Atrial L-Type Ca^{2+} Currents and Human Atrial Fibrillation

David R. Van Wagoner, Amber L. Pond, Michelle Lamorgese, Sandra S. Rossie, Patrick M. McCarthy, Jeanne M. Nerbonne

Abstract—Chronic atrial fibrillation (AF) is characterized by decreased atrial contractility, shortened action potential duration, and decreased accommodation of action potential duration to changes in activation rate. Studies on experimental animal models of AF implicate a reduction in L-type Ca^{2+} current (I_{Ca}) density in these changes. To evaluate the effect of AF on human I_{Ca}, we compared I_{Ca} in atrial myocytes isolated from 42 patients in normal sinus rhythm at the time of cardiac surgery with that of 11 chronic AF patients. I_{Ca} was significantly reduced in the myocytes of patients with chronic AF (mean -3.35 ± 0.5 pA/pF versus -9.13 ± 1.0 pA/pF in the controls), with no difference between groups in the voltage dependence of activation or steady-state inactivation. Although I_{Ca} was lower in myocytes from the chronic AF patients, their response to maximal β-adrenergic stimulation was not impaired. Postoperative AF frequently follows cardiac surgery. Half of the patients in the control group (19/38) of this study experienced postoperative AF. Whereas chronic AF is characterized by reduced atrial I_{Ca}, the patients with the greatest I_{Ca} had an increased incidence of postoperative AF, independent of patient age or diagnosis. This observation is consistent with the concept that calcium overload may be an important factor in the initiation of AF. The reduction in functional I_{Ca} density in myocytes from the atria of chronic AF patients may thus be an adaptive response to the arrhythmia-induced calcium overload. **(*Circ Res*. 1999;85:428-436.)**

Key Words: atrial fibrillation ■ postoperative atrial fibrillation ■ Ca^{2+} channel ■ β-adrenergic antagonist ■ cardiac surgery

Atrial fibrillation (AF) is the most common chronic arrhythmia, afflicting nearly 2 million Americans.[1] In spite of a growing recognition of its prevalence and associated morbidity and mortality, the mechanisms involved in the initiation, maintenance, and termination of AF remain poorly understood. Altered action potential characteristics (decreased response to verapamil and decreased resting potential) were noted in microelectrode recordings from diseased and fibrillating atrial tissue in 1976.[2] More than a decade ago, a shortening of the effective refractory period and diminished adaptation to changes in rate were demonstrated in patients vulnerable to the development of AF.[3] In microelectrode recordings from atrial tissue removed from chronic AF patients, a later study demonstrated a similar loss of rate adaptation and concomitant shortening of atrial action potential duration.[4] In 1995, Wijffels et al[5] demonstrated that initiation of AF in electrically stimulated goat atria caused consistent and relatively rapid electrophysiological changes. This pivotal study rekindled scientific interest in identifying the mechanisms involved in the initiation of AF, as well as the mechanisms responsible for the adaptation of the atria to the high-rate rhythm.

To evaluate the cellular mechanism(s) responsible for these changes, we previously evaluated the hypothesis that an increased density of repolarizing K^+ currents in the atrial myocytes of chronic AF patients could explain the reported electrophysiological changes.[6] In contrast to this hypothesis, the densities of both the transient and sustained outward K^+ currents were found to be significantly reduced in myocytes from chronic AF patients, combined with a decreased expression of the Kv1.5 K^+ channel protein. To explain this result, we inferred that the reduction in action potential duration was most likely the result of a simultaneous greater reduction in the density of voltage-dependent L-type Ca^{2+} current (I_{Ca}). A reduction in I_{Ca} has been documented in the rapidly paced canine atria.[7]

Since the publication of our initial results, several studies have reported that the electrophysiological remodeling accompanying episodes of AF could be prevented by pretreatment with calcium channel blockers,[8,9] suggesting that calcium overload was a critical factor in the electrophysiological remodeling process. Calcium overload might initiate the changes in gene expression that eventually lead to a downregulation of atrial K^+ and Ca^{2+} current densities.

In the rapidly paced canine atria model of AF, there has been shown to be both a decrement in functional I_{Ca} and a decrease in the number of dihydropyridine binding sites.[10] In

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recent studies on patients with AF, it has been demonstrated that both mRNA[11,12] and protein[12] for the L-type Ca^{2+} channel are reduced in patients with established AF. However, there has been no systematic evaluation of the functional L-type Ca^{2+} current density in patients with AF. The goal of the present study was to directly evaluate and compare the density of I_{Ca} in myocytes from patients in normal sinus rhythm with that of myocytes from chronic AF patients undergoing the Maze procedure for surgical treatment of the arrhythmia. Because of the implicit link between calcium overload and the initiation of electrophysiological remodeling, we also sought to analyze the relationship between preoperative I_{Ca} and the subsequent development of postoperative AF.

Materials and Methods

Patient Population

Atrial myocytes in the present study were isolated from the atrial appendage of 3 distinct patient groups: (1) patients in normal sinus rhythm undergoing first-time coronary artery bypass graft and/or valve repair surgery (n=40); (2) nonfailing hearts from unmatched organ donors in normal sinus rhythm (n=3); and (3) chronic AF patients undergoing the Maze III surgery (n=14). The study protocol was approved by the Cleveland Clinic Foundation Institutional Review Board. Experiments were performed from January 1997 to May 1999.

Tissue from group 1 patients was excised from the tip of the right atrial appendage at the time of bypass cannulation, placed in saline, and taken to the laboratory. Similarly, atrial appendages from group 3 patients were excised, placed in saline, and brought to the laboratory. Hearts from group 2 patients were perfused with cardioplegia before removal, chilled, and returned to the laboratory (within ≈1 hour of explant). Clinical characteristics of the control patients (groups 1 and 2) are summarized in Table 1, along with a notation about their subsequent status with respect to the development of postoperative AF. Clinical characteristics of the chronic AF patients are summarized in Table 2.

Atrial Myocyte Isolation Protocol

Atrial myocytes were dissociated using the protocol previously described,[6] with one change in the composition of the dissection buffer (DB). The DB in the present study contained (mmol/L) sucrose 134, NaCl 35, $NaHCO_3$ 25, Na_2HPO_4 16, KCl 4.75, KH_2PO_4 1.2, HEPES 10, and glucose 10 (pH 7.4) with NaOH. The myocytes were kept oxygenated at room temperature until used, within 8 hours of isolation. Yields were in the range of 10% to 40% for viable calcium-tolerant myocytes. Only well-striated, rod-shaped myocytes were used in the electrophysiological studies.

