Cellular Mechanisms of Atrial Contractile Dysfunction Caused by Sustained Atrial Tachycardia

Hui Sun, MD, PhD; Rania Gaspo, PhD; Normand Leblanc, PhD; Stanley Nattel, MD

Background—Transient atrial contractile dysfunction ("atrial stunning") follows conversion of atrial fibrillation (AF) to sinus rhythm and has significant clinical implications; however, the underlying mechanisms are poorly understood. We investigated the hypothesis that rapid atrial activation (as during AF) impairs cellular contractility and affects cellular Ca\(^{2+}\) handling.

Methods and Results—Edge detection and indo 1 fluorescence techniques were used to measure unloaded cell shortening and intracellular Ca\(^{2+}\) transients in atrial myocytes from control (Ctl) dogs and dogs subjected to atrial pacing at 400 bpm for 7 (P7) or 42 (P42) days. Atrial tachycardia reduced fractional cell shortening (0.1 Hz) from 7.3±0.4% (Ctl) to 4.3±0.3% and 2.0±0.3% in P7 and P42 dogs, respectively (P<0.01 for each). Resting [Ca\(^{2+}\)]\(_o\) was not altered in paced dogs, but the systolic Ca\(^{2+}\) transient was significantly reduced. Furthermore, cells from paced dogs showed slowed relaxation and use-dependent decreases of Ca\(^{2+}\) transients and cell shortening compared with cells from Ctl dogs. To determine whether changes in Ca\(^{2+}\) transients account fully for alterations in contractility, we varied [Ca\(^{2+}\)]\(_o\) to evaluate the relation between Ca\(^{2+}\) transients and cell shortening. Reductions in Ca\(^{2+}\) transients in Ctl cells reduced shortening to the level of paced cells; however, when Ca\(^{2+}\) transients in P42 cells were elevated to the range of Ctl cells, a significant reduction in cell shortening remained. Similar results were obtained in dogs that maintained 1:1 capture throughout the monitoring period and dogs that developed sustained AF over the course of the study.

Conclusions—Sustained atrial tachycardia causes important reductions in cellular contractility, in part by impairing cellular Ca\(^{2+}\) handling and decreasing systolic Ca\(^{2+}\) transients. These results provide direct evidence for the concept that AF induces atrial contractile dysfunction by causing a tachycardia-induced atrial cardiomyopathy. (Circulation. 1998;98:719-727.)

