Relation Between Ventricular and Myocyte Function With Tachycardia-Induced Cardiomyopathy

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Chronic supraventricular tachycardia (SVT) causes left ventricular (LV) dilatation and dysfunction. Changes in myocyte function and structure may be important factors in the development of SVT cardiomyopathy. Accordingly, LV function and isolated myocyte structure and function were examined in six pigs with pacing-induced SVT cardiomyopathy (3 weeks at 240 beats per minute) and six control pigs. LV function was examined by simultaneous echocardiography and catheterization, and isolated myocyte function was studied using computer-assisted video microscopy. Indexes of isolated myocyte contractile performance were examined in the unloaded, unattached state (31 control and 24 SVT cells) and after attachment to a basement membrane substrate (65 control and 45 SVT cells). LV fractional shortening and peak +dP/dt significantly decreased in SVT cells compared with control cells (12±2% versus 28±2%, and 842±61 versus 1,216±119 mm Hg/sec, respectively; p<0.05). Isolated myocyte percent shortening and normalized peak velocity of shortening of SVT myocytes adherent to a basement membrane were significantly lower than attached control myocytes (1.2±0.2% versus 4.3±0.3%, and 15±2 versus 37±5% resting cell length/sec, respectively; p<0.05). Similarly, in the unattached state, the extent and velocity of shortening of SVT myocytes were reduced by over 50% from control values. Contractile properties of attached and unattached cardiocytes were also examined in the presence of 2–8 mM extracellular Ca++. For both attached and unattached SVT myocytes, responsiveness to increases in extracellular Ca++ were significantly blunted from control values. Ultrastructural examination of SVT myocytes revealed that the percent volume of myofibrils within isolated myocytes was reduced from control values (46±7% versus 65±2%, p<0.05). In summary, SVT cardiomyopathy is probably due to a primary defect in isolated myocyte contractile performance. The reduced contractile function of SVT cardiomyopathic myocytes was associated with abnormalities in cytoarchitecture and Ca2+ responsiveness. (Circulation Research 1992;71:174–187)

Key Words • cardiomyopathy • myocyte function • ultrastructure • ventricular function • tachycardia

Dilated cardiomyopathy can occur from a wide range of etiologies and is characterized by increased ventricular volumes, filling pressures, and wall stress, with significantly compromised systolic function.1,2 One particular etiology that has been the focus of recent clinical and experimental studies is that of tachycardia-induced cardiomyopathy.3–14 Previous experimental studies have demonstrated that tachycardia-induced dilated cardiomyopathy is associated with a significant decline in ventricular contractile performance.7–9 In addition, it has been reported that tachycardia-induced cardiomyopathy is associated with significant alterations in both cellular and extracellular structure.5,7,12,13 The myocardial structural abnormalities that accompany this form of dilated cardiomyopathy include reduced collagen tethering with increased perivascular fibrosis and significant interstitial edema.5,12,13 Furthermore, tachycardia-induced cardiomyopathy has been shown to cause marked changes in neurohormonal status, myocardial blood flow, and mechanical loading conditions.6–14 In light of the complex spectrum of changes that occur with the development of tachycardia-induced cardiomyopathy, it remains unclear whether specific contractile abnormalities intrinsic to the myocyte itself may contribute to this overall ventricular dysfunction. Measurements of the contractile properties of isolated adult myocytes have distinct advantages, which include the ability to examine contraction and relaxation properties of myocytes independent from the effects of the extracellular matrix and to remove in vivo hemodynamic and neurohormonal influences. Many pathological processes such as pressure-overload hypertrophy cause complex changes in both myocyte and extracellular struc-
The contractile behavior of isolated myocytes in various experimental models of hypertrophy and heart failure has been the focus of several recent reports. Mann et al. reported that the extent and velocity of sarcomere shortening were reduced with pressure-overload hypertrophy. Jones et al. reported an attenuation of isolated myocyte function after adriamycin-induced heart failure in rabbits. In light of the fact that parallel changes between ventricular and isolated myocyte function have been observed with hypertrophy and heart failure, we suspected that significant abnormalities in the contractile properties of isolated myocytes would occur with the development of tachycardia-induced cardiomyopathy. Accordingly, the overall objective of this project was to examine the structure and function of isolated myocytes subsequent to the development of tachycardia-induced cardiomyopathy.

The present study was designed to accomplish the following specific objectives: 1) to measure baseline left ventricular (LV) and isolated myocyte function of the normal and cardiomyopathic swine LV, 2) to measure the contractile properties of normal and cardiomyopathic isolated porcine myocytes to increasing concentrations of extracellular calcium, and 3) to examine the ultrastructure of these isolated myocytes to determine whether changes in myocyte function are associated with changes in cell structure.

Materials and Methods

Experimental Preparation

Twelve age- and weight-matched pigs (Yorkshire, 23–25 kg, 5 months) were used in the study. Six animals were randomly assigned to each of two groups: 1) pigs subjected to rapid atrial pacing (240 beats per minute) for 3 weeks (supraventricular tachycardia [SVT] group) and 2) sham-operated pigs (control group). Pacemakers were implanted, or sham procedures were performed; all animals were anesthetized with isoflurane (2.0%/1.5 l/min) and nitrous oxide (0.5 l/min) and intubated. Through a left thoracotomy, a shielded stimulating electrode was sutured onto the left atrium, connected to a programmable pacemaker modified for programming heart rates up to 300 beats per minute (Spectrax, Medtronic Inc., Minneapolis, Minn.), and buried in a subcutaneous pocket. The pericardium was left open, the thoracotomy was closed, and the pleural space was evacuated of air. Seven to 10 days after recovery from the surgical procedure, the protocols described above were begun. Cardiac auscultation and an electrocardiogram were performed frequently during the pacing protocol to ensure proper operation of the pacemaker and the presence of 1:1 conduction. The sham-operated control pigs were cared for in an identical fashion with the exception of the pacing protocol. All animals were treated and cared for in accordance with the “Guide for the Care and Use of Laboratory Animals” of the National Institutes of Health (National Research Council publication No. [NIH] 86-23, revised 1985).

