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ARTICLE *in* JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY · APRIL 1999

Impact Factor: 16.5 · DOI: 10.1016/S0735-1097(99)00008-X

Journal of the American College of Cardiology
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Published by Elsevier Science Inc.

Vol. 33, No. 5, 1999
ISSN 0735-1097/99/$20.00
PII S0735-1097(99)00008-X

Down-Regulation of L-Type Calcium Channel and Sarcoplasmic Reticular Ca^{2+}-ATPase mRNA in Human Atrial Fibrillation Without Significant Change in the mRNA of Ryanodine Receptor, Calsequestrin and Phospholamban

An Insight Into the Mechanism of Atrial Electrical Remodeling

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OBJECTIVES We investigated the gene expression of calcium-handling genes including L-type calcium channel, sarcoplasmic reticular calcium adenosine triphosphatase (Ca^{2+}-ATPase), ryanodine receptor, calsequestrin and phospholamban in human atrial fibrillation.

BACKGROUND Recent studies have demonstrated that atrial electrical remodeling in atrial fibrillation is associated with intracellular calcium overload. However, the changes of calcium-handling proteins remain unclear.

METHODS A total of 34 patients undergoing open heart surgery were included. Atrial tissue was obtained from the right atrial free wall, right atrial appendage, left atrial free wall and left atrial appendage, respectively. The messenger ribonucleic acid (mRNA) amount of the genes was measured by reverse transcription–polymerase chain reaction and normalized to the mRNA levels of glyceraldehyde 3-phosphate dehydrogenase.

RESULTS The mRNA of L-type calcium channel and of Ca^{2+}-ATPase was significantly decreased in patients with persistent atrial fibrillation for more than 3 months (0.36 ± 0.26 vs. 0.90 ± 0.88 for L-type calcium channel; 0.69 ± 0.42 vs. 1.21 ± 0.68 for Ca^{2+}-ATPase; both $p < 0.05$, all data in arbitrary unit). We further demonstrated that there was no spatial dispersion of the gene expression among the four atrial tissue sampling sites. Age, gender and underlying cardiac disease had no significant effects on the gene expression. In contrast, the mRNA levels of ryanodine receptor, calsequestrin and phospholamban showed no significant change in atrial fibrillation.

CONCLUSIONS L-type calcium channel and the sarcoplasmic reticular Ca^{2+}-ATPase gene were down-regulated in atrial fibrillation. These changes may be a consequence of, as well as a contributory factor for, atrial fibrillation. (J Am Coll Cardiol 1999;33:1231–7) © 1999 by the American College of Cardiology

Atrial fibrillation is the most common tachyarrhythmia in humans. It causes palpitations, decreased cardiac output, heart failure and systemic thromboembolism, and is associated with a significant morbidity and mortality (1–3). Current treatment modalities for atrial fibrillation are far from satisfactory. Despite aggressive treatment, the recurrence rate of atrial fibrillation is still high, and many patients finally develop chronic persistent atrial fibrillation refractory to any treatment including electrical cardioversion. There is evidence showing that atrial fibrillation begets atrial fibrillation, and through this vicious cycle atrial fibrillation becomes incessant (4). This phenomenon was thought to be due to atrial electrical remodeling, which results in progressive shortening of the atrial refractory period after rapid atrial depolarization (5–7). This is an important contributing factor for the maintenance of atrial fibrillation.

The underlying mechanism of atrial electrophysiologic remodeling remains unknown and has been the focus of

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Manuscript received July 7, 1998; revised manuscript received November 18, 1998, accepted December 24, 1998.

JACC Vol. 33, No. 5, 1999
April 1999:1231–7

Abbreviations and Acronyms

Ca^{2+}-ATPase	=	calcium adenosine triphosphatase
cDNA	=	complementary deoxyribonucleic acid
GAPDH	=	glyceraldehyde 3-phosphate dehydrogenase
I_{Ca}	=	ionized calcium
mRNA	=	messenger ribonucleic acid

many studies (5–11). There has been evidence showing that intracellular calcium overload plays an important role in atrial electrical remodeling. Morillo and coworkers investigated the mechanism of atrial remodeling using a rapid atrial pacing canine model (8). They showed that the atrial effective refractory period shortened after artificial pacing, and the atrial tissue showed sarcoplasmic reticulum disruption and mitochondria swelling under electron microscope after rapid atrial pacing for 6 weeks. Similar findings have been reported by Ausma et al. in goats (9). These findings are suggestive of intracellular calcium overload. Subsequent studies further proved the effectiveness of L-type calcium channel blocking agents in preventing electrical remodeling and mitochondria swelling in a rapid atrial pacing canine model (11–13).