Conventional Whole-Cell Patch-Clamp Technique

Conventional (ruptured patch) recording techniques were used to measure whole-cell I_{Ca}. The pipette solution contained (mmol/L) CsCl 125, tetraethylammonium chloride (TEA-Cl) 20, MgATP 5, creatine phosphate 3.6, EGTA 10, and HEPES 10 (pH 7.2) with CsOH. The bath solution contained (mmol/L) TEA-Cl 157, $CaCl_2$ 1, $MgCl_2$ 0.5, and HEPES 10 (pH 7.4) with CsOH. The junction potential with these solutions was 7.5 mV (calculated using Axoscope, version 1.1, Axon Instruments) and was not corrected.

Data were acquired using a Pentium computer that controlled data acquisition hardware and software (pClamp 6.03+, Axon Instruments) connected to either Axopatch 1C or Axopatch 200 amplifiers (Axon Instruments). Currents were filtered at 2 kHz and sampled at 4 to 10 kHz. A holding potential of −50 mV was used to inactivate Na^+ current. I_{Ca} was elicited with step depolarization protocols, using test potentials in the range of −40 to +30 mV. I_{Ca} densities were computed by dividing current amplitudes by the whole-cell capacitance. Peak Ca^{2+} current density refers to I_{Ca} density at the peak of the current-voltage (*I*-V) curve.

Voltage-Clamp Recordings

Myocytes were superfused with test solutions in a 35-mm culture dish mounted in a thermal stage controller (Bioptech ΔT system), maintained at 35°C, and gassed with 100% O_2. Solutions were changed via a 6-port gravity flow system. Patch pipettes were prepared from Corning 8161 glass (WPI), and the shanks were covered with Sylgard. Tips were fire-polished immediately before use to an access resistance of 2 to 3 MΩ, when filled with the pipette solution. After patch rupture, series resistance values were in the range of 4 to 8 MΩ and were electronically compensated (30% to 80%) to minimize voltage-clamp errors, while maintaining stable recordings. Recordings were begun after 4 to 5 minutes of dialysis, after the Ca^{2+} current amplitudes and access resistance had stabilized. When using the above solutions, the rundown of I_{Ca} was negligible (<5%) over the time course of the experiments (<20 minutes).

Current-Clamp Recordings

Action potentials were recorded from a subset of the myocytes, to clarify the functional effect of the changes in I_{Ca}. Action potentials were recorded using conventional whole-cell recording conditions, with a pipette solution containing (mmol/L) KCl 140, MgATP 4, creatine phosphate 4, sodium pyruvate 3, $MgCl_2$ 1, and 50 μmol/L EGTA (pH 7.2) with KOH. The bath solution for these experiments contained (mmol/L) NaCl 140, sodium acetate 3, KCl 5, HEPES 5, glucose 5, $MgCl_2$ 1, and $CaCl_2$ 2 (pH 7.4) with NaOH. Action potentials were recorded at cycle lengths of 2, 1, 0.5, 0.4, and 0.3 seconds, under constant flow conditions, at a temperature of 35°C. At least 12 steady-state action potentials were recorded at each cycle length. These were averaged, and action potential amplitude and duration (50% and 90% repolarization [APD_{50} and APD_{90}, respectively]) were measured from the averaged traces.

Statistical Analysis

All data summaries are presented as mean±SEM. Statistical differences were evaluated using appropriate *t* tests (paired or unpaired). Differences were deemed to be significant for values of $P<0.05$.

Results

Assessment of I_{Ca} Density

Our initial goal was to compare the I_{Ca} density of atrial myocytes from the patients in normal sinus rhythm with that of the patients in chronic AF. We measured I_{Ca} in a total of 86 myocytes isolated from the atria of 42 patients in normal sinus rhythm and 28 myocytes isolated from the atria of 11 patients (patients 1 through 11, Table 2) in chronic AF. The age of the patient populations was not different: the mean age of the patients in normal sinus rhythm was 63.8±2 years, whereas the mean age of the patients undergoing the Maze procedure was 61.4±3.5 years. The voltage-clamp protocol is shown in Figure 1A. Figure 1 also illustrates representative current traces from right atrial myocytes isolated from either a patient in normal sinus rhythm (Figure 1B) or a patient in chronic AF for 10 years (Figure 1C). Mean *I*-V relations for all of the myocytes in the present study are plotted in Figure 1D. Myocytes from the patients in normal sinus rhythm had a significantly greater peak I_{Ca} density (−9.13±1.0 pA/pF, n=86) than those in chronic AF (−3.35±0.5 pA/pF, n=28, $P<0.002$). The potential at which I_{Ca} was greatest was somewhat variable (either 0 or +10 mV) from myocyte to myocyte. However, there was no systematic difference in the voltage dependence of activation of I_{Ca} between groups.

TABLE 1. Clinical Characteristics of the Patients in Normal Sinus Rhythm at the Time of Cardiac Surgery

Patient Number	Age, y	Sex	Diagnosis	Presurgical Drugs	Postoperative AF
1	77	M	CABG	GLY	−
2	78	M	CABG	AI	+
3	82	F	CABG	AI	−
4	26	F	DN	...	NA
5	83	M	CABG	...	+
6	69	F	AVR/CABG	CC, BB	+
7	75	M	CABG	AI, CC, BB	−
8	69	M	CABG	BB	+
9	74	M	CABG	BB, GLY	+
10	32	M	CABG	...	ND
11	71	M	CABG	BB	−
12	70	M	CABG	BB, CC	+
13	67	M	AVR/CABG	...	−
14	74	M	CABG	GLY	+
15	73	M	CABG	...	−
16	67	F	CABG	BB	+
17	75	M	CABG	BB	−
18	70	M	AVR/CABG	BB, GLY	+
19	67	M	AVR/CABG	CC, BB, GLY	+
20	81	F	CABG	BB, CC	−
21	73	M	CABG	...	+
22	49	M	CABG	BB	−
23	51	F	CABG	AM	−
24	68	F	AVR	...	−
25	74	M	CABG	...	+
26	59	M	CABG	BB	−
27	70	M	CABG	BB, GLY, NTG	+
28	66	M	CABG	BB, CC	+
29	49	M	CABG	...	−
30	81	F	CABG	BB, GLY	−
31	74	M	CABG	BB	+
32	62	M	CABG	AI, BB	−
33	51	F	AVR/CABG	CC	−
34	45	F	AVR/CABG	DIG	+
35	59	F	DN	BB, CC	NA
36	54	M	CABG	DIG	+
37	38	M	DN	N/A	NA
38	61	M	CABG	...	+
39	47	M	CABG	...	−
40	59	M	CABG	...	−
41	45	M	CABG	AI	−
42	66	M	CABG	...	−
43	26	M	CABG	BB, NTG	−