Data Acquisition

On the day of study, the animals were brought to the laboratory, an electrocardiogram was established, and the pacemaker was deactivated (SVT group only). After a 30-minute stabilization period, the animal was anesthetized with isoflurane (0.5%1.5 l/min), and ventilated through a nonrecirculating anesthesia circuit. Ventilation parameters were adjusted to maintain a pH of 7.38–7.45, a P CO2 of 35–40 mm Hg, and a P O2 of 200–300 mm Hg. Both carotid arteries were exposed. An externally calibrated micromanometer-tipped transducer (PPG Biomedical Systems, Pleasantville, N.Y.) and a fluid-filled catheter were placed into the LV. Internal calibration of the micromanometer was performed using the fluid-filled catheter. After calibration, the fluid-filled catheter was placed in the aorta. Between studies, the micromanometer-tipped catheter was immersed in a water bath at 37°C under constant electrical excitation, then balanced, and calibrated immediately before use. Pressures from the fluid-filled aortic catheter were obtained using an externally calibrated transducer (Statham P23ID, Gould, Oxnard, Calif.) and a pressure amplifier (model 78304A, Hewlett-Packard Corp., Andover, Mass.). The electrocardiographic and pressure waveforms were recorded using a multichannel recorder (model FWR3701, Western Graphitec, Inc., Irvine, Calif.). Two-dimensional and M-mode echocardiographic studies (Ultramark VI, 2.25-MHz transducer, ATL, Bothell, Wash.) were used to image the LV from a right parasternal approach. Measurements were made using the American Society of Echocardiography criteria. Simultaneous dimensions, thickness, and pressure recordings were obtained at 100 mm/sec for subsequent digitization and analysis. LV fractional shortening was computed as (end-diastolic dimension–end-systolic dimension)/end-diastolic dimension and was expressed as a percent. Peak +dF/dt was computed by differentiating LV pressure with respect to time. Circumferential global average wall stress was computed at end systole and end diastole using a spherical model:

\[
\sigma = \frac{PD}{4h(1 + h/D)} \times 1.36
\]

where \( \sigma \) is wall stress (g/cm²), P is pressure, D is the minor axis dimension, and h is wall thickness.

Myocyte Isolation

After the collection of echocardiographic and catheterization measurements, a sternotomy was performed, and the heart was quickly extirpated and placed in an oxygenated Krebs’ solution. The great vessels were rapidly removed at the aortic and pulmonary valves, and the region of the LV free wall incorporating the circumflex artery (5×5 cm) was excised and used for myocyte isolation. This region was isolated to obtain myocytes strictly from the LV free wall. The left circumflex coronary artery was cannulated, the distal branches were ligated, and the tissue was rinsed free of blood with 35 ml modified Kraft-Bruhe (KB) solution consisting of (mM) KCl 80, K2HPO4 30, MgSO4 5, glucose 10, Na2ATP 5, taurine 20, creatine 5, succinate 5, and HEPES 5, supplemented with 5 mM nitrotriatic acid and 0.1% salt-free bovine serum albumin. Collagenase (0.5 mg/ml, type II, 146 units/mg, Worthington Biochemical Corp., Freehold, N.J.) was then added to 75 ml modified KB solution, and the tissue was perfused with the collagenase solution for 35 minutes. All perfusion procedures were performed with the tissue maintained at 37°C and perfusion solutions continuously aerated.
with 95% O_2-5% CO_2. The tissue was then minced into 2-mm sections and added to an oxygenated trituration solution of fresh KB solution containing 2% bovine serum albumin, deoxyribonuclease II (DNase, 51 units/ml, type IV, Sigma Chemical Co., St. Louis, Mo.), 300 μM CaCl_2, and collagenase (0.5 mg/ml). The tissue and trituration solution were transferred to a centrifuge tube and gently agitated. After 15 minutes, the supernatant was removed and filtered, and the cells were allowed to settle. The myocyte pellet was then resuspended in Dulbecco’s modified Eagle medium/nutrient mixture F-12 HAM (2 mM Ca^{2+}, GIBCO Laboratories, Grand Island, N.Y.).

The number of viable myocytes was counted at ×100 magnification using a hemocytometer (Reichert-Jung, Cambridge Instruments Inc., Buffalo, N.Y.) and resuspended to a final concentration of 5×10^6 cells/ml. Viable myocytes were defined as those cells maintaining a rod shape and quiescence in culture. Previous studies have reported the contractile properties of isolated myocytes in an unattached state or after attachment to a basement membrane substrate.18-21,23-25 To be able to compare the results obtained from the present study with results of these past studies, an aliquot of the isolated porcine myocytes was plated onto a basement membrane substrate. An aliquot (2 ml) of the isolated myocyte suspension was plated on coverslips previously coated with a laminin/fibronectin matrix (Matrigel, Collaborative Research Inc., Bedford, Mass.) and incubated at 37°C for 1 hour. A second aliquot of unattached myocytes was simply allowed to incubate at 37°C for 1 hour. Finally, a third aliquot of isolated cells was fixed for 30 minutes in a buffered sodium cacodylate solution containing 2% paraformaldehyde and 2% glutaraldehyde (pH 7.4, 325 mosmol) and prepared for morphometric and ultrastructural studies.

**Isolated Myocyte Function**

Isolated myocytes (attached and unattached) were placed in a thermostatically controlled chamber (37°C) that was fitted with a coverslip on the bottom for imaging on an inverted microscope (Axiovert IM35, Zeiss Inc., Oberkochen, FRG). The volume of the chamber was 2.5 ml and contained two stimulating platinum electrodes and a miniature thermocouple (model CN7100, Omega Engineering, Inc., Stamford, Conn.). The medium within the chamber was preoxygenated, and a miniature pump system (733100 Reglo, Ismatec, Switzerland) changed the medium within the chamber every 15 minutes. The myocytes were imaged using a ×20 long-working-distance Hoffmann modulation contrast objective (Modulation Optics, Inc., Greenvale, N.Y.). Myocyte contractions were elicited by field stimulating the tissue chamber at 1 Hz (model S11 stimulator, Grass Instrument Co., Quincy, Mass.) using current pulses of 5-msec duration and voltages 10% above the contraction threshold. The polarity of the stimulating electrodes was alternated at every pulse to prevent the buildup of electrochemical by-products.26 Myocyte contractions were imaged using a charge-coupled device with a noninterlaced scan rate of 240 Hz (model GPCD60, Panasonic, Secaucus, N.J.). Myocyte motion signals were captured with the cell parallel to the video raster lines and with the video signal input through an edge detector system (Crescent Electronics, Sandy, Utah). The changes in light intensity at the myocyte edges were used to track myocyte motion.27

The distance between the left and right myocyte edges were converted into a voltage signal, digitized, and input to a computer (80286, model ZBV2526, Zenith Data Systems, St. Joseph, Mich.) for subsequent analysis.

Stimulated myocytes were allowed a 5-minute stabilization period, after which contraction data for each myocyte were recorded from a minimum of 20 consecutive contractions. Parameters computed from the digitized contraction profiles included the following: percent shortening (%), peak velocity of shortening (μm/sec), peak velocity of relengthening (μm/sec), total contraction duration (msec), and time to peak contraction (msec). Myocyte percent shortening (%) was determined as the percent difference between maximum and minimum cell length for each contraction. Myocyte velocity computations were obtained by differentiating the digitized contraction profiles. The time to peak contraction was computed by calculating the time required for the velocity profile to reach zero velocity after the start of contraction. These parameters were calculated for each contraction, and the results were averaged for the 20 contractions. After the collection of baseline indexes of myocyte function, measurements were performed in the presence of incremental increases in extracellular Ca^{2+} (3–8 mM). The identical stimulation and Ca^{2+} protocol were performed for both attached and unattached cells.