Key Words: arrhythmia ■ calcium ■ sarcoplasmic reticulum ■ cardiomyopathy

A delay in the return of atrial mechanical function after cardioversion of AF to sinus rhythm has been well recognized since the 1960s\(^{1-6}\) and most likely accounts for a delayed improvement of functional capacity.\(^7\) Recently developed pulsed Doppler techniques have shown that several weeks may be required for full atrial functional recovery after cardioversion\(^{8-11}\) and that the degree of contractile dysfunction and the time required for recovery are related to the duration of AF.\(^9\)\(^-\)\(^11\) Contractile dysfunction is mild after short-duration AF and recovery of atrial function occurs rapidly after cardioversion, whereas chronic AF produces severe dysfunction that may require up to 1 month to achieve maximal recovery.\(^9\)\(^-\)\(^11\) Prospective studies have confirmed a delayed restoration (days to weeks) of atrial contractile function after the return of normal electrical activity in almost all patients with chronic AF.\(^7\)\(^-\)\(^11\) Transient atrial dysfunction may be important in the process of atrial stunning, which is believed to play a role in thromboembolic complications among AF patients after rhythm reversion.\(^12\)\(^-\)\(^14\) The mechanisms of atrial contractile dysfunction after conversion of AF are poorly understood. Leistad et al\(^5\) showed that even short-term AF (5 minutes) was followed by impaired atrial contractility in pigs. Atrial dysfunction was reduced by exposure to the Ca\(^{2+}\) antagonist verapamil and increased by the Ca\(^{2+}\) agonist Bay K8644, suggesting that Ca\(^{2+}\) overload may play a role. Atrial ischemia did not appear to be involved. Morillo et al\(^6\) showed that rapid atrial pacing (400 bpm) for 42 days causes atrial dilation and permits the induction of sustained AF. These findings raise the possibility that the chronic rapid atrial activation of AF induces an atrial cardiomyopathy much like the well-recognized ventricular cardiomyopathy caused by chronically elevated ventricular rates.\(^15\) We have shown that atrial pacing at 400 bpm causes time-dependent reductions in the transient outward K\(^+\) current (I\(_{\text{to}}\)) and L-type Ca\(^{2+}\) current (I\(_{\text{Ca}}\)), with the latter appearing to be particularly important in causing action potential changes associated with the ability to maintain sustained AF.\(^16\) Because I\(_{\text{Ca}}\) is important in providing the
trigger for SR Ca\textsuperscript{2+} release and in maintaining the loading state of SR Ca\textsuperscript{2+} stores,\textsuperscript{19} abnormalities in IC\textsubscript{0} and/or other elements of Ca\textsuperscript{2+} handling that result from atrial tachycardia may cause contractile dysfunction. The present study was designed to use video edge-detection and microfluorimetric cellular Ca\textsuperscript{2+} measurements to determine (1) whether sustained atrial tachycardia causes impaired cellular contractility, (2) whether the systolic Ca\textsuperscript{2+} transient is altered in atrial myocytes from dogs subjected to chronic rapid atrial pacing, and (3) whether abnormal Ca\textsuperscript{2+} handling in itself can explain the cellular contractile dysfunction in rapidly paced dogs.

Methods

Preparation of the Animal Model

Adult mongrel dogs of either sex (25 to 30 kg) were anesthetized with sodium pentobarbital (30 mg/kg IV, followed by boluses of 4 mg/kg IV as needed). Artificial respiration was maintained via an endotracheal tube connected to a mechanical ventilator. Under sterile conditions, a unipolar screw-in Medtronic J pacing lead (Medtronic Inc) was inserted through the right jugular vein, and the distal end was fixed in the right atrial appendage under fluoroscopic guidance. The proximal end of the lead was connected to an implantable Medtronic pacemaker unit (model 8084) inserted into a subcutaneous pocket in the neck. After 24 hours for lead stabilization, the pacemaker was programmed to capture the atria at 400 bpm (150-ms cycle length) with 4-ms square-wave pulses at twice threshold current. The right atrium was stimulated at this rate for 7 (P7 dogs, n=6) or 42 (P42 dogs, n=6) days. The surface ECG was verified after 24 hours and then weekly to ensure atrial capture. Dogs who developed AF were not cardioverted; they were left in AF until the end of the study. Rapidly paced dogs were compared with a Ctl group (n=9), including 3 sham-operated dogs (pacemaker inserted but not activated) monitored for 7 days.

Cell Isolation and Solutions

On study days, dogs were anesthetized with morphine (2 mg/kg SC) and α-chloralose (120 mg/kg IV) and ventilated with room air supplemented with oxygen. A median sternotomy was performed, and a surface ECG and (in paced dogs) an atrial electrogram were recorded. The implanted pacemaker was then deactivated and the heart rapidly removed after an intra-atrial injection of 10,000 U heparin. The heart was immersed in Ca\textsuperscript{2+}-containing Tyrode’s solution aerated with 100% O\textsubscript{2} and warmed to 37°C. The tissue was then placed in a Tyrode’s storage solution containing 10 μmol/L Ca\textsuperscript{2+}, and the cells were dispersed by gentle trituration. The concentration of Ca\textsuperscript{2+} in the cellular supernatant was then increased to 100 μmol/L by addition of a small volume of storage solution containing 1 mmol/L Ca\textsuperscript{2+}. The cells were kept in this solution at room temperature for use within 12 hours after isolation.

