Relation Between Ventricular and Myocyte Remodeling With the Development and Regression of Supraventricular Tachycardia–Induced Cardiomyopathy

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Chronic supraventricular tachycardia (SVT) causes left ventricular (LV) dilatation and dysfunction. Termination of SVT appears to reduce LV size and improve function. However, changes in myocyte structure and morphology that accompany the development and regression of SVT-induced cardiomyopathy have not been studied. Accordingly, we measured LV function using echocardiography and catheterization in three groups of six pigs each: 3 weeks of atrial pacing (SVT; 240 beats/min), 4-week recovery from SVT (PST), and sham-operated controls. At each of these three end points, isolated myocyte dimensions and nuclear number were measured using fluorescence, and the volume percent of myocytes and myofibrils was computed from tissue sections using stereological techniques. SVT resulted in reduced LV fractional shortening (15±3% versus 31±2%, p<0.05), increased end-diastolic dimension (5.6±0.8 versus 3.8±0.2 cm, p<0.05), and no change in mass (2.6±0.1 versus 2.6±0.2 g/kg, p=NS) compared with controls. Myocyte length significantly increased with SVT (171±9 versus 109±11 μm, p<0.05), without significant changes in cell width (28±2 versus 26±2 μm). Nuclear number did not change with SVT; however, nuclear area/myocyte area significantly increased compared with controls (9.5±0.8 versus 8.7±0.8x10^{-2}, p<0.05). The volume percent of myocytes within the ventricular wall and the volume percent of myofibrils within myocytes decreased with SVT compared with controls (72±3% versus 80±3% and 45±5% versus 63±4%, respectively, p<0.05), with no change in total myocyte volume (54.2±2.7 versus 54.3±1.8 μm^{3}x10^{12}). In the PST group, LV fractional shortening returned to control values; however, there was persistent dilatation (end-diastolic dimension: 4.2±0.1 cm, p<0.05), and LV hypertrophy developed (3.3±0.3 g/kg, p<0.05). Increased myocyte length (158±5 μm, p<0.05) and width (33±2 μm, p<0.05) were observed in the PST group. The volume percent of myocytes and myofibrils returned to control values, with total myocyte volume significantly increased in the PST group compared with the control and SVT groups (74.5±2.6 μm^{3}x10^{12}, p<0.05). In addition, the number of nuclei per myocyte in the PST group significantly increased from control values (5.1±0.1 versus 4.0±0.1, p<0.05). In summary, significant myocyte remodeling occurred concomitant with changes in LV morphology and function. Despite significant LV dilatation and increased myocyte size with chronic SVT, a reduction in total myocyte volume and myofibril content resulted in no LV hypertrophy. Recovery from SVT-induced cardiomyopathy resulted in LV and myocyte hypertrophy and nuclear hyperplasia. (Circulation Research 1991;69:1058–1067)

Chronic supraventricular tachycardia (SVT) results in dilated cardiomyopathy in humans and animals.1–5 Recent experimental studies suggest that recovery is associated with hypertrophy and persistent ventricular dysfunction.3,6,7 We have reported recently that chronic SVT causes a dilated congestive cardiomyopathy in swine.3 In addition, a preliminary report from our laboratory demonstrated...
that termination of SVT in these animals resulted in improved ventricular function but was associated with persistent ventricular dilatation and the development of hypertrophy. The myocyte is the cellular component of the ventricle responsible for active force production. Studies in animal models of hypertension or aortic stenosis have shown that in response to the chronic pressure overload, the myocyte hypertrophies, as manifested by an increase in cell surface area and volume. These changes in myocyte structure are closely associated with changes in ventricular structure and function. For example, pressure overload increases ventricular wall thickness, causes no change in ventricular chamber volume, and significantly increases ventricular mass.

Because parallel changes between ventricular and myocyte morphology occur during pressure overload, we suspected that cellular and ventricular changes would parallel each other during the development and regression of tachycardia-induced cardiomyopathy. However, the precise nature of the remodeling that occurs with respect to the ventricular myocytes with the development of ventricular dysfunction and dilatation due to chronic SVT and after termination of the tachyarrhythmia is not known. Therefore, the purpose of this study was to examine the relation between ventricular and myocyte structure during the development and regression of tachycardia-induced dilated cardiomyopathy.

Materials and Methods

Experimental Preparation

Eighteen age- and weight-matched pigs (Yorkshire, 23–25 kg, 4 months old) were used in the study. Six animals were randomly assigned to each of three groups: 1) rapid atrial pacing (240 beats/min) for 3 weeks (SVT); 2) atrial pacing at 240 beats/min for 3 weeks, followed by deactivation of the pacemaker and a 4-week recovery period (PST); and 3) sham-operated controls. All animals were treated and cared for in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Pacemakers were implanted or sham procedures were performed with animals anesthetized with isoflurane (2.0% at 1.5 l/min) and nitrous oxide (0.5 l/min). Animals were intubated, and 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/min (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 animals had recovered 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. Sham-operated controls were cared for in an identical fashion, with the exception of the pacing protocol.