All the above studies showed the important role of calcium homeostasis in atrial electrical remodeling. The cytosolic calcium homeostasis in myocytes involves the influx of extracellular calcium through L-type calcium channels and the release, uptake and storage of the sarcoplasmic reticulum calcium store. Therefore, we propose to investigate the gene expression changes of these calcium-handling proteins in atrial tissue from patients with atrial fibrillation. These proteins include L-type calcium channel, ryanodine receptor, calcium adenosine triphosphatase (Ca^{2+}-ATPase), calsequestrin and phospholamban.

METHODS

Patients. A total of 34 patients undergoing open heart surgery were included. There were 15 men and 19 women with a mean age of 55 ± 14 years (range 17 to 77). Atrial fibrillation was present in 26 of the patients and absent in eight. The underlying heart disease was rheumatic heart disease in 23 and nonrheumatic heart disease in the remaining 11. The clinical characteristics are shown in Table 1. A piece of atrial tissue of 0.1 to 0.5 g was obtained during the surgery from the right atrial appendage, the right atrial free wall, the left atrial appendage and the left atrial free wall, respectively. Since a left atrial tissue sampling was necessary, only surgical procedures involving a left atriotomy were included.

Clinical parameters. The onset and duration of atrial fibrillation were obtained by history taking and serial electrocardiograms. We scored atrial fibrillation by the following system: 0 = no atrial fibrillation, 1 = paroxysmal atrial fibrillation, 2 = persistent atrial fibrillation for less than 3 months, 3 = persistent atrial fibrillation for more than 3 months. The right atrial pressure and pulmonary wedge pressure were recorded during cardiac catheterization and represented the right and left atrial filling pressure, respectively. Use of calcium channel blockers was defined as long-term use of verapamil for the control of ventricular rate in atrial fibrillation.

Ribonucleic acid extraction and reverse transcription. Total cellular ribonucleic acid (RNA) extraction was performed immediately after obtaining the atrial tissue. The tissue was homogenized with a Polytrone-Aggregate (Dispergier und Mischtechnik, Littau, Switzerland), and Trizol solution (Gibco BRL, Grand Island, New York) was added for RNA extraction. The extracted RNA was dissolved in diethyl pyrocarbonate–treated distilled water. Spectrophotometry at 260 and 280 nm was performed to measure the amount and quality of RNA. The RNA was then converted to complementary deoxyribonucleic acid (cDNA) by reverse transcription with random hexanucleotides and avian myoloblastosis virus reverse transcriptase (Boehringer, Mannheim, Germany).

Polymerase chain reaction. Table 2 shows the DNA sequence for all the primers used in the study. All the primer pairs for calcium-handling genes were designed according to the human cardiac DNA sequence (14–18). The L-type calcium channel primer pair was designed to amplify the alpha subunit of the channel, and all the alternatively spliced messenger RNA (mRNA) was amplified to yield a single DNA band on agarose gel electrophoresis. The primer pairs were specific to their target genes, and a similarity search in the GenBank revealed no false priming to noncardiac isoforms of the calcium-handling genes or to any known human gene sequence. The polymerase chain reaction condition was 95°C for 5 s, 55°C for 30 s and 72°C for 60 s with a final extension time of 5 min at 72°C. The glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene was used as the housekeeping gene. For each of the genes of interest, cDNA from all patients underwent polymerase chain reaction at the same time. The primers of GAPDH were added for coamplification in all reactions (19). The reaction products were analyzed by agarose gel electrophoresis. Optic densitometry was performed after the gel was stained with ethidium bromide for measuring the DNA amount. Differing amounts of a standard DNA sample (0.2 mg/ml) was loaded on all gels, and a linear regression curve between the OD number and the known DNA amount was obtained.