CABG indicates coronary artery bypass graft; AVR, aortic valve repair/replacement; DN, nonfailing heart donor; AI, angiotensin-converting enzyme (ACE) inhibitor; AM, amiodarone; BB, β-adrenergic receptor blocker; CC, calcium channel blocker; DIG, digoxin; GLY, glybenclamide; NTG, nitroglycerin; N/A, not available; +, AF present; −, AF absent; ND, AF could not be determined; and NA, not applicable.

To determine whether changes in the availability of Ca^{2+} channels could account for the differences in peak I_{Ca}, we analyzed the steady-state inactivation characteristics in a series of myocytes from both groups (Figure 2). The voltage-clamp protocol for these experiments is shown in Figure 2A. Raw current traces from a representative control myocyte are shown in Figure 2B and from an AF myocyte in Figure 2C. The summary in Figure 2D shows that chronic AF, although lowering the peak I_{Ca} density, had no effect on the steady-state inactivation characteristics of I_{Ca} in the human atrial myocytes studied. Thus, the reduction in I_{Ca} is most likely due to a decrease in the number of functionally available channels, rather than to a modification of their voltage dependence.

Relationship Between Myocyte Capacitance and I_{Ca}

The mean capacitance of the AF myocytes studied was greater than that of the control myocytes (control 67.6±2.9 pF, n=86, versus AF 90.4±8.3 pF, n=28; $P<0.01$). In Figure 3, peak I_{Ca} amplitude (Figure 3A) and density (Figure 3B) for each myocyte are plotted as a function of myocyte size (capacitance). These plots illustrate that both the peak I_{Ca} amplitude and density were inversely related to myocyte size. This trend was observed in myocytes from both groups of patients. Importantly, however, these plots demonstrate that the reduction in I_{Ca} in the myocytes from the chronic AF patients occurred in all myocytes, regardless of capacitance. To emphasize this, Figure 3C plots the current density for control and AF myocytes for small (<60 pF), medium (60 to 100 pF), and large (>100 pF) atrial myocytes. The I_{Ca} density was significantly lower in the myocytes isolated from the AF patients, relative to the controls, in each capacitance range.

Responses to β-Adrenergic Stimulation

The adrenergic sensitivity of patients with heart failure is reduced.[13,14] To assess whether chronic AF is similarly characterized by a diminished response to β-adrenergic stimulation, the response of individual myocytes to maximal β-adrenergic stimulation (1 μmol/L isoproterenol) was assessed in a subgroup of myocytes from both the patients in normal sinus rhythm and those in chronic AF. Figure 4 plots the mean±SEM peak I_{Ca} densities for these myocytes.

Isoproterenol significantly increased I_{Ca} in myocytes from both the control patients (−6.6±1.4 pA/pF to −16.1±2.3 pA/pF, n=15 myocytes, from 7 patients) and the chronic AF patients (−2.6±0.6 pA/pF to −8.9±2.1 pA/pF, n=11 myocytes, from 4 patients). However, the relative response to maximal β-adrenergic stimulation (1 μmol/L isoproterenol) was greater (unpaired *t* test, $P<0.05$) in the myocytes isolated from chronic AF patients (4.3±0.7-fold increase, n=12) compared with patients in normal sinus rhythm (2.8±0.3-fold increase, n=15). This difference remained significant (unpaired *t* test, $P<0.01$) when the comparison was made by patient means (normal sinus rhythm 2.5±0.3 fold, n=7 patients, versus chronic AF 4.5±0.6 fold, n=4 patients).

Effect of Chronic AF on Action Potential Duration

Action potentials are recorded using physiological ion concentrations in both the pipette solution and the bath solution.

TABLE 2. Clinical Characteristics of the Chronic AF Patients in the Study

Patient Number	Age, y	Sex	Surgery	AF Duration, y	Presurgical Drugs	Left Atrial Size, cm
1	52	F	Maze/MVR	≈20	DIG, ALB	5.2
2	72	F	Maze/MVR/AVR	20	...	7.3
3	66	M	Maze/MVR	10	DIG	8.5
4	72	M	MVR/AVR/CABG	6–7	AI, NTG	3.7
5	63	M	Maze/MVR	>3	...	4.8
6	62	M	Maze/MVR/CABG	>5	AI, DIG	5.8
7	59	M	Maze/MVR	3	BB, NTG	4.5
8	37	F	Maze	5	CC, DIG	4.9
9	74	F	Maze/MVR	4	AM, BB	5.2
10	52	M	Maze	10–15	AM, BB	4.3
11	66	F	Maze/MVR/CABG	4	AI, CC, DIG	6.6

MVR indicates mitral valve repair/replacement; ALB, albuterol. Other abbreviations are the same as in Table 1. In addition to the cardiovascular drugs listed, all of the chronic AF patients were on Coumadin therapy for anticoagulation.

Because I_{Ca} is recorded under conditions in which K^+ currents are strongly suppressed (CsCl in the pipette, TEA in the bath), it is not possible to quantitatively assess I_{Ca} in the same myocytes from which action potentials are recorded. To qualitatively evaluate the effect of the observed changes in I_{Ca} on the atrial action potential, action potentials were recorded from right atrial myocytes from patients in normal sinus rhythm and from patients in chronic AF. Figure 5A plots representative action potentials recorded over a range of cycle lengths from a myocyte isolated from a 26-year-old patient in normal sinus rhythm. A clear, cycle length–dependent variation of action potential duration is evident. To illustrate the effect of a reduction in I_{Ca}, Figure 5B shows action potentials from the same myocyte recorded at the same cycle lengths in the presence of 10 μmol/L nifedipine. Figure 5C plots the action potential duration at APD_{50} and APD_{90}. In contrast, Figure 5D and Figure 5E shows action potentials recorded from atrial myocytes from 2 different chronic AF patients. Little cycle length–dependent change in action potential duration was evident, except at a 300-ms cycle length. Mean±SEM data for the APD_{90} and APD_{50} values of 5 myocytes isolated from 5 chronic AF patients are plotted in Figure 5F. Myocytes from chronic AF patients were characterized by shorter APD_{90} values, with less variation as a function of cycle length than the control myocyte in Figure 5A. APD_{50} values were also flatter across the range of cycle lengths tested.