Data Acquisition

On the day of study, 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% at 1.5 l/min) and ventilated through a nonrecirculating anesthesia circuit. Ventilation parameters were adjusted for maintenance of a pH of 7.38–7.45, a PCO2 of 35–40 mm Hg, and a PO2 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 left ventricle. Internal calibration of the micromanometer was performed with 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 with an externally calibrated transducer (Statham P231D, Gould Instruments, Oxnard, Calif.) and a pressure amplifier (78304A, Hewlett-Packard Co., Andover, Mass.). The electrocardiogram and pressure waveforms were recorded with a multichannel recorder (FWR3701, Western Graphtec, Inc., Irvine, Calif.).

Two-dimensional and M-mode echocardiographic studies (Ultramark VI, 2.25-mHz transducer, Advanced Technology Laboratories, Bothell, Wash.) were used to image the left ventricle from a right parasternal approach. Measurements were made with criteria from the American Society of Echocardiography. Simultaneous dimension, thickness, and pressure recordings were obtained at 100 mm/sec for subsequent digitization and analysis. Left ventricular (LV) fractional shortening was computed as (end-diastolic dimension–end-systolic dimension)/(end-diastolic dimension) and was expressed as a percent. Peak +dP/dt was computed by differentiating LV pressure with respect to time. Circumferential, global average wall stress (σ, in grams per square centimeter) was computed at end systole and end diastole with a spherical model:

\[ σ = \left( \frac{PD}{4h \sqrt{1 + h/D}} \right) × 1.36 \]

where P is pressure, D is the minor axis dimension, and h is wall thickness.

After echocardiographic and catheterization measurements were collected, a sternotomy was performed, the heart was quickly extirpated and placed in a phosphate-buffered ice slush, and the coronary arteries were flushed. The great vessels were removed at the aortic and pulmonary valves, and the left ventricle was weighed quickly. The region of the LV free wall incorporating the circumflex artery (5×5 cm) was excised
and prepared for myocyte isolation. From the remainder of the LV free wall, three full-thickness sections (2×2 cm) were taken, immersed in 10% buffered formalin for 3 hours, and then transferred to 70% ethanol. These sections were then processed for staining and stereological analysis. In addition, three full-thickness sections (2×2 mm) were minced finely, immersed for 2 hours in a buffered sodium cacodylate solution containing 2% paraformaldehyde and 2% glutaraldehyde solution (pH 7.4, 750 mosm), and were prepared for electron microscopy. Finally, a 2×4-cm section was taken for the measurement of water content. These sections were weighed, placed in a 37°C oven, dried for 48 hours, and reweighed. Water content was determined as (wt wt−dry wt)/(wt wt) and is expressed as a percent.

**Ventricular Section Analysis**

**Light microscopy.** The tissue was processed for light microscopic examination through graded ethanols, was cleared in xylenes, and was embedded in paraffin. Slices 4 μm in thickness were cut from the blocks, mounted on glass slides, and stained with hematoxylin and eosin for morphometric analysis. These sections were mounted on an inverted microscope (IM-35, Zeiss, Oberkochen, FRG), and circumferentially oriented myocytes along the subendocardium were imaged using an epifluorescence illuminator with a rhodamine filter at a magnification of ×630. The image then was analyzed with the computer image analysis system described above. Epifluorescence provided a high contrast between the myocytes and the extracellular space, allowing for simple and reliable digitization of the myocyte profiles. Ten random fields of circumferentially oriented myocytes along the subendocardial layer were examined from each slide and digitized. The sample field area was 7,600 μm², and the system was calibrated with a stage micrometer before each digitizing session. Three slices at 1, 2, and 3 mm deep from each tissue block were examined. With this method, nine full-thickness sections from the left ventricle of each animal were examined. With computer-aided stereology, the volume percent of myocytes occupying the LV free wall was computed.15

The total LV myocardial volume was computed by dividing LV weight by the specific gravity of muscle tissue: 1.06 g/ml.16 Total myocyte volume was computed as the product of LV myocardial volume and the morphometrically determined percent area of myocytes.15 Total water content was determined in a similar fashion.

**Electron microscopy.** Tissue sections fixed for electron microscopy were rinsed in 0.1 M phosphate buffer, postosmicated for 1 hour in 1% osmium tetroxide, dehydrated in an ascending series of alcohols, and embedded in Spurr’s resin (Ladd Research Industries, Inc., Burlington, Vt.). 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 and three blocks in the longitudinal orientation from the left ventricle of each pig then were used to obtain thin sections for electron microscopy. 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 a 100S electron microscope (JEOL U.S.A., Inc., Peabody, Mass.). The central portion of each section was photographed at a calibrated magnification of ×10,000. These electron micrographs then were coded, and this code was not broken until the completion of the study. From the circumferentially oriented micrographs, the percent volume of myofibers within myocytes was analyzed morphometrically by using a stereology sampling grid consisting of 140 sampling points.8,15 The total volume of LV myofibers was computed as the product of total myocyte volume (computed from light microscopic morphometry) and the percent volume of myofibers.