The Tyrode’s solution contained (in mmol/L) NaCl 136, KCl 5.4, MgCl\textsubscript{2} 1.0, CaCl\textsubscript{2} 2.0, Na\textsubscript{2}HPO\textsubscript{4} 0.33, glucose 10, and HEPES 10, pH adjusted to 7.4 with NaOH. The nominally Ca\textsuperscript{2+}-free Tyrode’s solution had the same composition except that CaCl\textsubscript{2} was omitted. The storage solution contained (in mmol/L) NaCl 136, KCl 5.0, MgCl\textsubscript{2} 1.0, Na\textsubscript{2}HPO\textsubscript{4} 0.33, glucose 10, HEPES 10, and CaCl\textsubscript{2} 0.01 or 1.0, plus BSA 0.2%, pH adjusted to 7.35 with NaOH. The normal bath solution for experiments was the same as Tyrode’s solution except for the CaCl\textsubscript{2} concentration (1.8 mmol/L). In some experiments, Tyrode’s solution modified to contain low (0.3 or 0.9 mmol/L) or high (2.7 or 5.4 mmol/L) Ca\textsuperscript{2+} concentrations were used as specified below.

Field Stimulation and Measurement of Cell Contraction

Isolated myocytes were field-stimulated via 10-ms square-wave pulses with 1.5 times threshold amplitude. Stimuli were delivered by a stimulator (S48, Grass Instruments) via a platinum bipolar electrode on a micromanipulator. CS was measured with a video edge detector (Crossed Electronics) coupled to a charge-coupled camera mounted on the side port of the microscope. The contraction signal was sampled at 200 Hz (TL-1 A/D Converter, Axon) and delivered to an IBM-compatible computer via Axotape software (Axon). The cursors for edge detection were generally positioned at both ends of the cell to measure whole-cell shortening. Examples for 1 cell of microscopic images, along with cursor placement and the corresponding length recording, are shown in Figure 1.

Intracellular CaTs

Intracellular CaTs were recorded by a fluorometric ratio technique (indo 1 fluorescence) similar to that previously described.\textsuperscript{20} The fluorescent indicator indo 1 was loaded by incubating the myocytes at room temperature for 10 to 12 minutes with 5 μmol/L of the acetoxymethyl ester form (indo 1-AM, Molecular Probes) in 100 μmol/L Ca\textsuperscript{2+} storage Tyrode’s solution. Myocytes were then perfused with normal bath solution at room temperature for at least 40 minutes to wash out extracellular indicator and to allow for intracellular deesterification of indo 1. The loading procedure did not significantly alter CS (Table) but reduced maximal shortening and relaxation rates (+dL/dt and −dL/dt) in Ctl cells. Consequently, contractility was analyzed primarily on the basis of studies without indo 1 loading, with CS studied during stimulations to verify qualitatively comparable contractile behavior of the cells used to study CaTs. Background and cell autofluorescence were cancelled out by zeroing the output of the photomultiplier tubes using cells without indo 1 loading.

In this study, R\textsubscript{400/500} was used as the index of [Ca\textsuperscript{2+}]i, as previously described.\textsuperscript{20} This approach avoids uncertainties related to in vivo calibration of fluorescent Ca\textsuperscript{2+} indicators.\textsuperscript{21} Ultraviolet light emanating from a mercury arc lamp was used to excite indo 1. Exposure of the cell to UV light was controlled by an electronic shutter (Optikon, model T132, Vincent Associates) anchored between the arc lamp and epifluorescence attachment of an inverted Nikon Diaphot inverted fluorescence microscope (×40 Nikon oil-immersion fluor objective; numeric aperture, 1.3). The shutter could be manually triggered in the open position to verify the level of loading of indo 1 or to perform actual [Ca\textsuperscript{2+}]i measurements. Only a portion of the cell was exposed to UV light (a circular beam ~15 μm in diameter). The dye was excited at 340 nm by means of a narrow-bandwidth filter (±10 nm) and a dichroic mirror (>380 nm). The emitted fluorescent light (>380 nm) was then relayed to the lateral port of the microscope and processed by a spectral microfluorometer (Sycamore Scientific) equipped with a charge-coupled camera (Pulnix America Inc, model TM-440) and a TV monitor (JVC, model TM-122U) to
Effects of Indo 1-AM Loading on the Amplitude and Kinetics of CS