Relation of I_{Ca} to the Occurrence of Postoperative AF

After cardiac surgery, many patients develop AF in the postoperative recovery period (typically 2 to 5 days after surgery). To determine whether the preoperative I_{Ca} was correlated with the occurrence of postoperative AF, we analyzed the control patient I_{Ca} recordings on the basis of the occurrence of postoperative AF during the in-hospital recovery period. Table 1 lists the clinical characteristics of the control patients and indicates whether the patient experienced

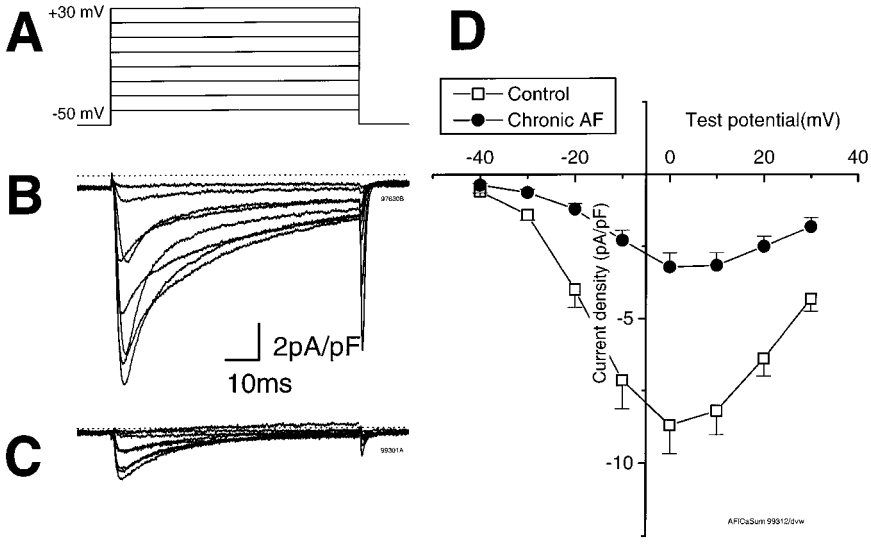

Figure 1. Representative whole-cell Ca^{2+} currents (I_{Ca}) from human right atrial myocytes. A, Voltage-clamp protocol used to record I_{Ca}. The interval between voltage steps was 5 seconds. B, Current traces recorded from an atrial myocyte obtained from a 74-year-old male patient undergoing coronary bypass graft surgery. The myocyte capacitance was 79.8 pF, and the access resistance was 5.4 MΩ. C, Recording from a myocyte obtained from a 66-year-old female patient undergoing the Maze III procedure and mitral valve repair. The myocyte capacitance was 73.5 pF, and the access resistance was 4.0 MΩ. The current amplitudes in both recordings were divided by the capacitance value, and the scale bar indicates the current density (pA/pF). D, Summary of the mean±SEM I_{Ca} densities for 86 myocytes obtained from 42 patients in normal sinus rhythm (□) and the mean±SEM current densities for the 28 myocytes obtained from 11 patients in chronic AF (●).

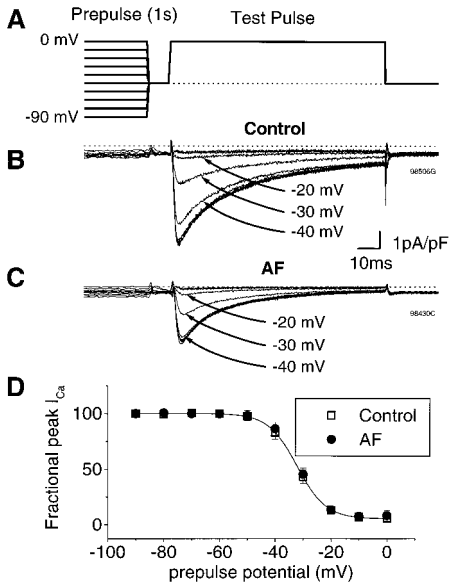

Figure 2. Steady-state inactivation of I_{Ca} is not altered by chronic AF. A, One-second prepulse protocol (−90 to 0 mV in 10-mV steps) was used to characterize the steady-state inactivation of the L-type Ca^{2+} current elicited by a step to 0 mV. B, Steady-state inactivation current traces from an atrial myocyte obtained from a patient in normal sinus rhythm. C, Steady-state inactivation current traces from a patient in chronic AF. D, No difference in the steady-state inactivation characteristics of myocytes isolated from patients in normal sinus rhythm (n=5, $V_{1/2}$=−31.7±0.1 mV, k=4.6±0.1 mV) vs the myocytes isolated from those patients in chronic AF (n=5, $V_{1/2}$=−32.1±0.1 mV, k=5.2±0.1 mV).

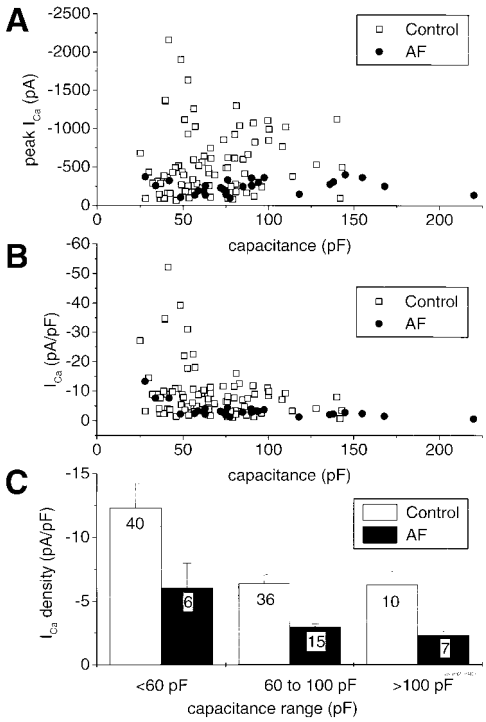

Figure 3. Relations between cell capacitance and Ca^{2+} current amplitude or current density in myocytes isolated from patients in normal sinus rhythm (□) or chronic AF (●). A, Peak I_{Ca} amplitude for each myocyte as a function of its size (capacitance). B, Peak current density (peak current amplitude divided by capacitance) for each myocyte. A and B, Data are from 86 myocytes from 42 patients in normal sinus rhythm and 28 myocytes from 11 patients in chronic AF. C, Histogram analysis of the distribution of I_{Ca} densities as a function of myocyte size (capacitance) for the myocytes from the control (open bars) and chronic AF (filled bars) patients.