**Myocyte Isolation and Examination**

Myocytes were isolated using a modified version of a previously described method.17 The left circumflex coronary artery was cannulated and the tissue rinsed free of blood with 35 ml of a modified Krebs-Ringer (KB) solution (millimolar concentration: KCl 80, K2HPO4 30, MgSO4 5, glucose 10, Na2ATP 5, taurocholate 20, creatine 5, succinate 5, and HEPES 5, supplemented with 5 mM nitritotriacetic acid and 0.1% salt-free bovine serum albumin).18 Collagenase (0.5 mg/ml, Worthington type II; 146 units/mg) then was added to 75 ml of the modified KB solution, and the tissue was perfused with the collagenase solution for 35 minutes. The tissue then was minced into 2-mm sections and was added to an oxygenated trituration solution of fresh KB solution containing 2% bovine serum albumin, deoxyribonuclease II (DNase, 51 Kunitz units/ml, type IV, Sigma Chemical Co., St. Louis, Mo.), 300 μM CaCl2, and 0.5 mg/ml collagenase. The tissue and trituration solution was 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 then was resuspended in Dulbecco’s modified Eagles medium: nutrient mixture F-12 (GIBCO Laboratories, Grand Island, N.Y.) supplemented with transferrin (5 μg/ml), insulin (5 μg/ml), sodium selenite (5 ng/ml), 3,3',5-triiodo-L-thyronine (4 pg/ml), hydrocortisone (36 ng/ml), bovine serum albumin (3%), and DNase (71.4 Kunitz units/ml). The number of cells was counted at ×100 magnification with a hemocytometer (Reichert-Jung, Cambridge Instruments Inc., Buffalo, N.Y.).

Three coverslips were coated with Matrigel (Collaborative Research Inc., Bedford, Mass.) and incubated at 37°C for 1 hour. Isolated cells (5×10⁶ cells/ml) were plated onto the treated coverslips and incubated at 37°C. After 1 hour, the coverslips were irrigated with modified KB solution to remove unattached myocytes. Only those cells attached to the
substrate media were examined to minimize orientation and imaging artifacts. Morphometric measurements then were performed with an IBAS 2000 Automatic Image Analysis System (Zeiss/Kontron, Oberkochen, FRG). The myocytes were imaged with the Zeiss IM-35 inverted microscope with a phase-contrast objective (×10, PlanAchromat). The image was input into the image analysis system through a series 68 high-resolution monochrome video camera (Dage-MTI, Inc., Michigan City, Ind.). The images were digitized at a line resolution of 512×512 and 256 gray levels. Individual cell profiles were automatically discriminated by gray level. Discriminated cell profiles were edited to separate adjoining cells and exclude artifacts before measurement of myocyte length, diameter, and profile surface area. The volume of the digitized myocyte profile then was computed with methods previously described.\(^{19,20}\) The myocyte was digitally sectioned into 1-μm increments perpendicular to the long axis of the cell. The volume of each of these segments then was computed based on a cylindrical frame of reference. Total myocyte volumes were estimated as the sum of these individual volumes of rotation about the long axis of the cell.\(^{20}\) From this computed isolated myocyte volume, the total number of LV myocytes was determined by dividing the total myocyte volume by the isolated myocyte volume.\(^{15,19}\)

After the myocyte measurements, the plated coverslips were immersed in 4′,6-diamidino-2-phenylindole (DAPI, 50 μg/ml) for 30 minutes, washed, and imaged using 420 nm epifluorescence. DAPI is a polycationic fluorescent stain that binds strongly to the adenine-thymidine–rich regions of nuclear DNA.\(^{21–23}\) Using this DNA fluorophore and image analysis techniques, nuclear number and surface area were computed. Nuclear area was normalized as the ratio of nuclear surface area to myocyte surface area. A minimum of 50 randomly selected myocytes from each coverslip was analyzed. In a pilot study, the number of nuclei per myocyte was manually counted in 50 control cells and then subjected to DAPI staining and computer analysis. The computed number of nuclei per myocyte was identical with both methods.