Statistical analysis. The mRNA amount of the gene was correlated with atrial fibrillation score, sampling sites, age, gender, right atrial pressure, pulmonary wedge pressure, use of calcium channel blockers and underlying cardiac disease. Linear regression was used for correlation between the mRNA amount and atrial fibrillation score, age, right atrial

Table 1. Demographic and Clinical Characteristics of the Study Patients

Patient No.	Gender/ Age (yr)	Cardiac dz	RAP	PWP	Af Score	CCB Use	Atrial Sampling Site
1	M/55	IE	1	17	0	−	LAA, RAA, RAF
2	M/55	RHD	7	23	3	−	LAA, RAA, RAF
3	M/41	MVP	NA	NA	0	−	LAA, RAA, RAF
4	M/64	AR	3	7	0	−	LAF, RAA, RAF
5	M/49	RHD	6	30	1	−	LAA, LAF, RAA, RAF
6	M/54	RHD	8	18	3	−	LAA, LAF, RAA, RAF
7	F/31	RHD	3	20	1	−	LAA, RAA, RAF
8	F/67	VSD, RCT	4	13	2	+	LAA, LAF, RAA, RAF
9	F/58	RHD	7	17	2	−	LAA, LAF, RAA, RAF
10	F/44	RHD	13	14	2	−	LAA, LAF, RAA, RAF
11	F/67	RHD	5	15	3	−	LAF, RAF
12	F/75	RHD	7	16	3	−	LAF, RAA, RAF
13	M/51	RHD	8	22	3	−	LAF, RAA, RAF
14	F/35	RHD	9	31	1	+	LAA, LAF, RAA, RAF
15	F/65	RHD	NA	NA	2	−	LAA, RAA, RAF
16	M/17	RHD	0	8	1	−	LAF, RAA, RAF
17	F/65	RCT	19	22	3	−	RAA
18	M/72	MVP	9	12	3	+	LAA, LAF, RAF
19	F/38	RHD	NA	NA	3	−	LAA, LAF, RAA, RAF
20	F/77	RHD	6	16	3	−	LAA, LAF, RAA, RAF
21	F/46	RHD	5	16	0	−	LAA, LAF, RAA, RAF
22	F/51	RHD	10	30	3	−	LAA, LAF, RAA, RAF
23	F/52	RHD	9	22	3	−	LAA, LAF, RAA, RAF
24	F/59	RHD	6	23	2	+	LAA, LAF, RAA, RAF
25	M/58	RCT	6	13	0	−	LAA, LAF, RAA, RAF
26	F/64	RHD	6	20	3	−	LAA, LAF, RAA, RAF
27	M/51	RHD	NA	NA	3	−	LAA, LAF, RAA, RAF
28	M/71	RHD	8	16	3	−	LAA, LAF, RAA, RAF
29	M/61	RHD	4	20	3	−	LAA, LAF, RAA, RAF
30	F/76	AR	6	30	0	−	RAA, RAF
31	F/47	RHD	7	25	1	−	LAA, LAF, RAA, RAF
32	M/61	MVP	5	19	0	−	LAA, LAF, RAA, RAF
33	F/50	RCT	4	7	3	+	LAA, LAF, RAA, RAF
34	M/36	MVP	5	15	0	−	LAA, LAF, RAA, RAF

Af = atrial fibrillation; AR = aortic regurgitation; CCB = calcium channel blockers; dz = disease; F = female; IE = infective endocarditis; LAA = left atrial appendage; LAF = left atrial free wall; M = male; MVP = mitral valve prolapse; NA = not available; PWP = pulmonary wedge pressure in mm Hg; RAA = right atrial appendage; RAF = right atrial free wall; RAP = right atrial pressure in mm Hg; RCT = rupture of chordae tendineae; RHD = rheumatic heart disease; VSD = ventricular septal defect.

pressure and pulmonary wedge pressure. Student *t* test was used for comparing the mRNA amount and gender, atrial fibrillation (less than three months or more than three months), use of calcium channel blockers and underlying cardiac disease (rheumatic or nonrheumatic). Analysis of variance was used for comparing the mRNA amount from four different sampling sites.

RESULTS

Validation of the polymerase chain reaction. Polymerase chain reaction using each specific primer pair yielded only one DNA band of predicted size. Coamplification of each of the calcium-handling protein genes together with GAPDH showed no interference between the primer pairs, and the two DNA bands could be well separated by agarose gel electrophoresis (Fig. 1). For measuring the DNA amount of the polymerase chain reaction products, 0.01, 0.02, 0.04, 0.08, 0.12 and 0.16 μg of sample DNA was loaded on each gel. Linear regression of the known DNA amount and the OD number by ethidium bromide ultraviolet fluorescence showed a good linear relationship ($r^2 > 0.95$). The DNA amount of all polymerase chain reaction bands was estimated by the linear regression formula, and the mRNA amount of the calcium-handling genes was normalized to the mRNA amount of GAPDH.