postoperative AF. Half (19/38) of the surgical patients experienced postoperative AF. Figure 6A shows that there was no significant difference in either myocyte capacitance or the age between the patient groups, separated by postoperative AF occurrence. Figure 6B shows the mean±SEM current density–voltage relations for the patients who experienced postoperative AF (●) versus those that did not (□). There was no difference in the voltage dependence of I_{Ca} between groups, but the current density was significantly lower ($P<0.01$) in those patients who did not experience postoperative AF (peak I_{Ca} −5.96±0.6 pA/pF, n=37) versus those who did (−12.6±2.1 pA/pF, n=35).

Figure 6C plots the peak I_{Ca} density values of each myocyte, with symbols indicating the presence (●) or absence (□) of postoperative AF. Although there was a great deal of overlap at low I_{Ca} densities, the myocytes with the greatest I_{Ca} were isolated from patients who experienced postoperative AF. To account for the fact that different numbers of myocytes were studied per patient, we further analyzed the data by comparing the mean I_{Ca} densities for each patient. Figure 6D shows that analysis of the data by patient means yielded the same result. The mean I_{Ca} of the patients who did not experience postoperative AF (−6.12±0.9 pA/pF, n=19) was significantly lower compared with patients who did (−10.3±1.9 pA/pF, n=19; $P<0.05$).

Discussion

The present study demonstrates that atrial myocytes isolated from chronic AF patients have a significantly lower I_{Ca} density than myocytes isolated from patients in normal sinus rhythm (Figure 2). The mean reduction in peak I_{Ca} (−3.35 versus −9.13 pA/pF) was 63%. This is similar to the reduction that we previously detected in the transient outward K^+ current (≈60%) and somewhat greater than the reduction in the sustained outward K^+ current (≈50%) in myocytes from the same patient populations.[6] Figure 2 shows that, although the I_{Ca} density was reduced, there was no change in

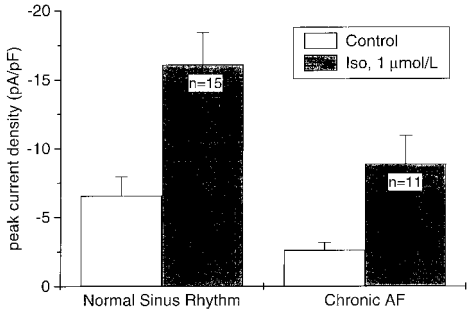

Figure 4. β-Adrenergic responsiveness of atrial myocytes from patients with chronic AF is not impaired. This plot shows mean±SEM values of current density before and after superfusion of the myocytes with 1 μmol/L isoproterenol (Iso). Data were gathered from 15 myocytes isolated from 8 patients in normal sinus rhythm at the time of cardiac surgery and 11 myocytes isolated from 4 chronic AF patients.

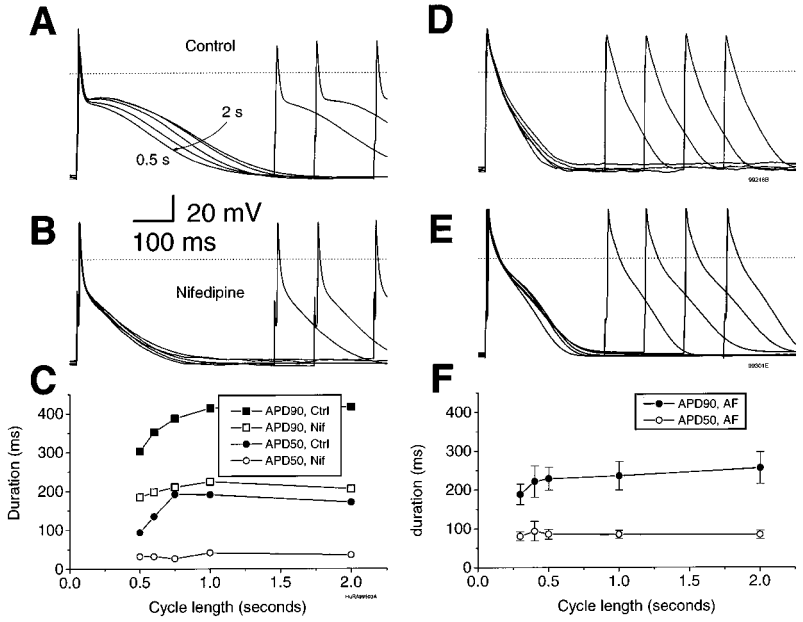

Figure 5. Reduced Ca^{2+} current attenuates the rate-dependent accommodation of human atrial action potentials. A and B, Steady-state action potentials recorded from a myocyte isolated from a 26-year-old patient in normal sinus rhythm at the time of cardiac bypass surgery. A, Action potentials recorded in a control bath solution. B, Action potentials from the same myocyte after superfusion with the calcium channel blocker nifedipine (10 μmol/L). APD_{90} and APD_{50} values for both sets of action potentials from this myocyte are plotted in panel C. D and E, Steady-state action potentials at a range of cycle lengths recorded from 2 different chronic AF patients. F, Summary of the mean±SEM action potential durations measured at APD_{90} and APD_{50} for 5 myocytes isolated from 5 patients in chronic AF.

the voltage dependence of activation or steady-state inactivation of I_{Ca} in the myocytes isolated from chronic AF patients.

We previously showed that the reduction in outward K^+ current density was present only in those patients in whom AF was persistent.[6] Patients with paroxysmal AF or dilated cardiomyopathy had normal outward K^+ current densities. In the present study, Figure 3 demonstrates that, although I_{Ca} was consistently low in chronic AF patients, there was a significant overlap with many of the patients in normal sinus rhythm who also had low I_{Ca} densities. Thus, whereas a low I_{Ca} density is characteristic of myocytes from patients with chronic fibrillation, it is possible for patients to have myocytes with low I_{Ca} density in the absence of AF.