**Isolated Myocyte Structure**

Isolated myocyte measurements were obtained using an automatic image analysis system (model IBAS 2000, Zeiss/Kontron, Oberkochen/Munich, FRG). The myocytes were imaged using an inverted microscope (model IM-35, Zeiss) with a phase-contrast objective (×10, PlanAchromat). The image was input into the image analysis system through a high-resolution monochrome video camera (model CCD72, Dage-MTI, Inc., Michigan City, Ind.). The images were digitized at 512×512 line resolution and 256 gray levels. Individual cell profiles were automatically discriminated by gray level. Discriminated cell profiles were edited to separate adjoining cells and exclude artifact before measurement of myocyte length, diameter, and profile surface area.

The fixed myocytes for transmission electron microscopy were rinsed in 0.1 M phosphate buffer, postosmicated for 1 hour in 1 % osmium tetroxide, and dehydrated in an ascending series of alcohols. The dehydrated cells were centrifuged at 1,000 rpm to form a pellet, and the pellet was then carefully transferred to freshly prepared Spurr’s resin (Ladd Research Industries, Inc., Burlington, Vt.). The pellet was infiltrated with the resin overnight, and the final pellet was embedded in 0.5×0.5-cm blocks. Thick sections were taken from these tissue blocks at a thickness of 1 μm, stained with toluidine blue, and viewed at ×100 to obtain areas of the tissue blocks where myofibers were oriented in a longitudinal or circumferential direction. Six tissue blocks in the circumferential orientation and three blocks in the longitudinal orientation from each pig were then used to obtain thin sections for electron microscopy. The long axis of the myocyte was considered to be where both edges of the myocyte could be clearly distinguished and the sarcomere lengths...
throughout the cell were uniform. The short axis was considered to be the orientation where the cut was made perpendicular to the long axis revealing the myofibrils in a circumferential orientation. Three grids, containing three thin sections each, were prepared from each block. Thin sections were stained with uranyl acetate and lead citrate and examined with an electron microscope (model 100S, JEOL U.S.A., Peabody, Mass.). The central portion of each section was photographed at a calibrated magnification of ×10,000. From the circumferentially oriented micrographs, the percent volume of myofibrils within myocytes was analyzed morphometrically by using a stereology sampling grid consisting of 140 sampling points. From the longitudinally oriented sections, composite electron micrographs of isolated myocytes were constructed at a precalibrated magnification of ×5,000 to measure sarcomere length, examine the organization of adjoining sarcomeres, and survey overall myocyte integrity.

A 0.25-ml aliquot of the fixed myocytes was also prepared for scanning electron microscopy. The cells were dehydrated and critical point–dried (Ladd Research Industries). The cells were mounted on 10×10-mm stubs using conductive adhesive tape (Scotch commercial tape, 3M Inc., St. Paul, Minn.) and gold sputter–coated (Hummer II, Technics, Va.). The sections were examined in a JEOL JSM-25S scanning electron microscope at an accelerating voltage of 15 kV.

Data Analysis

Hemodynamic and LV function parameters were compared between the sham control and SVT pigs using Student’s t test. Analysis of the myocyte function data was performed using the average measurements obtained for each pig, and the control and experimental groups were compared using analysis of variance. If the analysis of variance revealed significant differences, pairwise tests of individual group means were compared using Tukey’s procedure. In addition, to determine whether the myocyte function data were affected by differences in myocyte dimensions, analysis of covariance was performed on the myocyte contractile data using myocyte dimensions as covariates. Myocyte dimensions and morphometric parameters were compared between the two groups using Student’s t test. Before performing the analysis on the myocyte morphometric data, homogeneity of variances of each variable for the two groups was confirmed using Bartlett’s test. Results are presented as mean±SEM. Values of p<0.05 were considered to be statistically significant.

Results

All of the animals in the pacing protocol (SVT) group developed congestive heart failure as evidenced by dyspnea, ascites, and peripheral edema within 20–23 days of activating the pacemaker.

Left Ventricular Function

Indexes of LV function and hemodynamics at a spontaneous heart rate for the control and chronic SVT group are summarized in Table 1. There was no significant difference in the LV mass to body weight ratio between the control and SVT group. Heart rate was significantly higher and LV peak systolic pressure and peak dP/dt were significantly lower in the SVT group compared with the control group. Chronic SVT resulted in increased LV end-diastolic pressure, significantly increased LV dimensions, reduced LV wall thickness, and significantly lower fractional shortening. SVT caused significantly increased end-systolic wall stress compared with the control group. Thus, consistent with previous reports, chronic pacing-induced tachycardia produced a dilated cardiomyopathy.

Isolated Myocyte Structure

LV myocytes were successfully isolated from both SVT and sham control LVs. The average yield of viable, quiescent, rod-shaped myocytes for study in these preparations for both groups was 62±4%. There was no significant difference in the percent of rod-shaped myocytes isolated from the control or SVT LVs (66±4% versus 61±5%, respectively; p>0.35). Measurements of length, width, and surface area were obtained from 758 control and 763 SVT isolated myocytes (average, 125 cells per pig) and are shown in Table 2. SVT myocytes were 29% longer than control cells. Myocyte diameter and surface area were greater in the cells taken from the SVT LVs compared with sham control LVs.

Representative scanning electron micrographs of isolated control and SVT myocytes are shown in Figure 1. Scanning electron microscopy of control myocytes revealed a homogeneous distribution of sarcomeres.

| TABLE 1. Left Ventricular Function in Swine After Chronic Supraventricular Tachycardia |
|---------------------------------------------|-------------|-------------|
| LV wt/body wt (g/kg)                     | Control     | SVT         |
|                                           | (n=6)       | (n=6)       |
| 2.7±0.2                                   | 2.9±0.2     |
| Intrinsic heart rate (bpm)                | 94±5        | 152±6*      |
| Peak systolic pressure (mm Hg)            | 107±4       | 90±6*       |
| +dP/dt (mm Hg/sec)                        | 1,216±119   | 842±61*     |
| End-diastolic pressure (mm Hg)            | 8±2         | 28±3*       |
| End-diastolic dimension (cm)              | 3.8±0.2     | 5.3±0.2*    |
| End-diastolic wall thickness (cm)         | 0.72±0.03   | 0.65±0.02*  |
| Fractional shortening (%)                 | 28±2        | 12±2*       |
| End-systolic wall stress (g/cm²)          | 45±3        | 132±20*     |

n, Number of pigs; SVT, 3 weeks of supraventricular tachycardia at 240 beats per minute (bpm); LV, left ventricular; +dP/dt, peak positive LV dP/dt. Values are mean±SEM.

*p<0.05 vs. control.

| TABLE 2. Isolated Porcine Myocyte Morphology With Chronic Supraventricular Tachycardia |
|---------------------------------------------|-------------|-------------|
|                                             | Control     | SVT         |
|                                             | (n=6)       | (n=6)       |
| Length (μm)                                | 121±2       | 156±4*      |
| Width (μm)                                 | 21±4        | 28±5*       |
| Profile surface area (μm²)                 | 2,095±48    | 3,530±117*  |
| Myofibrillar volume (%)                    | 65±2        | 46±7*       |
| Mitochondrial volume (%)                   | 21±3        | 23±4        |

n, Number of pigs; SVT, 3 weeks of supraventricular tachycardia at 240 beats per minute (bpm). Values are mean±SEM.