**Data Analysis**

Indexes of ventricular function were compared among the three groups with a one-way analysis of variance. Analysis of the morphological data was performed with the average measurements obtained for each animal, and the three groups were compared with analysis of variance. A pilot study of three control hearts that used the morphometric sampling techniques described above resulted in less than an 8% coefficient of variation for the parameters computed in this study. Before the analysis of variance on the morphometric data was performed, homogeneity of variances of each morphometric variable for the three groups was confirmed with Bartlett’s test.\(^{24}\) If the analysis of variance revealed significant differences, pairwise tests of individual group means were compared using Tukey’s procedure.\(^{24}\) Nuclear number per myocyte is a discrete variable, and therefore a frequency distribution for the total number of myocytes analyzed for this parameter was constructed. Changes in the number of nuclei per myocyte among the three groups were examined using \(\chi^2\) analysis.\(^{24}\) Results are presented as mean±SEM. Values of \(p<0.05\) were considered statistically significant.

**Results**

All of the animals in the pacing protocol developed congestive heart failure as evidenced by dyspnea, ascites, and peripheral edema within 17–20 days of pacemaker activation. These symptoms and signs resolved in all of the animals in the PST group. Echocardiographic–catheterization studies were performed successfully in all of the animals.

**Ventricular Function**

Table 1 summarizes the ventricular function data obtained from the three groups. LV fractional shortening significantly decreased after 3 weeks of chronic SVT, and after a 4-week recovery period, fractional

**Table 1. Left Ventricular Function With Chronic Supraventricular Tachycardia and Recovery**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>SVT</th>
<th>PST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intrinsic heart rate (beats/min)</td>
<td>108±6</td>
<td>143±12</td>
<td>110±6</td>
</tr>
<tr>
<td>LV wt/body wt (g/kg)</td>
<td>2.6±0.2</td>
<td>2.6±0.1</td>
<td>3.3±0.3†</td>
</tr>
<tr>
<td>End-systolic pressure (mm Hg)</td>
<td>85±4</td>
<td>81±6</td>
<td>87±5</td>
</tr>
<tr>
<td>+dP/dt (mm Hg/sec)</td>
<td>1,425±118</td>
<td>800±147*</td>
<td>1,575±254†</td>
</tr>
<tr>
<td>End-diastolic pressure (mm Hg)</td>
<td>11±1</td>
<td>32±2*</td>
<td>15±1†</td>
</tr>
<tr>
<td>End-diastolic dimension (cm)</td>
<td>3.8±0.2</td>
<td>5.6±0.8*</td>
<td>4.2±0.1†</td>
</tr>
<tr>
<td>Fractional shortening (%)</td>
<td>31±2</td>
<td>15±3*</td>
<td>31±5</td>
</tr>
<tr>
<td>End-diastolic wall thickness (cm)</td>
<td>0.8±0.1</td>
<td>0.6±0.1*</td>
<td>1.1±0.2†</td>
</tr>
<tr>
<td>End-systolic wall stress (g/cm²)</td>
<td>37±3</td>
<td>120±20*</td>
<td>35±3†</td>
</tr>
<tr>
<td>End-diastolic wall stress (g/cm²)</td>
<td>14±1</td>
<td>66±13*</td>
<td>17±3†</td>
</tr>
</tbody>
</table>

Values are mean±SEM. SVT, 3 weeks of supraventricular tachycardia; PST, 3 weeks of supraventricular tachycardia and 4-week recovery period; LV, left ventricular; +dP/dt, peak positive LV dP/dt.

\(*p<0.05\) vs. control.

†\(p<0.05\) vs. SVT.
shortening returned to control values. LV end-diastolic pressure, dimension, and wall stress all were significantly increased after 3 weeks of chronic SVT compared with controls. In the PST group, LV end-diastolic pressure, dimension, and wall stress decreased compared with SVT values ($p<0.05$). However, end-diastolic dimension remained significantly higher than controls. Although chronic SVT resulted in significant chamber dilatation, wall thickness decreased, with no change in the LV wt/body wt ratio. In contrast, termination of SVT and a 4-week recovery period resulted in a significant increase in LV wall thickness and mass compared with controls.

**Myocardial and Myocyte Morphology**

Table 2 summarizes results from the morphological examination of LV sections taken from the three groups. Absolute LV myocardial volume significantly increased in the SVT group as compared with controls. However, this increase in myocardial volume with SVT was accompanied by a significant reduction in the percent volume of myocytes occupying the LV wall. Thus, the increased LV myocardial volume and the decreased percent volume of myocytes in the SVT group resulted in no significant change in total LV myocyte volume from control values. The percent volume of myofibrils significantly decreased with SVT compared with control values. This reduction in myofibrillar percent volume in the SVT group resulted in a significant decline in total LV myofibril volume compared with the control group. Myocardial water content significantly increased with SVT as compared with controls. Therefore, the increased absolute LV myocardial volume with chronic SVT was associated with significantly reduced LV myofibril volume and significantly increased myocardial water content.