Our results are consistent with the observation of Le Grand et al,[15] who demonstrated a significant reduction in inward Ca^{2+} and outward K^+ current densities in the myocytes of patients with dilated (but not necessarily fibrillating) atria. The presence of very large myocytes in the AF patient population (Figure 3A or 3B) is consistent with the presence of atrial dilation. However, we clearly demonstrate that the reduction in I_{Ca} density is not solely due to a shift in myocyte size (Figure 3C). Both the I_{Ca} amplitudes and current densities were reduced in the chronic AF patients, regardless of myocyte size.

Most of the chronic AF patients in the present study were undergoing mitral valve repair. Mitral regurgitation increases

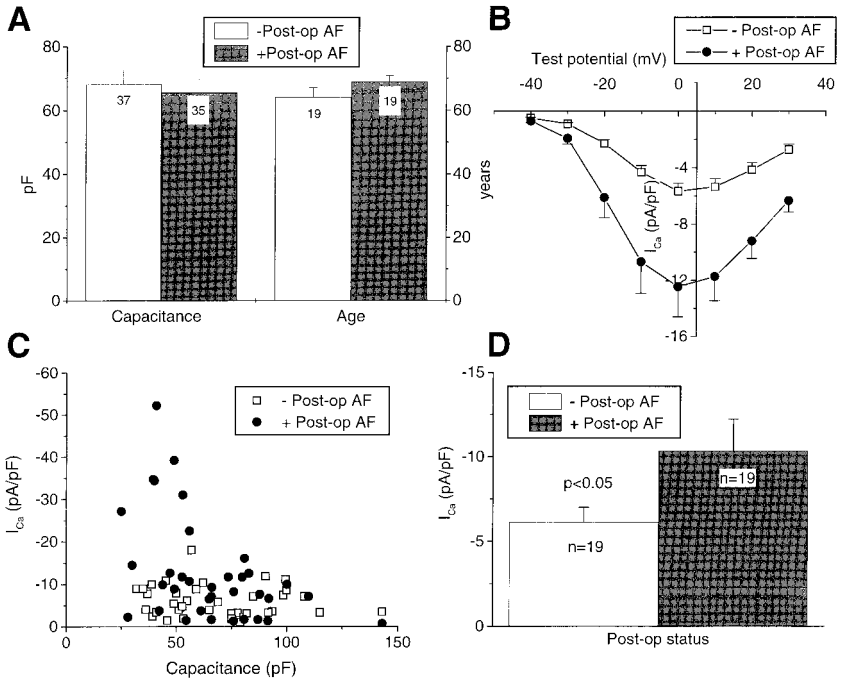

Figure 6. Preoperative I_{Ca} density is positivpely correlated with the development of postoperative AF. A, Mean±SEM capacitance of individual myocytes and mean±SEM age of the patients who either did (+) or did not (−) experience postoperative AF. B, Mean±SEM *I*-V relations for all myocytes in the control group (37 myocytes from 19 patients who did not develop postoperative AF and 35 myocytes from 19 patients who did develop postoperative AF). C, Scatter plot of I_{Ca} density as a function of myocyte capacitance. D, Comparison of mean±SEM I_{Ca} density values for each patient group analyzed by patient mean.

left atrial pressure and can directly cause significant left atrial dilation, in the absence of AF. However, the changes in I_{Ca} that we detected are not solely due to atrial dilation as a result of valvular disease. Two of the chronic AF patients (8 and 10) had no underlying valvular disease. The left atria of these patients were only modestly dilated (4.9 and 4.3 cm; normal left atrial dimension is 2 to 4 cm), and the mean I_{Ca} density of these patients (−2.9 pA/pF and −3.3 pA/pF) was indistinguishable from that of those AF patients with valvular heart disease. Thus, it is likely that both atrial dilation and AF have a similar effect on atrial I_{Ca}.

Whereas heart failure is characterized by diminished adrenergic responsiveness, the present study shows that chronic AF does not diminish the I_{Ca} response to a maximal β-adrenergic stimulus. As shown in Figure 4, the response to maximal β-adrenergic stimulation was somewhat enhanced in the myocytes from the chronic AF patients. Neither the mechanistic basis for this response nor its significance is clear at this time. One might speculate that a greater fraction of the Ca^{2+} channels in the atrial myocytes from the chronic AF patients may be unavailable under basal conditions but can be recruited in the presence of adrenergic stimulation (perhaps as a result of altered phosphorylation status of the L-type Ca^{2+} channels). In any case, this result demonstrates that chronic AF does not result in a functional downregulation of all membrane proteins or signal transduction pathways.

In 1976, Hordof et al[2] demonstrated that the plateau of action potentials recorded from atrial tissue of normal patients, but not those with AF, was attenuated in response to verapamil. Boutjdir et al[4] demonstrated that action potentials recorded from atrial tissue isolated from patients with chronic AF had a shorter duration (APD_{90}) and refractory period than tissue isolated from patients in normal sinus rhythm. The mechanisms responsible for these changes were not identified.

In rapidly paced canine atria, a significant reduction in the density of I_{Ca} has been reported, similarly resulting in an abbreviated action potential and decreased accommodation to changes in rate.[7] In that study, superfusion of control myocytes with nifedipine (10 μmol/L) largely mimicked the changes observed after rapid atrial pacing. We have shown that nifedipine has the same effect on normal human atrial action potentials (Figure 5A through 5C) and that similar reductions in action potential duration and rate-dependent modulation of duration occur in atrial myocytes isolated from patients with chronic AF (Figure 5D through 5F). Our action potential recordings (Figure 5) are qualitatively similar to those recorded by Hordof et al[2] and Boutjdir el al[4] from intact, excised atrial tissue. Thus, in combination with the finding of decreased I_{Ca} density in the atrial myocytes from chronic AF patients, our study supports the evolving concept that I_{Ca} is a critical component of the normal cycle length–dependent modulation of atrial action potential duration.[16,17]

Time Course of Human Atrial Electrophysiological Remodeling

It is now evident that AF causes electrophysiological remodeling of the atria. Studies have demonstrated that these changes are rapid and are at least initially reversible.[18] In addition to electrophysiological remodeling, there is also evidence for structural remodeling of the atria after prolonged periods of AF.[19] In humans, structural changes may be due to both the underlying cardiac disease (eg, valvular problems, atherosclerosis, or cardiomyopathy) and the direct effects of the fibrillatory rhythm. Patients in the present study had AF for a long time, with a minimum duration of 3 years (Table 2). Given the duration of AF in the patients in the present study, no information about the time course of changes in I_{Ca} can be determined. Studies based on animal experiments in either fibrillating goats[5,20] or rapidly paced canine atria[7] suggest that significant electrophysiological remodeling can occur within a week of high-rate atrial activity.

