\(^{2+}\) channel, lacks this alternative positive inotropic mechanism and becomes ineffective in the atrial myocardium of AF patients.

**No Dysfunction of the Sarcoplasmic Reticulum**

As reported in human failing ventricular myocardium and in experimental tachycardia-induced heart failure,\(^1\)\(^2\) dysfunction of the sarcoplasmic reticulum might also contribute to the reduced atrial contractility after the cessation of AF. Postrest potentiation of contractile force critically depends on the ability of the sarcoplasmic reticulum to store and release Ca\(^{2+}\) and reflects the functional capacity of the sarcoplasmic reticulum.\(^2^5\) In atrial myocardium of AF patients, the postrest potentiation was fully preserved, indicating that in fibrillating atria, FC is not limited by the capacity of the sarcoplasmic reticulum to release and take up Ca\(^{2+}\) again. This observation is in accordance with the finding of Sun et al,\(^1^2\) who reported that the postrest potentiation was still present in atrial cardiomyocytes from dogs with tachycardia-induced atrial contractile dysfunction.

**Limitations of the Study**

Although the present study has demonstrated that in atrial muscle bundles from AF patients, contractility is reduced by 75%, a causative relationship between AF and the atrial contractile dysfunction has not been studied. We cannot exclude the possibility that a common underlying factor causes both the atrial contractile dysfunction and AF. However, the L-type Ca\(^{2+}\) current is the main determinant of contractile force and becomes downregulated by AF. Therefore, it is reasonable to assume that electrical remodeling goes hand in hand with the atrial contractile dysfunction. This hypothesis is supported by the study of Sun et al,\(^1^2\) who showed that in dogs undergoing rapid atrial pacing, the decrease in atrial contractility follows a time course similar to the shortening of the action potential duration and the decrease of the Ca\(^{2+}\) inward current.

In all patients, the atria showed histological changes that were probably due to the underlying structural heart disease. Although the structural differences in atria from patients in AF and SR were similar to the changes found in the goat model of AF (resembling the situation in lone AF), extrapolation to AF in less diseased hearts should be performed with great caution. Further studies on atrial preparations from patients with lone AF would certainly be worthwhile.

A further limitation concerns the heterogeneity of our study population. Digitalis and Ca\(^{2+}\) antagonists were more frequently taken by the AF patients, and some patients from our study population were on \(\beta\)-blockers. The subgroup analysis revealed that drug treatment did not change contractile force and the response to positive inotropes in the atrial muscle preparations. Thus, although we cannot exclude the possibility that differences in medication might have influenced the contractile behavior of the muscle preparations, the marked differences in atrial contractility between SR and AF patients could not be explained by differences in drug therapy.

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**References**


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