Relationship between mRNA levels and atrial fibrillation. Table 3 shows the comparison of mRNA levels between patients with persistent atrial fibrillation for more than three months (score 3) and patients without persistent atrial fibrillation for more than three months (score 0, 1 and 2). We found that the mRNA levels of L-type calcium channel and levels of Ca^{2+}-ATPase were significantly

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Table 2. The Nucleotide Sequence of All Primers Used in the Study

Genes	Primer Sequence	Reference
L-type calcium channel		
Sense primer	5′-GTACAAGAACTGTGAGCTGG	14
Antisense primer	5′-GATCACCGCGTAGATGAAGA	14
Ca^{2+}-ATPase		
Sense primer	5′-TTGCATTGCAGTCTGGATCA	17
Antisense primer	5′-TCCAAAGCAGAGTCATTACA	17
Ryanodine receptor		
Sense primer	5′-AAGTGCCAGAGTCAGCATTC	15
Antisense primer	5′-AGTAGTATCCAATGATGCAG	15
Calsequestrin		
Sense primer	5′-ATAGGCTTTGTGATGGTGGA	18
Antisense primer	5′-GCAACAAGCAGAGGAAAGTC	18
Phospholamban		
Sense primer	5′-ACCTCTAGATCTGCAGCTTG	16
Antisense primer	5′-GTTAGGCTGGAATGGAAGAC	16
GAPDH		
Sense primer	5′-TCCATGACAACTTTGGCATCGTGG	19
Antisense primer	5′-GTTGCTGTTGAAGTCACAGGAGAC	19

Ca^{2+}-ATPase = calcium adenosine triphosphatase; GAPDH = glyceraldehyde 3-phosphate dehydrogenase.

reduced in patients with persistent atrial fibrillation for more than three months. In contrast, the mRNA levels of calsequestrin, ryanodine receptor and phospholamban showed no significant change (Fig. 1). Figure 2 shows the mRNA levels of the Ca^{2+}-ATPase and L-type calcium channel in patients with different atrial fibrillation score. The mRNA was significantly reduced in patients with atrial fibrillation for >3 months, whereas the mRNA amount among patients with atrial fibrillation 0, 1 and 2 was not significantly different. This indicated that down-regulation of Ca^{2+}-ATPase and L-type calcium channel was observed only when the duration of atrial fibrillation was greater than three months but was not observed in patients with paroxysmal atrial fibrillation or with atrial fibrillation of shorter duration.

Dispersion of mRNA levels. The mRNA levels were correlated with the sites of atrial tissue sampling. We found that the mRNA levels were not significantly influenced by the site of atrial tissue sampling. Figure 1 shows a representative polymerase chain reaction result. Despite a large variation of L-type calcium channel and Ca^{2+}-ATPase mRNA levels between patients, the mRNA levels of the genes were not significantly different among the four atrial tissue sampling sites in the same patient. Therefore, we did not find spatial dispersion of the gene expression among the right atrial free wall, right atrial appendage, left atrial free wall and left atrial appendage.

Analysis of other parameters. The influence of all clinical parameters was analyzed. The results are summarized in Table 4. We showed that gender, age, right atrial pressure, pulmonary wedge pressure, the use of calcium channel blocker and the underlying disease (analyzed as rheumatic and nonrheumatic) had no significant effects on the expres-