<table>
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<th>Parameters</th>
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<th>Indo 1 (+)</th>
<th>Indo 1 (+)</th>
<th>Indo 1 (+)</th>
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<td>ΔL/Lmax, %</td>
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<tr>
<td>dL/dt, μm/ms</td>
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<td>0.083±0.006*</td>
<td>0.087±0.006</td>
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<tr>
<td>−dL/dt, μm/ms</td>
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<td>0.064±0.005*</td>
<td>0.078±0.008</td>
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ΔL/Lmax indicates cell shortening normalized to resting cell length; TR1/2, time for 50% relaxation; and dL/dt, −dL/dt, maximum rate of length change during contraction and relaxation, respectively.

*P<0.01 vs indo 1–free Ctl group.
Effects of Sustained Atrial Tachycardia on Intracellular Ca²⁺ Signals

To understand the mechanisms underlying the contractile dysfunction observed in myocytes isolated from P7 and P42 dogs, we examined the effects of rapid atrial pacing on the CaT, an important determinant of cardiac contractility. Figure 3A shows representative CaT recordings from Ctl, P7, and P42 cells loaded with indo 1 and stimulated at 0.1 Hz. Resting \( R_{400:500} \) levels (numbers to left of recordings) were similar in these cells; however, the amplitude of the CaT was slightly reduced in the P7 cell and substantially reduced in the P42 cell. Overall, the mean CaT (Figure 3C) was reduced by 19% in 26 P7 cells compared with results in 27 Ctl cells (\( P<0.01 \)) and further reduced in P42 cells (\( P<0.01 \) versus Ctl), with a similar reduction for ST dogs (\( n=10 \)) and dogs that developed AF (\( n=11 \)). Resting Ca²⁺ levels were not different among groups (Figure 3D). The kinetics of the CaT were also altered by rapid pacing, as can be seen from the CaT recordings in Figure 3B, in which 5 recordings from each of the cells shown in Figure 3A have been normalized to the maximum value of each recording to control for differences in amplitude and averaged. Both the rising and relaxation phases of the Ca²⁺ signal are slowed in paced cells, particularly from the P42 dog. As shown by mean data for the TTP of the CaT corrected for the CaT amplitude (TTP/\( \Delta R_{400:500} \)) in F. Results for P42 cells are provided separately for ST dogs (\( n=10 \) cells) and for dogs that developed AF (\( n=11 \) cells). *\( P<0.01 \) vs Ctl; **\( P<0.01 \) vs P7.

Results

Effects of Sustained Atrial Tachycardia on Cellular Contractility

Figure 2A shows representative recordings of steady-state CS at 0.1 Hz from a Ctl, a P7, and a P42 cell. The amplitude of CS (\( \Delta L/L_{\text{max}} \)) is clearly decreased in the P7 cell and further reduced in the P42 cell. Overall, \( \Delta L/L_{\text{max}} \) was decreased by \(~40\%\) in P7 cells (\( n=16 \)) compared with Ctl (\( n=22 \)) and was reduced by \(~70\%\) in P42 cells, with similar results for cells from ST dogs (\( n=20 \)) and from dogs that developed AF (\( n=13 \), Figure 1B, \( P=NS \) for P42 ST versus AF cells). The maximum velocity of CS (Figure 2C) and relaxation (Figure 2D) decreased progressively in cells from paced dogs. No significant differences were noted in P42 dogs that developed chronic AF compared with those that maintained 1:1 atrial capture. These results indicate that chronic rapid activation reduced the extent of CS and decreased the rate of both contraction and relaxation.
tachycardia reduced the amplitude of Ca\(^{2+}\) release and slowed the kinetics of Ca\(^{2+}\) release and reuptake.