An average of 125 cells per pig (758 control and 763 SVT isolated myocytes) were analyzed. Mean values for each pig were computed, and these values were used for statistical analysis and data presentation.

*p<0.05 vs. control.
invaginations and a sarcolemmal festooning pattern clearly evident along the sarcolemmal interface, similar to that reported previously.\textsuperscript{13,31} Although chronic VT caused significant changes in isolated myocyte dimensions from control values, scanning microscopy revealed an intact sarcolemma with no apparent cell membrane vacuolization or blebbing (Figure 1). High-power scanning electron micrographs of control and VT myocytes revealed an evenly distributed sarcolemma between sarcomeres with no apparent distortions or rupture (Figure 2).

Isolated myocytes were embedded for transmission electron microscopy and examined in both longitudinal and short-axis profiles. Composite electron microscopic profiles of representative control and VT myocytes obtained from this study are shown in Figure 3. In the control cells, the sarcomeres were in register throughout the myocyte with a high degree of uniformity. Ultrastructural examination of these control myocytes revealed normal-appearing myocytes with densely packed mitochondria between the myofibers. In the VT myocytes, the sarcomeres were in register throughout the cell, but a diminished number of sarcomeric units were observed (Figure 3). In addition, the well-organized cytoarchitecture observed in the control myocytes was lost; i.e., abnormal mitochondrial distribution and normal myofilament banding patterns could not be readily resolved, and swelling of transverse tubules was observed. The average sarcomere length was determined by using 180 precalibrated electron micrographs taken in the longitudinal orientation from both the control and VT groups (30 micrographs per pig). Sarcomere length in the control group was 1.85±0.03 \( \mu \)m; there was no significant difference in this value for the VT group (1.89±0.05 \( \mu \)m, \( p>0.25 \)). The values obtained in the present study are in close agreement with resting sarcomere lengths reported previously for isolated quiescent myocytes.\textsuperscript{13,18,21} A total of 330 calibrated electron micrographs of control myocytes and 350 micrographs of VT cells (minimum of 55 per pig) were prepared in circumferential orientation, and the volume percent of mitochondria and myofibrils was computed (Table 2). Within the VT myocytes, the volume percent of myofibrils was significantly reduced with no change in the volume percent of mitochondria.

A subset of 30 control and 30 VT myocytes was examined as described above after incubation in the standard growth medium (2 mM Ca\textsuperscript{2+}) for a period of 6 hours at 37°C. There were no significant differences between control or VT myocyte cell dimensions or morphology after this incubation period and those reported in Table 2 (\( p>0.55 \)). Thus, myocyte preparations from both control and VT LVs were stable for the duration of the study period. Furthermore, the cytoarchitecture and volume percent of myofibrils and mitochondria of the control and VT isolated myocytes obtained in the present study were very similar to those reported previously for intact perfusion-fixed myocardium.\textsuperscript{13,32,33} Thus, the changes in isolated myocyte structure and composition observed with VT cardiomyop-
athy were not artifactually due to the isolation procedure.

**Isolated Myocyte Contractile Properties**

*Myocytes adherent to a basement membrane substrate.* Figure 4 presents representative baseline (2 mM Ca\(^{2+}\)) contractions of a control and an SVT myocyte. Both the extent and velocity of shortening were significantly blunted in the SVT cells compared with control cells. Indexes of contractile performance of control \((n=65)\) and SVT \((n=45)\) isolated myocytes adherent to a basement membrane substrate at baseline and in the presence of increasing extracellular Ca\(^{2+}\) concentrations are summarized in Table 3. All baseline indexes of SVT myocyte function were significantly depressed from control values. The percent shortening of control and SVT adherent myocytes in the presence of increased extracellular Ca\(^{2+}\) is presented in Figure 5. Control myocytes responded in a dose-dependent fashion to increased Ca\(^{2+}\) and appeared to plateau after 6 mM Ca\(^{2+}\). In contrast, the responsiveness of the SVT myocytes to extracellular Ca\(^{2+}\) was significantly blunted as compared with the responsiveness of control cells. Although higher concentrations of extracellular Ca\(^{2+}\) provoked an increase in the percent shortening of the SVT myocytes, this parameter remained significantly lower than in control myocytes. In light of the fact that SVT myocyte length was greater than the control length, velocity of shortening and relengthening were normalized by expressing these parameters as a percent of the resting cell length.\(^{21,24,26}\) The normalized peak velocity of shortening for control and SVT adherent myocytes in the presence of increasing concentrations of extracellular Ca\(^{2+}\) is presented in Figure 6. The peak velocity of shortening increased in a dose-dependent fashion for both the control and SVT myocytes. However, the responsiveness of the SVT myocytes to Ca\(^{2+}\) was significantly reduced from control values. The peak velocity of SVT myocyte relengthening and time to peak contraction were significantly lower than control values at all Ca\(^{2+}\) concentrations. In addition, although all of the control cells responded with brisk contractions in the presence of 8 mM Ca\(^{2+}\), only 65% of the SVT myocytes that responded to electrical stimulation at 6 mM Ca\(^{2+}\) would contract in the presence of 8 mM Ca\(^{2+}\). This observation suggests that the SVT myocytes could not maintain ionic homeostasis at higher concentrations of extracellular Ca\(^{2+}\).

*Unattached myocytes.* To examine the issue that myocytes adherent to a basement membrane substrate are externally loaded and the fact that contractile parameters of myocytes have been reported in the attached and unattached state,\(^{18,21,23-27,34}\) contractile parameters were collected from control \((n=31)\) and SVT \((n=24)\) unattached myocytes. Indexes of control and SVT unattached myocyte function at baseline and in the presence of increased concentrations of extracellular Ca\(^{2+}\) are presented in Table 4. Baseline percent shortening and velocity of shortening for unattached control and SVT cells were higher than those observed at baseline for adherent myocytes \((p<0.05)\). However, baseline unattached myocyte function remained markedly lower in the SVT group than in the control group, with myocyte percent shortening reduced by 62% and peak velocity reduced by 48%. The percent shortening of unattached control and SVT myocytes in the presence of increased extracellular Ca\(^{2+}\) is presented in Figure 7. For control unattached myocytes, the extent of shortening dramatically increased after the addition of 8 mM Ca\(^{2+}\). In contrast, although the percent shortening of SVT myocytes increased with 8 mM Ca\(^{2+}\), this was not a statistically significant increase from baseline values \((p>0.25)\). At all extracellular Ca\(^{2+}\) concentrations, the percent shortening of SVT myocytes was over 50% lower than control values. The normalized peak velocity of shortening for control and SVT unattached myocytes is presented in Figure 8. Although the unattached SVT myocytes remained responsive to extracellular Ca\(^{2+}\), the normalized peak velocity of shortening remained significantly lower than the control value at all Ca\(^{2+}\) concentrations. Similar to the attached SVT myocytes, 35% of
the unattached SVT myocytes failed to respond to electrical stimulation in the presence of 8 mM Ca\(^{2+}\).