In the PST group, absolute LV myocardial volume significantly increased from both control and SVT values. The percent volume of myocytes increased from SVT values and was not significantly different from controls. The increased myocardial volume in conjunction with a normalization of the percent volume of myocytes resulted in a 37% increase in total myocyte volume in the PST group. Similarly, the percent volume of myofibrils in the PST group returned to control values and resulted in a significantly increased total LV myofibril volume compared with the control and SVT groups. Despite the significant increase in LV myocardial volume, there was no significant difference in myocardial water content between the PST and SVT groups. Thus, in contrast to the SVT group, the significantly increased absolute LV myocardial volume in the PST group was associated with significantly increased LV myocyte and myofibril volumes, with no change in myocardial water content.

Myocytes were isolated successfully from all hearts obtained in this study, yielding an average of 62±6% quiescent, rod-shaped myocytes for study from each group. Figure 1 shows representative photomicrographs of myocytes harvested from control, SVT, and PST left ventricles. Table 3 summarizes data from the myocyte morphometric analysis. Myocytes from SVT hearts were significantly longer, with no significant difference in cell diameter. The increased myocyte length in the SVT group resulted in a higher cell surface area compared with control myocytes, but this difference did not reach statistical significance ($p=0.25$). This increased myocyte length resulted in significant increased computed myocyte volume in the SVT group compared with controls. The increased isolated myocyte volume and reduced total LV volume of myocytes with chronic SVT resulted in a significant reduction in the computed number of myocytes within the LV wall compared with controls. After termination of SVT (PST), cell length fell significantly from SVT values but remained significantly higher than controls. In the PST group, myocyte diameter and profile surface area all were significantly higher than control values. Myocyte diameter and surface area were greater in the PST group compared with the SVT group, but this did not reach statistical significance ($p=0.18$). These changes in myocyte diameter and length in the PST group resulted in a significantly increased cell volume from both the control and SVT groups. The total number of LV myocytes in the PST group was very similar to the SVT value and was significantly lower than control values. Thus, chronic SVT resulted in an increase in isolated myocyte volume but a reduction in the total number of myocytes within the LV wall.

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**Table 2. Left Ventricular Myocardial Morphology With Chronic Supraventricular Tachycardia and Recovery**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>SVT</th>
<th>PST</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventricular weight (g)</td>
<td>72±3</td>
<td>79±4</td>
<td>97±3*</td>
</tr>
<tr>
<td>Total myocardial volume (cm³)</td>
<td>67.9±2.3</td>
<td>74.4±3.4*</td>
<td>89.7±3.1†</td>
</tr>
<tr>
<td>Myocyte percent area (%)</td>
<td>80±3</td>
<td>72±3*</td>
<td>83±5†</td>
</tr>
<tr>
<td>Total myocyte volume (µm³×10³)</td>
<td>54.3±1.8</td>
<td>54.2±2.7</td>
<td>74.5±2.6*†</td>
</tr>
<tr>
<td>Myofibril percent area (%)</td>
<td>63±4</td>
<td>45±5*</td>
<td>64±5†</td>
</tr>
<tr>
<td>Total myofibril volume (µm³×10³)</td>
<td>33.6±2.8</td>
<td>23.8±3.2*</td>
<td>46.7±3.9†</td>
</tr>
<tr>
<td>Myocardial percent water (%)</td>
<td>73±4</td>
<td>82±6*</td>
<td>75±5†</td>
</tr>
<tr>
<td>Total myocardial water (ml)</td>
<td>49.6±2.3</td>
<td>63.2±2.6*</td>
<td>65.6±3.1*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. SVT, 3 weeks of supraventricular tachycardia; PST, 3 weeks of supraventricular tachycardia and 4-week recovery period.

*p<0.05 vs. control.

†p<0.05 vs. SVT.
Early recovery from SVT cardiomyopathy resulted in a further increase in myocyte volume, with no change in myocyte number from SVT values.

Nuclear number and the nuclear area/myocyte area ratio are shown for the three groups in Table 3. In control myocytes, the mean number of nuclei per

<table>
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<tr>
<th>Table 3. Isolated Myocyte Morphology With Chronic Supraventricular Tachycardia and Recovery</th>
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<tr>
<td></td>
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<tr>
<td>----------------------------</td>
</tr>
<tr>
<td>Length (µm)</td>
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<tr>
<td>Diameter (µm)</td>
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<tr>
<td>Profile surface area (µm²)</td>
</tr>
<tr>
<td>Computed volume (µm³×10³)</td>
</tr>
<tr>
<td>Total number myocytes (×10⁸)*</td>
</tr>
<tr>
<td>Number nuclei per myocyte</td>
</tr>
<tr>
<td>Nuclear area/myocyte area (×10⁻²)</td>
</tr>
</tbody>
</table>

Values are mean±SEM. SVT, 3 weeks of supraventricular tachycardia; PST, 3 weeks of supraventricular tachycardia and 4-week recovery period.

*Myocyte number was computed using calculated isolated myocyte volume and total myocyte volume value from Table 2 (see “Materials and Methods” section for details).