**Effects of Sustained Atrial Tachycardia on Postrest Staircase Phenomena**

Figure 4A shows representative recordings of CS (top) and CaT (bottom) obtained from a Ctl cell (left), a P7 cell (middle), and a P42 cell (right) stimulated at 1 Hz after a 1-minute rest period. Both the CS and CaT of the first postrest beat are larger than those at steady state, indicating postrest potentiation. All but 1 of 10 Ctl cells showed a progressive increase (positive staircase) in the amplitude of CS and the CaT after an initial decrease (negative staircase). In contrast, most P7 cells (10 of 12 cells) and all P42 cells (n=10) exhibited a monotonic and much more dramatic decrease in the amplitude of both CS and CaT during 1 minute of continued stimulation. Two P7 cells showed a small biphasic pattern, with a much lower amplitude of steady-state CS and CaT compared with Ctl cells. Mean data for CS (Figure 4B) show a significant and similar postrest reduction in CS in P7 and P42 cells compared with Ctl and a much more marked decrease in steady-state CS. The results for CaTs (Figure 4B, right) were qualitatively similar to those for CS, but the reductions in paced cells were not as striking. These observations suggest that rapid atrial activation causes abnormalities in time-dependent Ca\(^{2+}\) uptake, translocation, and/or release mechanisms, so that with repeated activation, Ca\(^{2+}\) cycling does not permit maintenance of the Ca\(^{2+}\) transient and the linked contraction process.

**Effects of Sustained Atrial Tachycardia on the Frequency Dependence of Contraction and Ca\(^{2+}\) Cycling**

Figure 5 shows representative recordings of steady-state CS (top) and CaTs (bottom) at 0.1, 0.5, and 1 Hz from a Ctl (top), a P7 (middle), and a P42 cell (bottom). At 0.1 Hz, both CS and the CaT were reduced in P7 and P42 cells compared with Ctl, and a P42 cell (bottom). At 0.1 Hz, both CS and the CaT were reduced in P7 and P42 cells compared with Ctl, as described above. When the stimulation frequency was...
increased, CS was slightly decreased in the Ctl cell (by \( \sim 25\% \)), and the CaT was unchanged. In P7 and P42 cells, however, increasing the stimulation frequency caused striking reductions in CS and the CaT. Figure 6 shows mean values of CS (Figure 6A) and the CaT (Figure 6B) obtained at 5 stimulation frequencies for each cell for 9 Ctl, 14 P7, and 10 P42 cells. CS and CaTs were moderately reduced in paced cells at low frequencies, and reductions became much more dramatic at higher frequencies, with near-steady-state values reached at 0.5 Hz. Neither CS nor the CaT was significantly frequency dependent in Ctl cells; however, both decreased substantially in paced cells as frequency increased from 0.1 to 0.5 Hz, with an overall frequency dependence that was highly significant \( (P<0.001) \). Cells from dogs subjected to longer durations of pacing showed greater abnormalities at low frequencies, but as frequency increased, CS and the CaT were very strongly and similarly reduced relative to Ctl in both P7 and P42 cells.