Two recent biochemical studies on tissue isolated from patients with chronic AF both show that significant downregulation of mRNA levels for the L-type Ca^{2+} channel was detectable after only 6 months of persistent AF.[11,21] Evidence from both an animal model of AF[10] and patients undergoing Maze surgery[12] suggests that mRNA changes result in reduced expression of the α_1 subunit of the L-type Ca^{2+} channel. Our study complements these results and directly demonstrates that there is a significant (63%) reduction in the density of I_{Ca} in atrial myocytes isolated from patients with chronic AF. This decrement in I_{Ca} is likely to contribute to the changes in action potential morphology and to the loss of adaptation of action potential duration as a function of activation rate that are characteristic of recordings from chronic AF patients and from those patients vulnerable to arrhythmia induction.

Structural Remodeling

In addition to electrophysiological remodeling, chronic AF is also associated with structural changes in the atria. In atrial tissue from many of the patients studied, there was a significant accumulation of fatty deposits in the atria and a loss of the trabeculation of the left atria. These changes may have a significant effect on the pathways of atrial excitation and the tendency to maintain AF. We have not yet begun to quantitatively assess the structural remodeling, but we note that it was quite variable from patient to patient. It could not be easily correlated with the duration of chronic AF, the age of the patient, or the presence of valvular regurgitation. Thus, regardless of the time course of the electrophysiological remodeling, it is likely that once a patient is restored to sinus rhythm, the time required for structural remodeling of the atria (if it is even possible) will vary greatly and will likely be much slower than the electrophysiological remodeling of the individual atrial myocytes. These considerations reinforce the concept that early intervention in terminating AF may be desirable.

Postoperative AF

AF in the postoperative period is a common complication of cardiac surgery, occurring in 30% to 60% of all patients. Postoperative AF increases the risk of embolic events and stroke to the patient. It also increases the length of stay and, thus, the costs associated with being in the hospital.[22] The causes of postoperative AF are not well understood and may be mechanistically different from nonsurgically induced AF,

reflecting, in part, the response to a variety of intraoperative and/or postoperative factors associated with the overall surgical trauma. In the present study, the incidence of postoperative AF was 50%. When comparing those patients in the present study who did and who did not experience postoperative AF, we found that myocytes in both groups were of similar size and that the patients who experienced postoperative AF tended to be older but were not significantly different in age (Figure 6A). We initially anticipated that patients with a lower I_{Ca} would be more likely to sustain reentrant activity, owing to a predicted shorter wavelength. On the contrary, our results revealed (Figure 6B) a positive correlation between I_{Ca} measured at the time of surgery and the occurrence of postoperative AF. As demonstrated in Figure 6C, the myocytes with the greatest I_{Ca} were all from patients who experienced postoperative AF. By averaging the mean I_{Ca} densities for all myocytes from each patient (Figure 6D), we reduced the possibility that sampling bias from a few patients shifted the means artificially. This analysis yielded the same result. In view of the significant overlap in the Ca^{2+} current density data for most patients, we suggest that the preoperative Ca^{2+} current density is an additional factor (but clearly not the only factor) that modulates the propensity of the patient to develop postoperative AF after cardiac surgery.

It has recently been demonstrated that administration of calcium channel blockers before the initiation of AF can blunt or prevent the electrophysiological remodeling that accompanies AF.[8,9] Calcium overload has also been suggested to be an important factor in the initiation of arrhythmias.[23] The postoperative setting is one of high sympathetic tone.[24] As shown in Figure 4, catecholamines significantly increase calcium influx through L-type Ca^{2+} channels. Thus, we speculate that patients suffering from postoperative AF (with greatest I_{Ca}) may be more easily subjected to atrial calcium overload. Conversely, we speculate that patients with lower basal I_{Ca} may have a lower risk of calcium overload, potentially reducing their incidence of afterdepolarizations that could trigger abnormal activity, premature atrial contractions, or the activation of latent atrial pacemakers.[25] Finally, many of the patients in the present study had been administered beta-blocker therapy preoperatively (Table 1). Postoperative beta-blocker therapy (which would blunt the catecholamine effects on I_{Ca}) has been shown to significantly reduce the incidence of postoperative AF.[26]

Summary/Implications

Calcium influx via L-type Ca^{2+} channels plays a crucial role in atrial excitation-contraction coupling. Atrial mechanical function is impaired in chronic AF patients and returns slowly (and only partially) after the Maze procedure (as assessed with echocardiography).[27] Atrial mechanical function, as well as the amplitude of the cytosolic calcium transient, is also impaired in a canine model of AF.[28] We suggest that the electrophysiological remodeling that develops during chronic AF, resulting in significantly decreased I_{Ca}, is likely to contribute to the impairment of atrial mechanical function.

In contrast to the effects of chronic AF, some surgical patients in normal sinus rhythm with greater I_{Ca} may be predisposed to the development of postoperative AF. We suggest that calcium overload may contribute to the arrhythmogenesis in this setting, in response to the high sympathetic tone in the postoperative setting. For patients who have good cardiac function, prophylactic maintenance of these patients with beta-blocker therapy during the postoperative period would thus be logical and unlikely to present significant risk. In contrast, treatment of these patients with digoxin (for ventricular rate control after AF has appeared) seems counterintuitive, because it would further exacerbate the calcium overload of the atria. A combination of beta-blockers and/or calcium channel blockers (to slow the ventricular rate) would be a more logical treatment.