**Myocyte dimensions and function.** In addition to normalizing the extent and peak velocity of shortening and relengthening to resting myocyte length as described in the preceding paragraphs and in other studies,\(^{24,26}\) analysis of covariance was performed on these indexes of contractile function using myocyte dimensions as covariates. The analysis of covariance adjusted group mean differences for the extent of shortening and peak velocity of shortening and relengthening with respect to myocyte length, width, and surface area. When adjusted group means for control and SVT myocytes were used, this analysis revealed statistical results identical to those reported in Tables 2 and 3. Thus, a significant depression in the contractile performance of SVT myocytes was observed despite the normalization or covariate adjustment for the dimensional differences between SVT and control cardiocytes.

**Discussion**

This study examined the relation between ventricular function and isolated myocyte function with the development of SVT-induced cardiomyopathy. To our knowledge, this is the first report describing isolated myocyte function in this form of dilated cardiomyopathy. The most important findings of this study were as follows: 1) LV dilatation and dysfunction caused by chronic SVT were accompanied by a concomitant reduction in myocyte contractility. 2) This reduction in contractile performance of SVT myocytes occurred whether these cardiocytes were adherent to a basement membrane substrate or externally unloaded (unattached). 3) Probable contributory factors toward the reduction in the contractile properties of isolated SVT cardiomyopathic myocytes were alterations in cytoarchitecture. 4) The depression in SVT myocyte function...
Dilated cardiomyopathy can result from a multiplicity of factors as well as from unknown causes. The functional findings of dilated cardiomyopathy are an enlarged ventricle with little increase in wall thickness and a depressed contractile state. With increased chamber volumes, pressures, and diminished wall thickness, dilated cardiomyopathy results in increased wall stress. In the present study, chronic SVT caused a dilated cardiomyopathy with similar changes in hemodynamics and ventricular geometry seen in patients with dilated cardiomyopathy. The present study has clearly demonstrated for the first time that the depressed ventricular performance in this form of dilated cardiomyopathy is associated with reduced contractile performance of isolated myocytes. With SVT-induced cardiomyopathy, the indexes of depressed LV function (fractional shortening) and force development (peak + dP/dt) were associated with concomitant reductions in the extent and velocity of isolated myocyte shortening. In addition, results from the present study suggest that the depressed in vivo contractile state that has been reported with tachycardia-induced cardiomyopathies by this laboratory and others is probably due to a primary defect in the contractile properties of the myocyte itself.

**Myocyte Structure and Function in Other Cardiomyopathies**

Changes in ventricular performance and myocardial structure have been reported in several genetic and familial cardiomyopathies. For example, dilated cardiomyopathy results in a large ventricle with little increase in wall thickness. This pathology in the heart is associated with depressed contractile state. With increased chamber volumes, pressures, and diminished wall thickness, dilated cardiomyopathy results in increased wall stress.  

**Table 3. Indexes of Contractile Function of Attached Porcine Myocytes: Calcium Response**

<table>
<thead>
<tr>
<th>Extent of shortening (μm)</th>
<th>Baseline (2 mM Ca(^{2+}))</th>
<th>Calcium (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>5.1±0.5</td>
<td>6.6±0.4</td>
</tr>
<tr>
<td></td>
<td>1.6±0.3*</td>
<td>1.9±0.2†</td>
</tr>
<tr>
<td>Percent shortening (%)</td>
<td>Control</td>
<td>4.3±0.3</td>
</tr>
<tr>
<td></td>
<td>SVT</td>
<td>1.2±0.2†</td>
</tr>
<tr>
<td>Velocity of shortening (μm/sec)</td>
<td>Control</td>
<td>44.7±7.0</td>
</tr>
<tr>
<td></td>
<td>SVT</td>
<td>21.3±2.1†</td>
</tr>
<tr>
<td>Percent velocity of shortening (%L/sec)</td>
<td>Control</td>
<td>36.9±4.8</td>
</tr>
<tr>
<td></td>
<td>SVT</td>
<td>15.3±2.0†</td>
</tr>
<tr>
<td>Velocity of relengthening (μm/sec)</td>
<td>Control</td>
<td>47.8±5.4</td>
</tr>
<tr>
<td></td>
<td>SVT</td>
<td>21.5±1.7†</td>
</tr>
<tr>
<td>Percent velocity of relengthening (%L/sec)</td>
<td>Control</td>
<td>39.4±3.4</td>
</tr>
<tr>
<td></td>
<td>SVT</td>
<td>15.8±1.8†</td>
</tr>
<tr>
<td>Total duration (msec)</td>
<td>Control</td>
<td>453.3±17.6</td>
</tr>
<tr>
<td></td>
<td>SVT</td>
<td>393.9±52.1</td>
</tr>
<tr>
<td>Time to peak contraction (msec)</td>
<td>Control</td>
<td>236.3±14.7</td>
</tr>
<tr>
<td></td>
<td>SVT</td>
<td>146.9±19.6†</td>
</tr>
</tbody>
</table>

SVT, myocytes with supraventricular tachycardia–induced cardiomyopathy; L, resting cell length. Values are mean±SEM; n=65 for control, and n=45 for SVT.

*†p<0.05 vs. baseline.

†p<0.05 vs. control.

The velocity of shortening and the velocity of relengthening were normalized to the resting length of the myocyte.
toxin-induced animal models of cardiomyopathy. Jones et al. reported that isolated myocyte responsiveness to extracellular Ca\(^{2+}\) was reduced by over 20% after adriamycin-induced cardiomyopathy in rabbits. In myocytes isolated from the hereditary cardiomyopathic Syrian hamster, Sen et al. reported a reduction in both the extent and velocity of shortening of cardiomyopathic cells compared with control cells. In addition, these investigators reported that cardiomyopathic hamster cardiocytes responded poorly to increased extracellular Ca\(^{2+}\) and exhibited evidence of Ca\(^{2+}\) intolerance at high concentrations of extracellular Ca\(^{2+}\). Ventricular performance and myocyte structure have been reported by several investigators in naturally occurring congestive cardiomyopathy in turkeys. Changes in ventricular geometry and myocyte structure in these cardiomyopathic turkeys are very similar to those observed in the present study of SVT-induced cardiomyopathy. However, there are no data available on the contractile performance of isolated myocytes from the turkey model of cardiomyopathy. In the present study, dilated cardiomyopathy was produced in swine by chronic tachycardia, an etiology that has been clearly shown to cause cardiomyopathy in humans. Chronic pacing-induced tachycardia in this animal model produced LV chamber dilatation and dysfunction that was accompanied by increased myocyte dimensions with a reduction in the volume percent of myofibrils and a reduction in the contractile performance of these isolated myocytes. Thus, dilated cardiomyopathy induced by rapid-pacing tachycardia is associated with significant structural and functional alterations to the myocyte itself.