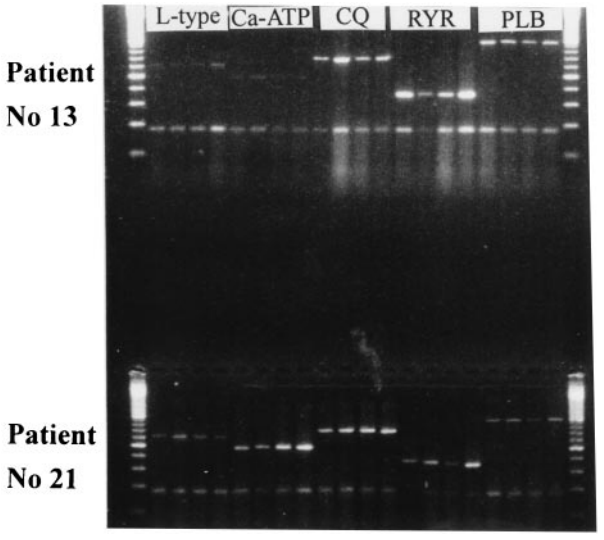

Figure 1. Representative figures of the polymerase chain reaction products after agarose gel electrophoresis. Coamplification of each of the calcium-handling protein genes with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) showed no interference between the primer pairs, and the two deoxyribonucleic acid bands could be well separated by gel electrophoresis (**upper band** for calcium-handling protein and **lower band** for GAPDH). The **upper panel** shows the polymerase chain reaction results from Patient No. 13 and the **lower panel** from Patient No. 21, and the samples for each calcium-handling gene were right atrial appendage, right atrial free wall, left atrial appendage and left atrial free wall from left to right. Despite a large variation of messenger ribonucleic acid (mRNA) levels of L-type calcium channel and calcium adenosine triphosphatase (Ca^{2+}-ATPase) between patients, the mRNA levels of the genes among the four atrial sampling sites were not significantly different in the same patient. Lane specification: **lanes 1 and 22** = molecular weight marker; **lanes 2 to 5** = L-type calcium channel; **lanes 6 to 9** = Ca^{2+}-ATPase; **lanes 10 to 13** = calsequestrin (CQ); **lanes 14 to 17** = ryanodine receptor (RYR); **lanes 18 to 21** = phospholamban (PLB).

Table 3. The Messenger Ribonucleic Acid Levels of Calcium-Handling Proteins in Patients With and Without Persistent Atrial Fibrillation for More than 3 Months

	Af <3 Months or No Af (n = 18)	Af >3 Months (n = 16)	p Value
Ca^{2+}-ATPase	1.21 ± 0.68	0.69 ± 0.42	< 0.05
L-type calcium channel	0.90 ± 0.88	0.36 ± 0.26	< 0.05
Ryanodine receptor	2.01 ± 1.17	1.96 ± 0.90	NS
Phospholamban	3.78 ± 3.36	4.25 ± 3.28	NS
Calsequestrin	2.66 ± 1.50	2.56 ± 1.60	NS

Af = atrial fibrillation; Ca^{2+}-ATPase = calcium adenosine triphosphatase.

sion of the genes. We showed that atrial fibrillation was the only factor that influenced the mRNA amount of L-type calcium channel and Ca^{2+}-ATPase.

DISCUSSION

In this study, we found that the mRNA levels of L-type calcium channel and sarcoplasmic reticular Ca^{2+}-ATPase were down-regulated in human atrial fibrillation. We further showed that these changes were independent of the underlying cardiac disease, atrial filling pressure, gender, age and use of calcium channel blockers. A decrease of mRNA level was observed for L-type calcium channel and Ca^{2+}-ATPase, whereas the mRNA levels for other genes such as ryanodine receptor, calsequestrin, phospholamban and GAPDH were not significantly altered. Therefore, these changes cannot be attributed to a dilutional effect caused by a decrease of atrial myocytes.

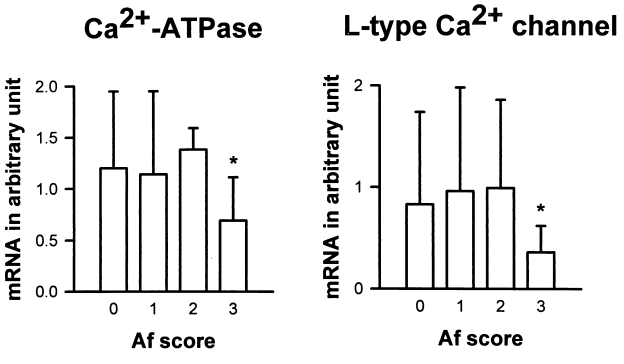

Figure 2. The messenger ribonucleic acid (mRNA) levels of L-type calcium channel and of calcium adenosine triphosphatase (Ca^{2+}-ATPase) in patients with different atrial fibrillation (Af) scores. The mRNA levels of both genes were significantly decreased in patients with an atrial fibrillation score of 3. However, the mRNA levels were not significantly different among patients with atrial fibrillation scores of 0, 1 and 2. *$p < 0.05$ when compared with atrial fibrillation score 0, 1 and 2. **Error bars** represent standard deviations.