†*p<0.05 vs. control.

‡*p<0.05 vs. SVT.
myocyte was four. Nuclear number did not increase significantly with SVT; however, the nuclear area/myocyte area ratio significantly increased. In the PST group, nuclear number significantly increased, and the nuclear area/myocyte area ratio was significantly reduced from both control and SVT values. Figure 2 presents a percentile frequency distribution of nuclear number per myocyte for the cells counted in this study. The percentage of cells with four nuclei significantly decreased, and the percentage of cells with eight nuclei significantly increased in the PST group \((p<0.05)\).

**Discussion**

The most important findings of this study were that 1) chronic SVT resulted in LV dysfunction and dilatation that was accompanied by significant changes in myocyte structure and myocardial composition, 2) termination of SVT subsequent to the development of dilated cardiomyopathy improved LV function but was accompanied by ventricular and myocyte hypertrophy, and 3) increased nuclear area occurred with SVT, but LV myocyte and myofibrillar volumes decreased, whereas regression from SVT-induced cardiomyopathy was associated with nuclear hyperplasia and significantly increased LV myocyte and myofibrillar volumes.

The combination of functional and morphometric studies made it possible to relate structural changes in the ventricular chamber to changes in cell structure. This study demonstrated that chronic SVT in swine resulted in LV dysfunction and dilatation that was accompanied by significant alterations in myocyte composition and morphology. Chronic SVT produced significant chamber dilatation and increased wall stress, but no change in the LV wt/body wt ratio occurred. The ventricular remodeling associated with SVT is manifested by increased LV chamber and myocardial volume, decreased wall thickness, and lengthening of the myocytes within the free wall. Although absolute LV myocardial volume increased with chronic SVT, total LV myocyte volume, number, and myofibrillar content decreased. Thus, the changes in myocyte composition and structure observed in this study may help explain the LV dilatation and dysfunction observed with chronic tachycardia.

The LV dilatation accompanying chronic SVT did not appear to be associated with chamber hypertrophy. Myocytes from SVT hearts were longer, with a significantly increased cell volume. Although isolated myocyte volume increased with SVT, total LV myocyte volume remained unchanged from control values. These changes in isolated myocyte volume and chamber composition resulted in a significant reduction in the computed number of myocytes within the LV wall. These results suggest that chronic SVT resulted in significant myocyte loss. This laboratory previously presented that chronic SVT is associated with subendocardial fibrosis.\(^5\) This past finding and results from the present investigation suggest that chronic SVT caused myocyte injury and subsequent myocyte loss. In the present study, absolute LV myocardial mass and volume increased with chronic SVT. However, this increased LV mass was accompanied by a reduction in myofibrillar volume, no change in total LV myocyte volume, and increased myocardial water content. Thus, the increased LV mass with chronic SVT most likely was not due to an augmentation of contractile elements but to increased myocardial edema.

Chronic SVT resulted in an approximate 32% increase in myocyte volume from control cells. However, the increased cell volume with SVT did not result in chamber hypertrophy, as evidenced by reduced LV wall thickness and no change in total LV myocyte volume or in the LV wt/body wt ratio. The failure of this myocyte volume expansion with chronic SVT to be translated into significant LV hypertrophy probably was due to several factors, including 1) a significant reduction in myocyte number, 2) myocyte slippage within the LV free wall, and 3) reduced cellular composition of myofibrils. The significant reduction in myocyte number with chronic SVT most probably masked the increased myocyte volume at the organ level. The reduction in LV wall thickness with SVT concomitant with increased myocyte volumes suggests that cellular slippage within the ventricular wall also may have contributed to the LV architectural changes with SVT. Side-to-side slippage of myocytes in association with ventricular dilatation recently has been reported after myocardial infarction in rats.\(^{25}\) We recently have reported that SVT myocytes lose the capacity to bind to basement membrane components.\(^{26}\) These previous results provide a potential mechanism for the myocyte slippage that may have occurred with SVT cardiomyopathy. Finally, the increased myocyte volume with SVT probably was not due to an augmentation of contractile elements, because myofibrillar content significantly decreased. Rather, the volume expansion of the SVT myocytes probably was due to cellular edema as evidenced by the increased myocardial water content.

Termination of SVT subsequent to the development of LV dysfunction and dilatation resulted in the
development of LV hypertrophy and a normalization of LV function and wall stress. Although LV function improved with termination of SVT, there was persistent chamber dilatation. These changes in LV chamber architecture occurred concomitantly with changes in myocyte morphology. In the PST group, the increased LV mass was accompanied by a normalization of the volume percent of myocytes within the LV and the volume percent of myofibrils within the myocytes. These changes in LV mass and composition resulted in significantly increased LV myocyte and myofibrillar volumes in the PST group. In contrast to the SVT group, the increased LV mass in the PST group was due to an augmentation of myocyte volume and contractile elements. These increased LV myocyte and myofibrillar volumes provide a cellular basis for the improvement in LV function observed during early recovery from SVT-induced cardiomyopathy.