Resistive Load and Myocyte Function

The abnormalities in isolated contractile performance with SVT cardiomyopathy were present whether indexes of isolated myocyte contractile function were obtained for unattached myocytes or those adherent to a basement membrane substrate. The purpose of examining contractile performance of control and SVT myocytes under both conditions was twofold. First, previous studies have reported contractile properties of attached or unattached myocytes, making comparisons between studies difficult. Second, the attachment of isolated myocytes to a basement membrane substrate provided a means to determine whether isolated SVT myocytes would be able to contract under a resistive load. In a recent study by Pollack et al., feline ventricular myocyte function was examined in both unattached myocytes and cells adherent to a laminin substrate. Almost a 50% reduction in baseline myocyte percent shortening was observed for those cells adherent to the laminin substrate. This reduction in baseline myocyte function after basement membrane substrate adhesion that was observed in this past study as well as in the present work is to be expected. Isolated myocytes in suspension are externally unloaded, whereas those attached to a substrate result in some degree of loading and therefore will affect the extent and velocity of shortening. In the present study, all adherent control and SVT myocytes responded to field stimulation, demonstrating that these cells were capable of contraction under a load. However, baseline percent shortening of SVT myocytes adherent to a basement membrane substrate were 68% lower than those of attached control myocytes. Similarly, baseline peak velocity of shortening was 61% lower in SVT myocytes adherent to a basement membrane as opposed to attached control cells. In the unattached unloaded state, the baseline extent and velocity of shortening were higher in SVT myocytes than in the respective attached SVT myocytes. However, both baseline extent and velocity of shortening of these unattached SVT myocytes were reduced by over 50% from values obtained for unattached control myocytes. Although control and SVT myocytes contracted on electrical stimulation after attachment to a basement membrane substrate, this resistive load was probably
highly variable and could not be easily quantitated. Thus, the dynamic properties of myocyte contractile performance under alterations in resistive load could not be performed. Using specific changes in the viscosity of the cell media, Mann et al.\textsuperscript{18} recently reported the relation between contractile performance of isolated feline cardiomyocytes and well-defined changes in load. In light of the findings that SVT cardiomyopathy is associated with abnormalities in isolated myocyte contractile performance, a future study aimed at defining specific characteristics of SVT cardiomyopathic myocyte function with well-defined changes in loading conditions would be appropriate.

**Relation Between Ventricular and Myocyte Function**

Extrapolation of the findings of isolated myocyte function to changes in overall ventricular performance should be done with a great deal of caution. However, several findings of isolated myocyte function and structure with SVT cardiomyopathy may be paralleled to changes in ventricular function. First, the LV dilatation and wall thinning with SVT cardiomyopathy were accompanied by increased isolated myocyte length and surface area. Second, the reduced LV fractional shortening and pressure development with SVT cardiomyopathy were mirrored by a depressed extent of myocyte shortening and reduced velocity and time to peak shortening. Finally, the abnormalities in diastolic function previously reported with tachycardia-induced cardiomyopathy\textsuperscript{6,9} were associated with an attenuation in peak velocity of relengthening of isolated myocytes.

**Mechanisms for Reduced Myocyte Contractile Performance**

The potential mechanisms responsible for the depressed baseline function of SVT cardiomyopathic myocytes and the reduced inotropic responsiveness of these cells with increased extracellular Ca\textsuperscript{2+} are probably multifactorial and include 1) alterations in Ca\textsuperscript{2+} handling and homeostasis, 2) reduced myofibrillar content and alterations in cytoarchitecture, and 3) biochemical defects in sarcolemmal receptor/transport systems.

**Calcium.** There have been several studies examining the contractile properties and Ca\textsuperscript{2+} kinetics of cardiac muscle taken from patients with end-stage congestive cardiomyopathy.\textsuperscript{38,39} Using small trabeculae isolated from explanted human hearts with congestive cardiomyopathy, Gwathmey et al.\textsuperscript{38} reported a reduction in isometric peak tension development in muscle taken from these cardiomyopathic hearts. More important, these investigators reported reduced Ca\textsuperscript{2+} handling capacity of cardiac muscle taken from these cardiomyopathic hearts.\textsuperscript{38,39} Using the hereditary cardiomyopathic hamster model, Sen and colleagues\textsuperscript{20,34} have reported that

### Table 4. Indexes of Contractile Function of Unattached Porcine Myocytes: Calcium Response

<table>
<thead>
<tr>
<th>Extent of shortening (µm)</th>
<th>Baseline (2 mM Ca\textsuperscript{2+})</th>
<th>Calcium (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.6±0.8</td>
<td>7.1±0.6</td>
</tr>
<tr>
<td>SVT</td>
<td>3.2±0.4\dagger</td>
<td>4.0±0.9\dagger</td>
</tr>
<tr>
<td>Percent shortening (%)</td>
<td>5.5±0.6</td>
<td>5.4±0.5</td>
</tr>
<tr>
<td>Control</td>
<td>2.1±0.3\dagger</td>
<td>2.6±0.5\dagger</td>
</tr>
<tr>
<td>SVT</td>
<td></td>
<td>3.0±0.6\dagger</td>
</tr>
<tr>
<td>Velocity of shortening (µm/sec)</td>
<td>63.2±15.0</td>
<td>60.0±12.8</td>
</tr>
<tr>
<td>Control</td>
<td>33.0±2.9\dagger</td>
<td>40.6±6.3\dagger</td>
</tr>
<tr>
<td>SVT</td>
<td></td>
<td>45.5±7.8\dagger</td>
</tr>
<tr>
<td>Percent velocity of shortening (%/sec)\dagger</td>
<td>53.1±11.5</td>
<td>60.5±6.6</td>
</tr>
<tr>
<td>Control</td>
<td>22.0±2.8\dagger</td>
<td>28.8±5.6\dagger</td>
</tr>
<tr>
<td>SVT</td>
<td></td>
<td>33.1±7.8\dagger</td>
</tr>
<tr>
<td>Velocity of relengthening (µm/sec)</td>
<td>68.2±15.7</td>
<td>74.9±10.8</td>
</tr>
<tr>
<td>Control</td>
<td>31.9±3.7\dagger</td>
<td>39.8±6.7\dagger</td>
</tr>
<tr>
<td>SVT</td>
<td></td>
<td>45.4±10.5</td>
</tr>
<tr>
<td>Percent velocity of relengthening (%/sec)\dagger</td>
<td>56.6±12.5</td>
<td>62.2±9.1</td>
</tr>
<tr>
<td>Control</td>
<td>20.6±2.7\dagger</td>
<td>27.4±5.7\dagger</td>
</tr>
<tr>
<td>SVT</td>
<td></td>
<td>34.0±9.9\dagger</td>
</tr>
<tr>
<td>Total duration (msec)</td>
<td>523.7±55.5</td>
<td>501.6±41.4</td>
</tr>
<tr>
<td>Control</td>
<td>482.2±27.7</td>
<td>493.5±28.1</td>
</tr>
<tr>
<td>SVT</td>
<td></td>
<td>522.3±40.8</td>
</tr>
<tr>
<td>Time to peak contraction (msec)</td>
<td>251.6±30.1</td>
<td>241.2±16.2</td>
</tr>
<tr>
<td>Control</td>
<td>169.0±27.6\dagger</td>
<td>177.9±14.8\dagger</td>
</tr>
<tr>
<td>SVT</td>
<td></td>
<td>172.7±20.4\dagger</td>
</tr>
</tbody>
</table>

SVT, myocytes with supraventricular tachycardia–induced cardiomyopathy; L, resting cell length. Values are mean±SEM; n=31 for control, and n=24 for SVT.