Comparison with previous studies in atrial electrical remodeling. The shortening of the atrial refractory period in response to rapid atrial depolarization has been demonstrated in experimental animals as well as in human studies (5–7). However, studies with regard to the underlying mechanism and the associated cellular changes are mostly done in goats or dogs. Mitochondria swelling and disruption of sarcoplasmic reticulum have been demonstrated in dogs and goats with rapid atrial pacing (8,9,11), and these findings are characteristic of intracellular calcium overload. Yue et al. (20) further showed that I_{Ca} was decreased in the same animal model and might be responsible for the shortening of atrial refractory period. Van Wagoner et al. (21) investigated the changes of outward potassium current in atrial fibrillation. Although this study is exceptional in being a human study, the results showed a paradoxical decrease of outward potassium current, which should result in a prolongation of action potential duration and atrial refractory period (22).

All the above studies suggest that a decrease of I_{Ca} was responsible for the shortening of the atrial refractory period in atrial fibrillation. However, there are few reports that make a direct measurement of atrial tissue I_{Ca} in human atrial fibrillation. In the present study, we demonstrated that the mRNA amount of L-type calcium channel is reduced in atrial tissue from human subjects with atrial fibrillation. This would result in a decrease of the number of L-type calcium channels and therefore a decrease of I_{Ca}. Furthermore, a decrease of the mRNA of L-type calcium channel would result in a decrease of channel number without a change in channel properties. This finding is in agreement with the observations by Yue et al. that I_{Ca} decreased after rapid atrial pacing for 42 days in dogs and the decrease of I_{Ca} was not associated with a change of the gating or kinetic properties of the calcium channel (20).

The decrease of sarcoplasmic reticular Ca^{2+}-ATPase in atrial fibrillation is reported here for the first time. Since the Ca^{2+}-ATPase is responsible for calcium reuptake into the sarcoplasmic reticulum, the decrease of Ca^{2+}-ATPase should result in decreased reuptake and cytosolic calcium overload, which is characteristic in atrial electrical remodeling as suggested by several previous reports (8–13).

Calcium overload and atrial electrical remodeling. Since the most important electrophysiologic change of atrial cells in response to rapid depolarization is a shortening of the refractory period, early research effort was focused on the potassium currents, which are the major repolarization currents and are important determinants of action potential duration (22). However, many reports showed a paradoxical decrease of potassium channels, either at current density level or at gene expression level (21). These results excluded the possibility that an increase of repolarization outward current shortens the action potential duration. Further studies have demonstrated that calcium overload with a decrease of calcium current density might be responsible.

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Table 4. The p Values of Correlation Between Messenger Ribonucleic Acid Levels of Calcium-Handling Genes and Clinical Parameters

	Gender	Age	Af	Site	RAP	PWP	Dz	CCB
Ca^{2+}-ATPase	0.088	0.716	0.001*	0.410	0.630	0.164	0.264	0.182
L-type calcium channel	0.207	0.706	0.006*	0.271	0.847	0.265	0.550	0.256
Ryanodine receptor	0.606	0.746	0.167	0.487	0.223	0.985	0.086	0.502
Calsequestrin	0.277	0.840	0.365	0.613	0.912	0.308	0.520	0.843
Phospholamban	0.484	0.609	0.128	0.358	0.057	0.109	0.091	0.467

*$p < 0.05$.
Af = atrial fibrillation score; Ca^{2+}-ATPase = calcium adenosine triphosphatase; CCB = use of calcium channel blockers; Dz = underlying cardiac disease; PWP = pulmonary wedge pressure; RAP = right atrial pressure.

The accumulation of intracellular calcium may be a direct consequence of rapid atrial depolarization. Rapid depolarization results in cytosolic calcium overload since cells spend more time in the systolic phase and facilitate the influx of calcium through L-type calcium channels and further calcium-induced calcium release from the sarcoplasmic reticular store. With the use of L-type calcium channel blocker, the shortening of the refractory period can be prevented acutely. Wijffels et al. (10) have also reported that electrical remodeling in atrial fibrillation is not mediated by changes in autonomic tone, ischemia and atrial stretch. Therefore, calcium overload plays a pivotal role in the atrial electrical remodeling, and an understanding of the changes of calcium-handling proteins is important for the prevention or reversal of the atrial remodeling process.