Relationship Between Reductions in Contractility and Ca\(^{2+}\) Handling Caused by Sustained Atrial Tachycardia

The above results show that the alterations of CS and CaT caused by chronically rapid atrial activation are qualitatively similar in terms of kinetic changes during a beat, postrest behavior, and frequency dependence. We analyzed the relations between the CaT and CS to determine whether changes in contractility of paced cells can be explained fully by abnormalities in intracellular Ca\(^{2+}\) handling. We first determined the CS-CaT relation in a group of Ctl cells by changing the superfusate \([\text{Ca}^{2+}]_0\) from 0.3 to 2.7 mmol/L. This results in gradually increasing bath \([\text{Ca}^{2+}]_0\) over several minutes, thereby gradually increasing \([\text{Ca}^{2+}]_i\), until bath and superfusate \([\text{Ca}^{2+}]_i\) equilibrate. As bath \([\text{Ca}^{2+}]_0\) increases, numerous measurements of CS and CaT can be made simultaneously in each cell, defining the CS-CaT relation for that cell. Figure 7A shows graphs of CS versus the logarithm of the peak CaT measured at 0.1 Hz in each of 8 Ctl cells. CS increases with the CaT in each cell in a similar way. When all data from all 8 Ctl cells were analyzed together (Figure 7B), the Ctl CS–versus–log CaT relation was well fit by a sigmoid relation, as classically described, providing the relation (solid curve) and 95% confidence limits (dashed curves) shown. The results obtained from P7 and P42 cells were then compared with the Ctl CS-CaT relationship, as shown in Figure 7C and 7D, respectively. The solid symbols in these figures are results in Ctl cells (reproduced from Figure 7B), the open circles represent mean results obtained from all P7 cells (1 data point per cell), with the best-fit Ctl CS-CaT curve and 95% confidence limits (solid and dashed curves, respectively) as defined by experiments shown in B. The data obtained from all P7 cells (1 data point per cell) fell within the 95% confidence limits of the Ctl CS-CaT relation (Figure 7D), the CaTs covered the entire range, and most values fell within the 95% confidence limits of Ctl cells. Although results from \( \sim 75\% \) of P42 cells \( (n=20) \) fell within the 95% confidence limits of Ctl cells, the lack of cells with higher CaT values makes it difficult to know whether contractility would be normal in these cells if CaT were normal, i.e., whether all of their contractile abnormalities can be attributed to reduced activator Ca\(^{2+}\) release on stimulation.
concentration associated with 50% of maximum CS in Ctl cells (0.647 mmol/L) was very similar to that of P42 cells (0.644 mmol/L), suggesting that the intrinsic Ca\(^{2+}\) sensitivity of the contractile apparatus was not altered but the degree of CS at any given level of activator Ca\(^{2+}\) was reduced.

**Discussion**

The present study shows that chronic rapid atrial activation, as occurs during AF, impairs the contractile performance of atrial myocytes. In addition, atrial tachycardia reduces the systolic Ca\(^{2+}\) transient in a frequency-related fashion. Similar results were obtained in cells from dogs with AF compared with those from dogs maintaining 1:1 pacing throughout the study period. The reduced Ca\(^{2+}\) transient contributes to contractile dysfunction but does not account for it completely.

**Pathogenesis of Atrial Contractile Dysfunction**

Although the delayed restoration of effective left atrial contraction after reversion of AF to sinus rhythm has been recognized for >30 years, its underlying mechanisms remain unclear. Contractile dysfunction has been thought to be related to intrinsic myocardial abnormalities and possibly to the mode of cardioversion (pharmacological versus electrical). Depressed atrial mechanical function after cessation of AF does, however, occur in patients without identifiable heart disease (lone AF), although its recovery may be faster in patients without associated structural heart disease. Furthermore, a recent study of AF patients and disease-matched control subjects has confirmed that transient atrial contractile dysfunction is due to AF per se. The impact of the mode of cardioversion on atrial mechanical function remains controversial; however, a transient depression of atrial contractility has been well documented after both pharmacological and electrical conversion to sinus rhythm. Furthermore, transthoracic electrical cardioversion to terminate ventricular tachycardia does not impair atrial contractile function.