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References

1. Giardina EG. Atrial fibrillation and stroke: elucidating a newly discovered risk factor. *Am J Cardiol*. 1997;80:11D–18D, 35D–39D.
2. Hordof AJ, Edie R, Malm JR, Hoffman BF, Rosen MR. Electrophysiologic properties and response to pharmacologic agents of fibers from diseased human atria. *Circulation*. 1976;54:774–779.
3. Attuel P, Childers R, Cauchemez B, Poveda J, Mugica J, Coumel P. Failure in the rate adaptation of the atrial refractory period: its relationship to vulnerability. *Int J Cardiol*. 1982;2:179–197.
4. Boutjdir M, Le Heuzey JY, Lavergne T, Chauvaud S, Guize L, Carpentier A, Peronneau P. Inhomogeneity of cellular refractoriness in human atrium: factor of arrhythmia? *Pacing Clin Electrophysiol*. 1986;9:1095–1100.
5. Wijffels MC, Kirchof CJ, Dorland RD, Allessie MA. Atrial fibrillation begets atrial fibrillation: a study in awake chronically instrumented goats. *Circulation*. 1995;92:1954–1968.
6. Van Wagoner DR, Pond AL, McCarthy PM, Trimmer JS, Nerbonne JM. Outward K^+ current densities and Kv1.5 expression are reduced in chronic human atrial fibrillation. *Circ Res*. 1997;80:772–781.
7. Yue L, Feng J, Gaspo R, Li G-R, Wang Z, Nattel S. Ionic remodeling underlying action potential changes in a canine model of atrial fibrillation. *Circ Res*. 1997;81:512–525.
8. Tieleman RG, De Langen C, Van Gelder IC, de Kam PJ, Grandjean J, Bel KJ, Wijffels MC, Allessie MA, Crijns HJ. Verapamil reduces tachycardia-induced electrical remodeling of the atria. *Circulation*. 1997;95:1945–1953.
9. Daoud EG, Knight BP, Weiss R, Bahu M, Paladino W, Goyal R, Man KC, Strickberger SA, Morady F. Effect of verapamil and procainamide on atrial fibrillation-induced electrical remodeling in humans. *Circulation*. 1997;96:1542–1550.
10. Gaspo R, Sun H, Fareh S, Levi M, Yue L, Allen BJ, Hebert TE, Nattel S. Dihydropyridine and β-adrenergic receptor binding in dogs with tachycardia-induced atrial fibrillation. *Cardiovasc Res*. 1999;42:434–442.
11. Lai LP, Su MJ, Lin JL, Lin FY, Tsai CH, Chen YS, Huang SK, Tseng YZ, Lien WP. Down-regulation of L-type calcium channel and sarcoplasmic reticulum Ca^{2+}-ATPase mRNA in human atrial fibrillation without significant change in the mRNA of ryanodine receptor, calsequestrin and phospholamban: an insight into the mechanism of atrial electrical remodeling. *J Am Coll Cardiol*. 1999;33:1231–1237.
12. Brundel BJ, Van Gelder IC, Henning RH, Tuinenburg AE, Deelman LE, Tieleman RG, Grandjean JG, Van Gilst WH, Crijns HJ. Gene expression of proteins influencing the calcium homeostasis in patients with persistent and paroxysmal atrial fibrillation. *Cardiovasc Res*. 1999;42:443–454.

13. Bristow MR, Ginsburg R, Minobe W, Cubicciotti RS, Sageman WS, Lurie K, Billingham ME, Harrison DC, Stinson EB. Decreased catecholamine sensitivity and β-adrenergic-receptor density in failing human hearts. *N Engl J Med*. 1982;307:205–211.
14. Bristow MR. Changes in myocardial and vascular receptors in heart failure. *J Am Coll Cardiol*. 1993;22(4 suppl A):61A–71A.
15. Le Grand B, Hatem S, Deroubaix E, Couétil J-P, Coraboeuf E. Depressed transient outward and calcium currents in dilated human atria. *Cardiovasc Res*. 1994;28:548–556.
16. Li GR, Nattel S. Properties of human atrial I_{Ca} at physiological temperatures and relevance to action potential. *Am J Physiol*. 1997;272: H227–H235.
17. Courtemanche M, Ramirez RJ, Nattel S. Ionic mechanisms underlying human atrial action potential properties: insights from a mathematical model. *Am J Physiol*. 1998;275:H301–H321.
18. Wijffels MC, Kirchhof CJ, Dorland R, Allessie MA. Atrial fibrillation begets atrial fibrillation. A study in awake chronically instrumented goats. *Circulation*. 1995;92:1954–1968.
19. Ausma J, Wijffels M, Thone F, Wouters L, Allessie M, Borgers M. Structural changes of atrial myocardium due to sustained atrial fibrillation in the goat. *Circulation*. 1997;96:3157–3163.
20. Wijffels MC, Kirchhof CJ, Dorland R, Power J, Allessie MA. Electrical remodeling due to atrial fibrillation in chronically instrumented conscious goats: roles of neurohumoral changes, ischemia, atrial stretch, and high rate of electrical activation. *Circulation*. 1997;96:3710–3720.
21. Van Gelder IC, Brundel BJ, Henning RH, Tuinenburg AE, Tieleman RG, Deelman L, Grandjean JG, De Kam PJ, Van Gilst WH, Crijns HJ. Alterations in gene expression of proteins involved in the calcium handling in patients with atrial fibrillation. *J Cardiovasc Electrophysiol*. 1999;10:552–560.
22. Cooklin M, Gold MR. Implications and treatment of atrial fibrillation after cardiothoracic surgery. *Curr Opin Cardiol*. 1998;13:20–27.
23. Ravens U, Himmel HM. Drugs preventing Na^+ and Ca^{2+} overload. *Pharmacol Res*. 1999;39:167–174.
24. Kaul TK, Swaminathan R, Chatrath RR, Watson DA. Vasoactive pressure hormones during and after cardiopulmonary bypass. *Int J Artif Organs*. 1990;13:293–299.
25. Bassani RA, Bassani JW, Lipsius SL, Bers DM. Diastolic SR Ca efflux in atrial pacemaker cells and Ca-overloaded myocytes. *Am J Physiol*. 1997;273:H886–H892.
26. Ali IM, Sanalla AA, Clark V. Beta blocker effects on post-operative atrial fibrillation. *Eur J Cardiothorac Surg*. 1997;11:1154–1157.
27. Albirini A, Scalia GM, Murray RD, Chung MK, McCarthy PM, Griffin BP, Arheart KL, Klein AL. Left and right atrial transport function after the Maze procedure for atrial fibrillation: an echocardiographic Doppler follow-up study. *J Am Soc Echocardiogr*. 1997;10:937–945.
28. Sun H, Gaspo R, Leblanc N, Nattel S. Cellular mechanisms of atrial contractile dysfunction caused by sustained atrial tachycardia. *Circulation*. 1998;98:719–727.

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