In the PST group, isolated myocyte diameter significantly increased, and length remained significantly longer than controls. These changes in myocyte dimensions resulted in a significantly increased myocyte volume from control and SVT values. In contrast to SVT myocytes, the volume expansion of myocytes in the PST group was not due to cellular edema but to significantly increased myofibrillar content. The increased LV mass and total myocyte volume in the PST group probably was not due to significant myocyte hyperplasia, because there was no change in total LV myocyte number from SVT values. Results from this study revealed that augmentation of cell diameter and a persistent lengthening of the myocytes within the LV free wall occurred with the recovery from SVT-induced cardiomyopathy. Therefore, the cellular basis for the changes in LV architecture that occurred after termination of SVT probably were due to 1) increased LV wall thickness associated with an expansion of myocyte diameter and volume, and 2) the residual LV dilatation associated with a persistent increase in myocyte length.

Because the myocyte is the major component responsible for force production and shortening in the heart, changes in structure and volume of myocytes occupying the ventricular wall will result in changes in the capacity for force production and changes in ventricular function. The myocyte remodeling that accompanies changes in hemodynamic loading conditions has been a subject of a large body of research.8–11,27–35 To our knowledge, however, there have been no reports on myocyte remodeling with the development and regression from dilated cardiomyopathy. A mechanism for myocytes to meet an increased hemodynamic load is the addition of sarcomeres within existing cardiocytes. Most studies have focused on the relation of ventricular shape to myocyte morphology during the hypertrophic response associated with pressure- or volume-overload states.8–11,27–35 Marino et al11 observed significant cellular remodeling after chronic right ventricular pressure overload in cats. These investigators found that the proportional volume of myocytes was decreased and that of connective tissue increased with pressure-overload hypertrophy. Olivetti et al10 observed a significant increase in right ventricular wall thickness and myocyte diameter after pulmonary artery banding in rats. This previous report and others have suggested that hypertrophy may be mediated not only by increased cell volume but also by myocyte hyperplasia.10,35 Loud et al8 reported a significant increase in LV mass with a concomitant increase in myocyte volume after chronic hypertension in rats. Smith et al10 examined isolated myocyte morphology after renal hypertension in rats. These investigators found a significant increase in myocyte volume. In these studies, as well as others,27–30 ventricular compensation for a chronic, sustained increase in load was accomplished by an increase in chamber mass and wall thickness, lateral expansion of the myocytes, and perhaps, in certain instances, myocyte hyperplasia. In contrast, no compensatory hypertrophy occurred with chronic SVT, resulting in chamber dilatation and reduced wall thickness and a consequent increase in wall stress. Unlike pressure overload, the higher wall stress that occurred with chronic SVT was accompanied by reduced LV wall thickness, no change in total LV myocyte volume, reduced myofibrillar volume, and reduced myocyte number. It is unclear from this study why there is a failure of a hypertrophic response with chronic tachycardia despite the significant increases in ventricular wall stress. This lack of compensatory hypertrophy may act in an unending cycle in which continued increases in wall stress further exacerbate the chamber dysfunction. In contrast, termination of SVT resulted in the development of compensatory hypertrophy and normalization of wall stress. Marino et al21 reported regression of right ventricular hypertrophy after debanding of the pulmonary artery in cats. Termination of SVT subsequent to the development of dilated cardiomyopathy resulted in the development of myocyte and chamber hypertrophy and persistent LV dilatation. The chamber and myocyte architectural changes that occurred with resolution from SVT-induced cardiomyopathy are very similar to those changes seen during compensated volumo- or pressure-overload hypertrophy.28,29,32

In the present study, we used a fluorescent probe that specifically binds to DNA21–23 to examine nuclear number and area. With the development of SVT-induced cardiomyopathy, nuclear number remained unchanged; however, a significant increase in the nuclear area staining for DNA within the SVT myocytes occurred. Despite this apparent augmentation in nuclear material, there was an absence of chamber and myocyte hypertrophy. In volume or pressure overload, there may be an increase in nuclear area and number concomitant with an increase in myocyte dimensions and contractile elements, resulting in ventricular hypertrophy.28,34 The mechanism by which the initial mechanical message, such as wall stress, is translated by the myocytes into DNA synthesis remains an unresolved question. However, it is clear that increased load
on the myocardium will result in increased protein synthesis and growth of the existing myocytes and therefore must involve changes in nuclear DNA. Results from the present study suggest that SVT-induced cardiomyopathy resulted in increased nuclear area; however, a failure of a compensatory response of the SVT myocytes occurred. This increased nuclear area most probably resulted from both increased DNA content and increased nuclear number that occurred with SVT cardiomyopathy.