The present study is the first of which we are aware to examine the changes in cellular contractility caused by sustained atrial tachycardia. Our results suggest that the rapid atrial activation rates in AF (generally 400 to 600 bpm) are sufficient to cause substantial impairments in atrial contractile function that in and of themselves may account for transient atrial contractile depression on reversion from AF to sinus rhythm. This idea is consistent with recent work that has shown that atrial flutter, which produces atrial rates of the order we studied, causes significant impairments in atrial contractile function. The contractile impairments we observed most likely explain the significant atrial dilation observed by Morillo et al. in dogs subjected to 6 weeks of atrial pacing at 400 bpm. Short-term AF (15 minutes) has been shown by Leistad et al. to reduce atrial contractility, a phenomenon antagonized by verapamil infusion. It is difficult to relate these findings for very brief AF to the slower-developing changes we noted. The latter are likely to involve downregulation of mRNA expression, whereas the shorter-term changes may be due to [Ca\(^{2+}\)]-induced I\(_{\text{C}}\) inhibition.

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To address this issue, we exposed a group of P42 cells (n=6) to gradually increasing bath Ca\(^{2+}\) concentrations from 1.0 to 5.4 mmol/L by increasing superfusate Ca\(^{2+}\) concentration from 1.0 to 5.4 mmol/L and making repeated simultaneous measurements of CS and CaT in each cell as bath Ca\(^{2+}\) (CaT) in each cell as bath [Ca\(^{2+}\)] (CaT) was increased. The inset in Figure 8B shows the best-fit sigmoid relation obtained in Ctl cells with that in P42 cells. Solid circles and corresponding sigmoid fit represent Ctl data from Figure 7B, and open circles and corresponding fit are for P42 data from Figure 8A. Best-fit sigmoid relations shown in B were replotted with CS values normalized to maximum CS of each sigmoid to correct for differences in maximum response. Normalized curves superimpose, indicating that changes in CaT have same relative effect on CS in Ctl and P42 cells, ie, that intrinsic sensitivity to [Ca\(^{2+}\)] is not altered.

**Figure 8.** A, Percentage CS plotted against logarithm of peak CaT obtained from 6 P42 cells exposed to varying [Ca\(^{2+}\)]. Each symbol represents a set of data obtained from 1 cell exposed to bath Ca\(^{2+}\) concentrations between 1 and 5.4 mmol/L (by increasing superfusate Ca\(^{2+}\) concentration from 1 to 5.4 mmol/L and making repeated simultaneous measurements of CS and CaT in each cell as bath [Ca\(^{2+}\)] (CaT) gradually increased). B, Comparison of CS-CaT relation obtained in Ctl cells with that in P42 cells. Solid circles and corresponding sigmoid fit represent Ctl data from Figure 7B, and open circles and corresponding fit are for P42 data from Figure 8A. Inset, Normalized CS (ΔL/L\(_{\text{max}}\)) vs CaT relation for Ctl (solid curve) and P42 (dashed curve) cells. Best-fit sigmoid relations shown in B were replotted with CS values normalized to maximum CS of each sigmoid to correct for differences in maximum response. Normalized curves superimpose, indicating that changes in CaT have same relative effect on CS in Ctl and P42 cells, ie, that intrinsic sensitivity to [Ca\(^{2+}\)] is not altered.
Cellular Mechanisms Underlying Atrial Contractile Dysfunction Caused by Atrial Tachycardia