\*p<0.05 vs. baseline.

\dagger p<0.05 vs. control.

\dagger The velocity of shortening and the velocity of relengthening were normalized to the resting length of the myocyte.
abnormalities in Ca\(^{2+}\) homeostasis accompany the depressed contractile function of isolated cardiomyopathic myocytes. Specifically, these investigators identified that defects exist in the sequestration of the intracellular Ca\(^{2+}\) pool in cardiomyopathic hamster cardiocytes. In the present study, myocytes isolated from cardiomyopathic LVs caused by chronic SVT responded poorly to increased extracellular Ca\(^{2+}\). This altered responsiveness to extracellular Ca\(^{2+}\) subsequent to the development of SVT cardiomyopathy is consistent with these past reports of altered Ca\(^{2+}\) responsiveness in failing human and hamster hearts. In addition, a large percentage of SVT cardiomyopathic myocytes failed to contract on electrical stimulation with high extracellular Ca\(^{2+}\) concentrations, which is suggestive of abnormal Ca\(^{2+}\) handling. The blunted responsiveness of the SVT cardiomyopathic myocytes to increased extracellular Ca\(^{2+}\) observed in the present study may have also been partly due to the impaired baseline contractile performance of these myocytes. Thus, the reduced responsiveness to Ca\(^{2+}\) in SVT myocytes compared with control cells may have been a function of the differences in baseline contractile performance. However, in light of past studies clearly demonstrating a defect in Ca\(^{2+}\) homeostasis with the development of cardiomyopathy,\(^{20,34-38,39}\) the depressed Ca\(^{2+}\) responsiveness and tolerance of isolated myocytes with SVT cardiomyopathy is probably due to an impairment in normal Ca\(^{2+}\) kinetics. Future studies that focus on identifying the specific defects in isolated myocyte Ca\(^{2+}\) kinetics with the development of SVT cardiomyopathy would be appropriate.

**Myocyte structure.** Potential structural mechanisms responsible for the abnormalities in myocyte function with SVT cardiomyopathy are the concomitant changes that occurred in myofibrillar content and intracellular architecture. A mechanism by which myocytes are able to meet an increased hemodynamic load is the addition of sarcomeres within existing cardiocytes.\(^{40-42}\) After both pressure- and volume-overload hypertrophy in cats, Marino et al\(^{41}\) reported that myofibril percent volume was maintained or slightly increased. Olivetti et al\(^{42}\) reported that pressure-overload hypertrophy in rats caused no significant change in the volume fraction of myofibrils within myocytes. In the present study, SVT-induced cardiomyopathy was associated with increased hemodynamic load (such as increased wall stress) and isolated myocyte size. However, unlike past studies of volume- and pressure-overload hypertrophy,\(^{40-42}\) there was not a concomitant increase in the percent volume of myofibrils. Additional changes in the cytoarchitecture with SVT cardiomyopathy observed in the present study as well as in past studies\(^{7,13,33}\) included mitochondrial and transverse tubule swelling and regional areas of myofilament disorganization. In a recent study by Sen et al,\(^{20}\) similar cytoarchitectural findings were observed in isolated cells taken from cardiomyopathic hamster hearts. These changes in cardiocyte structure were associated with a reduction in the amplitude and velocity of myocyte shortening.

In the present study, the overall disruption in normal intracellular organization and reduced myofibrillar content of SVT myocytes probably contributed significantly to the reduced myocyte contractile performance observed. The biochemical defect responsible for the failure to maintain the volume percent of myofibrils within SVT cardiomyopathic cells is unclear. It has been reported previously that SVT cardiomyopathy does not cause a loss of nuclear material and is not associated with changes in DNA content.\(^{33}\) Thus, changes in the distribution of myofibrils within SVT myocytes is probably not due to nuclear disintegration and disruption. In the present study, the reduction in the percent volume of myofibrils with the development of SVT cardiomyopathy did not appear to be accompanied by a concomitant decline in mitochondrial percent volume. One possible reason for this finding is that mitochondria within the SVT myocytes revealed high-amplitude swelling and may have masked an absolute reduction in mitochondrial number within the myocytes. A second possibility for this observation is that SVT cardiomyopathy is accompanied by selective degradation and reduced synthesis of contractile proteins. Although the reduced myofibrillar content with SVT cardiomyopathy probably contributed to the reduced isolated myocyte

![Figure 7](http://circres.ahajournals.org/)

**Figure 7.** Graph showing isolated myocyte shortening of control myocytes and myocytes with supraventricular tachycardia (SVT)–induced cardiomyopathy that were examined in the unloaded state (unattached). For both the control and SVT unattached myocytes, baseline (2 mM Ca\(^{2+}\)) myocyte shortening was higher in the unattached state vs. the attached state (p<0.05). However, the percent shortening remained significantly lower in unloaded SVT myocytes than in unattached control myocytes (p<0.05). In addition, the responsiveness to extracellular Ca\(^{2+}\) was significantly blunted in unattached SVT myocytes compared with control myocytes (p<0.05).

![Figure 8](http://circres.ahajournals.org/)

**Figure 8.** Graph showing that the peak velocity of shortening of unattached control myocytes steadily increased with increased extracellular Ca\(^{2+}\). In contrast, the peak velocity of shortening of unloaded myocytes with supraventricular tachycardia (SVT)–induced cardiomyopathy responded poorly to extracellular Ca\(^{2+}\); this value remained significantly lower than that of control unattached myocytes (p<0.05).
function observed, other cytoarchitectural changes may have also contributed toward the reduced contractile function of the SVT myocytes. Specifically, increased cellular edema and alterations in cytoskeletal proteins may have caused increased internal load, thereby resisting shortening of the SVT myocytes. Thus, the structural basis for the alterations in isolated myocyte performance with the development of SVT cardiomyopathy may be due to both a reduction in contractile protein content and an increased internal resistive load.