To our knowledge, this is the first study reporting that nuclear hyperplasia occurs during the recovery from dilated cardiomyopathy. Grove et al.27 reported increased [3H]thymidine uptake during the development of myocardial hypertrophy in the rat. These investigators reported that the sites of increased DNA synthesis were in cells located in the interstitium and not within the myocytes themselves. These findings led to the belief that nuclear number and content within the myocyte remain unchanged during the development of hypertrophy. However, more recent studies have reported increased nuclear number, nucleoli, and area in various animal models of hypertrophy.28,33,34 In the spontaneously hypertensive rat, Clubb et al.30 reported that an increase in myocyte length and nuclear number occurred during spontaneous development of hypertrophy in the neonatal rat. Interestingly, these investigators reported these changes occurring before significant increases in systemic blood pressure. Olivetti et al.31 reported nuclear hyperplasia after pulmonary artery banding in rats. Anversa et al.32 reported that nuclear proliferation occurred after long-standing renal hypertension. Hatt et al.28 reported a significant increase in cell volume and the number of nucleoli per nucleus after creation of aorta-caval fistulae in rats. Although nuclear hyperplasia now appears to occur in animal models of hypertension, it also has been reported to occur in humans. Postmortem studies performed in humans have indicated that when the heart is subjected to a chronic load, hyperplasia of myocyte nuclei may occur.35 In the present study, recovery from SVT-induced cardiomyopathy resulted in nuclear hyperplasia concomitant with a significant increase in myocyte volume and LV mass. The nuclear hyperplasia that occurs with termination of SVT most likely is associated with increased DNA synthesis. Therefore, unlike in SVT-induced cardiomyopathy, the increased nuclear number was associated with a compensatory increase in myocyte and myofibrillar volumes, increased LV wall thickness, and a normalization of wall stress.

The findings of this study with respect to nuclear morphology were unexpected. First, it appears that normal swine myocytes contain four nuclei. This is in contrast to cytometric studies of human hearts, which have shown the myocytes to be primarily mononucleated and binucleated.36–38 The majority of studies examining nuclear number and DNA content have been performed in the rat heart during normal growth as well as in several disease states.8,27,28,33,34 In the normal rat heart, binucleated myocytes commonly are seen, and multinucleated cells also are observed.33,34,39 Most researchers examining nuclear content and number within the myocardium in normal and pathological states have used full-thickness sections and stereological techniques.8,10,28,33–39 In the present study, nuclei were counted from isolated myocytes with the use of a DNA-specific stain, thus allowing direct computation of nuclear number and density. A limitation of the present study is that isolated myocyte volumes were computed from digitized dimension data. This method may have overestimated actual myocyte volumes as compared with more accurate methods such as the Coulter systems.40 However, the important finding from this analysis was that the relative isolated myocyte volume significantly increased with the development and regression of SVT-induced cardiomyopathy.

Damiano and colleagues3 reported that chronic SVT resulted in significant LV chamber dilatation and reduced ejection fraction in dogs. Wilson and associates41 demonstrated that ventricular tachycardia in dogs produced increased left atrial pressures and increased chamber volumes when compared with controls. Recently, we serially examined ventricular function in swine after the induction of SVT and reported that biventricular failure occurred with no change in mass. This present study builds on these previous studies by demonstrating that LV dysfunction and dilatation is accompanied by significant myocyte remodeling. Howard et al.42 reported significant chamber dilatation based on echocardiography after rapid ventricular pacing in dogs. More importantly, these investigators reported that termination of tachycardia resulted in hypertrophy and residual chamber dilatation. Our study differs from these previous studies in that atrial pacing was used, and ventricular pressure and dimensions and myocyte composition and morphology were examined. This present study extends the work of previous studies by demonstrating that chronic SVT not only results in LV dilatation and reduced pump performance but is accompanied by significant alterations in myocyte composition and morphology within the ventricular wall. Furthermore, regression from SVT-induced cardiomyopathy results in chamber and myocyte hypertrophy and nuclear hyperplasia.

In summary, chronic SVT produced in swine results in LV dilatation and dysfunction, with elongation of the myocytes within the LV wall. Although increased nuclear area occurs with SVT, there is an absence of a hypertrophic response. Termination of SVT resulted in nuclear hyperplasia and the development of myocyte and ventricular hypertrophy. This hypertrophic response after termination of SVT resulted in normalization of LV function. Thus, changes in LV function and morphology that occur with the development and regression of SVT-induced cardiomyopathy are associated with significant changes in myocyte structure, morphology, and nuclear content.
References


Key Words • cardiomyopathy • myocytes • hypertrophy • nuclear hyperplasia
Relation between ventricular and myocyte remodeling with the development and regression of supraventricular tachycardia-induced cardiomyopathy.
F G Spinale, J L Zellner, M Tomita, F A Crawford and M R Zile

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