To gain insight into the cellular mechanisms of the atrial contractile dysfunction of paced dogs, we studied the cellular Ca\(^{2+}\) transient and the response of CS and the CaT to rate change. We found that the CaT is reduced in cells from paced dogs, almost certainly contributing to reduced contractility. This observation is compatible with previously described decreases in \(I_{Ca}\) of paced dogs.\(^{18}\) Our present observations, however, point to additional abnormalities in cellular contractility and Ca\(^{2+}\) handling. When CaTs were increased to normal values in P42 cells by increasing \([Ca^{2+}]_c\), CS failed to normalize, indicating an inability of the contractile apparatus to respond normally to the CaT. This abnormality is not due to an alteration in sensitivity to Ca\(^{2+}\) per se, because the normalized CS-CaT relations of P42 and Ctl cells can be superimposed (inset, Figure 8B). The results are consistent with abnormal function of the contractile apparatus. The detailed mechanisms underlying contractile apparatus dysfunction, in terms of their biochemical and structural bases, remain to be determined. In addition to abnormalities in the amplitude of the CaT, paced cells show slowed kinetics of relaxation and markedly altered responses to repetitive activation. Some of these findings resemble those previously reported in ventricular failure\(^{29}\) and point to abnormal SR function. Important ultrastructural abnormalities have been noted by electron microscopy in the SR of rapidly paced dog atria,\(^{46}\) in keeping with our functional observations. More recent ultrastructural observations in goats with sustained AF indicate a loss of myofibrils, which may underlie the intrinsic contractile abnormalities we noted even when the CaT was normalized, along with fragmentation of the SR.\(^{30}\) The slowed relaxation of CS and the abnormal response of CS to rate increases closely parallel those of CaT, suggesting that these elements of contractile abnormality are most likely due to altered cellular Ca\(^{2+}\) handling.

Potential Limitations

We used the membrane-permeant acetoxymethyl ester form of indo 1 (indo 1-AM) to avoid the dialysis of intracellular contents that inevitably accompanies the loading of the free acid form via the pipette. Intracellular dialysis controls the cytoplasmic milieu and thus might mask possible alterations in cytoplasmic homeostasis and function caused by atrial tachycardia. In addition, cell dialysis can accelerate rundown of CS and the CaT, limiting the stability of measurements and the time intervals over which they can be meaningfully measured. Potential shortcomings of the AM form include the possibilities of intracellular compartmentation and partial deesterification of the indicator, which can alter cellular functions and limit the sensitivity of CaT measurement.\(^{28}\) We were careful to ensure adequate and equivalent loading of indo 1-AM to obtain accurate and reproducible measurements of CaTs. Although the indicator had mild effects on cellular contractility (Table), our primary analyses of CS were performed on cells not exposed to indo 1, and the same changes in CS behavior were observed in paced cells whether or not they were loaded with the indicator.

It would have been interesting to know the absolute values of \([Ca^{2+}]_c\), rather than simply expressing \([Ca^{2+}]_c\), in terms of the \(R_{440/550}\) ratio. It is possible to calibrate for \([Ca^{2+}]_c\) in aqueous solutions and then to apply this calibration to provide \([Ca^{2+}]_c\) estimates; however, the properties of all Ca\(^{2+}\)-sensitive dyes in the cytoplasm can be very different from those in aqueous solution.\(^{21}\) This can lead to considerable error in estimated \([Ca^{2+}]_c\) values, and it has therefore become common practice to express the results of fluorescent Ca\(^{2+}\)-sensitive dye studies in terms of fluorescent emission ratios (as we did), instead of as potentially misleading intracellular Ca\(^{2+}\) concentrations.

To evaluate the effects of rapid atrial activation on myofilament Ca\(^{2+}\) responsiveness, the CS-CaT relations were established for Ctl and P42 cells (Figures 7 and 8). We were unable to attain the plateau of the sigmoid relation curve, because exposing cells to higher extracellular Ca\(^{2+}\) concentrations caused frequent irregular spontaneous contractions. Nevertheless, the data were well fitted by classically described sigmoid relations\(^{22}\) that allowed for quantitative comparisons of the dependence of CS on CaT in each group of cells.

Conclusions

We have shown that sustained rapid atrial tachycardia, as occurs during AF, impairs cellular contractile function and Ca\(^{2+}\) handling. Some of the contractile dysfunction is due to an impaired systolic Ca\(^{2+}\) transient, but additional abnormalities of contractile apparatus function are also present. These findings provide the first detailed insights at the cellular level into mechanisms of the clinically relevant atrial contractile dysfunction associated with sustained AF.

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References


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