In the present study, the suggestion that an absolute reduction in myofibrillar content occurs within SVT myocytes and is therefore a primary mechanism for reduced contractile performance is probably an oversimplification. Although the percent volume of myofibrils decreased with SVT cardiomyopathy relative to control cells, SVT isolated myocyte volumes were expanded, as evidenced by increased SVT cardiomyocyte dimensions. In a past report using geometric modeling, isolated SVT cardiocyte volume was increased by approximately 33% above control values. The 29% reduction in the volume percent of myofibrils within SVT myocytes observed in the present study may be solely due to a concomitant increase in SVT isolated myocyte volume. Thus, absolute myofibrillar content within SVT myocytes may be unchanged. A future study specifically designed to quantitate changes in contractile proteins within the SVT cardiomyopathic cell by using biochemical methods and by more carefully measuring isolated SVT myocyte volumes (e.g., by Coulter counter methodologies) would be appropriate. What is clear from the present study, however, is that SVT-induced cardiomyopathy resulted in an increased myocyte surface area with a reduction in relative myofibril content per unit area of myocyte. Although isolated myocyte size increased with SVT cardiomyopathy, the extent and velocity of shortening of these myocytes decreased. Thus, the capacity of these cells to generate force on a per unit basis was probably reduced.

Sarclemmal defects. It has been clearly established that tachycardia-induced cardiomyopathy is associated with significant abnormalities in neurohumoral systems. These changes include increased renin, angiotensin, and plasma catecholamines. Recent studies have clearly shown that tachycardia-induced heart failure is accompanied by significant alterations in the sarclemmal β-adrenergic system itself. Furthermore, this laboratory and others have identified that the Na⁺,K⁺-ATPase system is altered with the development of tachycardia-induced cardiomyopathy. Specifically, SVT cardiomyopathy is associated with a reduction in the number of sarclemmal cardiac glycoside binding sites and decreased catalytic activity of Na⁺,K⁺-ATPase. The Na⁺,K⁺-ATPase system is a major mechanism by which intracellular sodium Na⁺ is controlled. If intracellular Na⁺ homeostasis is altered with SVT cardiomyopathy, then this may in turn affect Ca²⁺ handling by way of the sarclemmal Na⁺/Ca²⁺ exchange system. Thus, alterations in the Na⁺,K⁺-ATPase system with SVT cardiomyopathy may contribute to the abnormal extracellular Ca²⁺ responsiveness and tolerance of the SVT myocytes that were observed in the present study. Future studies aimed at using this isolated myocyte preparation to examine specific abnormalities in sarclemmal receptor systems and ion channels subse-

quent to the development of SVT cardiomyopathy would seem appropriate.

Myocyte Dimensions and Function

It has been clearly established that changes in isolated myocyte dimensions occur after a wide variety of cardiac pathologies. For example, Mann et al reported a significant increase in myocyte surface area after pulmonary artery banding in cats. Similarly, Olivetti et al observed a significant increase in myocyte diameter and surface area after pulmonary artery banding in rats. Gerdes et al reported increased isolated myocyte length and volume after the development of volume-overload hypertrophy in rats. Isolated myocyte length and width have been reported to increase in cardiocytes taken from hereditary cardiomyopathic hamster hearts. To determine whether changes in isolated myocyte dimensions alone might have influenced cell function with SVT cardiomyopathy, the extent and peak velocity of shortening were normalized to resting cardiocyte length. These normalized values further amplified the depressed extent and peak velocity of shortening of the SVT myocytes compared with control cells. In addition, analysis of covariance was performed to adjust for any potential effects that changes in myocyte dimensions alone may have had on indexes of myocyte contractile performance with SVT cardiomyopathy. This analysis revealed that the depressed extent of shortening and peak velocity of shortening and relengthening with SVT cardiomyopathy were present independent of the changes in myocyte length, width, and surface area. The finding that abnormalities in contractile properties of isolated myocytes occur independent of changes in myocyte dimensions is not unique and has been reported previously.

Myocyte Isolation and Function

In the present study, the contraction velocities obtained for control porcine myocytes are very similar to those reported for isolated cardiocytes taken from human, rabbit, and sheep. The structure and composition of these isolated control myocytes are very similar to those reported previously for perfused intact swine myocardium. We have reported previously that the perfusion and isolation methods used in the present study provide a high yield of Ca²⁺-tolerant control and SVT myocytes for study. As in these past studies, the yield of Ca²⁺-tolerant myocytes isolated from SVT cardiomyopathic ventricles in the present study was similar to the yield obtained from control preparations. Scanning electron microscopy revealed that the sarclemma of these SVT myocytes was grossly intact and absent of blebbing and vacuolization. In addition, SVT myocytes remained quiescent and responded to electrical stimulation after 6 hours in a culture medium containing 2 mM Ca²⁺. Thus, it is unlikely that the changes in contractile performance of isolated myocytes taken from SVT cardiomyopathic ventricles was simply due to some artifact of the isolation procedure alone.
Extracellular Influences on Ventricular Function

Although alterations in myocyte function with tachycardia-induced cardiomyopathy may contribute significantly to the overall ventricular dysfunction, abnormalities in LV function with tachycardia-induced cardiomyopathy may also be due to alterations in loading conditions, changes in extracellular matrix composition, and myocardial blood flow. In the present study as well as previous reports, hemodynamic alterations such as increased end-diastolic pressure and end-systolic wall stress have been observed with the development of tachycardia-induced cardiomyopathy.\textsuperscript{7-9,12,13,33} However, analysis of end-systolic stress-volume relations with tachycardia-induced cardiomyopathy have suggested a reduction in contractile state independent of these changes in ambient loading conditions.\textsuperscript{8,9} We have reported previously that SVT myocytes have reduced adhesion capacity to specific basement membrane components.\textsuperscript{13} Further, tachycardia-induced cardiomyopathy is associated with disruption of the collagen struts between adjacent myocytes.\textsuperscript{5,12} These changes in basement membrane attachment and collagen tethering with SVT cardiomyopathy may result in a failure to translate myocyte shortening (albeit reduced) into a coordinated ventricular contraction. It has been reported previously that, although the determinants of myocardial oxygen demand (such as increased basal heart rate and LV wall stress) were higher than in control animals, myocardial blood flow was reduced.\textsuperscript{14} This reduction in myocardial blood flow may significantly affect ventricular performance with SVT cardiomyopathy. In the present study, the contractile performance of isolated SVT myocytes was depressed despite superfusion with oxygenated media and independent of neurohormonal and extracellular influences. Thus, although the changes described above may contribute to the overall decline in ventricular dysfunction with chronic tachycardia, the alterations in myocardial contractility observed in previous reports are probably primarily due to depressed contractile performance of the myocyte itself.

Summary

For the first time, the present study has directly demonstrated that the ventricular dilatation and dysfunction caused by chronic SVT is directly associated with a primary defect in isolated myocyte contractile function. These abnormalities in SVT myocyte contractile performance were present irrespective of whether the cells were adherent to a basement membrane substrate or externally unloaded (unattached). These abnormalities in isolated myocyte contractile performance with SVT cardiomyopathy were associated with blunted responsiveness to extracellular Ca\textsuperscript{2+} and alterations in cytoarchitecture.

References


Relation between ventricular and myocyte function with tachycardia-induced cardiomyopathy.

F G Spinale, B M Fulbright, R Mukherjee, R Tanaka, J Hu, F A Crawford and M R Zile

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