Time course of changes of calcium-handling proteins in atrial fibrillation. With regard to the time course of atrial electrical remodeling, the shortening of the atrial refractory period happens after a short period of atrial rapid depolarization. Daoud et al. have demonstrated that several minutes of atrial fibrillation is sufficient to shorten the atrial refractory period in humans (6). The time course is much shorter than the time needed for down-regulation of Ca^{2+}-ATPase and L-type calcium channel, which is not evident in patients with atrial fibrillation for less than three months. Since intracellular calcium overload is a direct consequence of rapid atrial depolarization, the increase of cytosolic calcium will cause a decrease of I_{Ca} due to a decrease of electrochemical gradient and an acceleration of I_{Ca} inactivation, which then result in a rapid shortening of action potential duration (23). Therefore, the decrease of I_{Ca} may occur before the down-regulation of L-type calcium channel. Besides, the activation of Ca^{2+}-activated potassium outward current as well as Ca^{2+}-activated chloride outward current may also contribute to the shortening of action potential duration (24). However, in the late phase of atrial fibrillation, the decrease of L-type calcium channel will further contribute to the shortening of the action potential duration, and the decrease of the calcium uptake Ca^{2+}-ATPase will further contribute to the cytosolic calcium overload and enhancement of Ca^{2+}-activated potassium and chloride currents. These changes will aggravate the shortening of the atrial refractory period or delay the recovery from remodeling after a long period of atrial fibrillation. It has been reported clinically that a longer duration of persistent atrial fibrillation predicts atrial fibrillation recurrence after a successful cardioversion (25). This may be caused by the down-regulation of L-type calcium channel and Ca^{2+}-ATPase after a long duration of atrial fibrillation and may aggravate the atrial remodeling. Tieleman et al. also reported the association between atrial electrical remodeling and early recurrence of atrial fibrillation after electrical cardioversion (26). Therefore, the down-regulation of L-type calcium channel and Ca^{2+}-ATPase, though occurring late in the course, still contributes significantly to the perpetuation of atrial fibrillation.

Atrial mechanical stretch and atrial electrical remodeling. It has been reported that atrial mechanical stretch may contribute to the change of electrical properties (27,28). The perpetuation of atrial fibrillation may also be attributed to the progression of underlying disease, worsening of the hemodynamic status and atrial stretch. Therefore we analyzed the correlation between the atrial filling pressure and the mRNA levels of calcium-handling proteins. The atrial filling pressure was represented by the pulmonary wedge pressure (for the left atrium) and the right atrial pressure (for the right atrium), which were available in 88% of the study patients. We demonstrated that the gene expression changes of L-type calcium channel and Ca^{2+}-ATPase were not related to these hemodynamic parameters. Therefore, these gene expression changes were a result of atrial fibrillation itself instead of being mediated by the hemodynamic deterioration caused by atrial fibrillation.

Dispersion of gene expression in atrial tissue. Dispersion of the atrial electrical properties may result in uneven conduction and repolarization, and therefore contributes to the occurrence and maintenance of atrial fibrillation. In the present study, dispersion of gene expression was assessed by obtaining atrial tissue from four different sites including the left atrial appendage, the left atrial free wall, the right atrial appendage and the right atrial free wall, respectively. We showed that the mRNA levels were not significantly different among these atrial sampling sites.

Study limitations. The changes of calcium-handling proteins were assessed at the mRNA level only. There was no

investigation about the changes at protein level or at ion current level. Since the amount of human atrial tissue obtained was small, especially for the left atrium, we chose to study the mRNA levels by reverse transcription and polymerase chain reaction. Although a change of gene expression is a very common way for regulating functional protein level, a parallel change between the mRNA level and the protein level was not demonstrated in the present study.

Patients with different atrial fibrillation scores were not matched for their age, gender and underlying disease. Besides, the number of patients in each atrial fibrillation score group was small. However, we analyzed the effects of all clinical parameters and showed that atrial fibrillation is the only factor correlated with a decrease of L-type calcium channel and of Ca^{2+}-ATPase mRNA.

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