Alterations in Myocyte Shape and Basement Membrane Attachment With Tachycardia-Induced Heart Failure


Chronic supraventricular tachycardia (SVT) results in left ventricular (LV) dilatation and dysfunction. However, the underlying mechanisms responsible for LV failure in this setting are not known. LV force production is dependent on the coupling of myocytes to the extracellular matrix, which is mediated through the basement membrane. This study was designed to determine whether alterations in myocyte geometry and basement membrane attachment are associated with LV failure in a pacing-induced model of cardiomyopathy. Echocardiographic measurement of LV function was performed in six pigs after 3 weeks of pacing-induced SVT (240 beats/min) and in eight sham-operated controls. Myocytes from these hearts were isolated, and attachment studies to specific components of the basement membrane were performed using laminin, fibronectin, and collagen IV. The SVT group when compared with the control group showed a significant reduction of LV fractional shortening (14±2% versus 31±2%, respectively; p<0.05), increased end-diastolic dimension (50±1 versus 35±1 mm, respectively; p<0.05), and lengthening of isolated myocytes (196±18 versus 142±9 μm, respectively; p<0.05). Myocyte attachment to laminin (50 μg/ml) was significantly decreased at 60 minutes in the SVT group compared with the control group (18.2±4.5 versus 60.9±4.5 cells/mm², respectively; p<0.05). Similar reductions in myocyte attachment to fibronectin and collagen IV were observed. Ultrastructural examination of LV sections revealed focal disruptions of the basement membrane–sarcolemmal interface and a reduced number of sarcolemmal festoons in SVT hearts compared with control hearts (0.8±0.6 versus 2.8±0.8/4 μm, respectively; p<0.05). These alterations in myocyte morphology and basement membrane attachment may contribute to the LV failure associated with chronic SVT. Further, these structural changes may play a significant role in the progression of ventricular dysfunction as well as recovery from chronic SVT. (Circulation Research 1991;69:590–600)

Chronic supraventricular tachycardia (SVT) has been previously linked to the development of congestive cardiomyopathy. Recent laboratory studies have shown that chronic SVT directly results in significant chamber dilatation, wall thinning, and biventricular dysfunction. However, the cause of this SVT-produced dilated cardiomyopathy remains unclear.

The myocyte is the elemental component of the heart that is responsible for force generation. Transduction of this force into mechanical pump performance is dependent on the interrelation between the myocyte and extracellular matrix. The extracellular matrix of the heart is a three-dimensional network that, until recently, was thought to serve primarily as a scaffolding for the myocyte. However, it is now evident that the role of the extracellular matrix is far more complex and dynamic. Specifically, the extracellular matrix ensures proper myocyte alignment during diastole, coordinates myocyte contraction during systole, and maintains capillary patency throughout the cardiac cycle.

Myocyte attachment to the extracellular matrix is mediated through the basement membrane, which surrounds the cell and serves as an extracellular anchor between the myocyte and the connective tissue latticework. Components of the basement

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membrane include collagen IV, laminin, and fibronectin. The importance of the basement membrane in maintaining myocyte shape and positional integrity was recently demonstrated in a study by Lundgren et al. in which enzymatic removal of the basement membrane resulted in cardiac myocytes becoming spherical in shape. Recent investigations have shown that connections among the myocyte, components of the basement membrane, and collagen network are colocalized around the Z band. This region has been suggested to be the site of mechanical transmission of forces across the sarcolemma.

Thus, alteration of the myocyte attachment to basement membrane components may produce myocyte slippage and misalignment within the ventricle, resulting in diminished force production.

The purpose of this study was to examine whether alterations in myocyte geometry and basement membrane attachment are associated with left ventricular (LV) failure in a pacing-induced model of cardiomyopathy.

Materials and Methods

Surgical Procedure

Sixteen age- and weight-matched pigs (Yorkshire, 28.0±1.58 kg) were used in this study. All pigs received humane care in accordance with the guidelines of the National Research Council for the care and use of laboratory animals (DHEW Publication No. [NIH] 78-23, revised 1978). Eight pigs were randomly assigned to each of two groups: 1) pigs subjected to rapid atrial pacing (240 beats/min) for 3 weeks (SVT group) and 2) sham-operated control pigs. The pigs were anesthetized with isoflurane (2.5%, 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/min (Spectrax, Medtronic Inc., Minneapolis, Minn.), and buried subcutaneously. The pericardium was left open, the thoracotomy was closed, and the pleural space was evacuated of air. Seven days after surgery, the SVT group had the pacemaker activated for atrial pacing at 240 beats/min for a duration of 3 weeks. An electrocardiogram (ECG) was obtained every 3 days during the pacing protocol to ensure that 1:1 conduction was present. The sham-operated controls (thoracotomy and pericardiotomy only) were fed and cared for in an identical fashion, with the exception of the pacing protocol.

Hemodynamic and ECG Data Acquisition

On the day of the study, ECGs were established, pacemakers were deactivated, and the pigs were allowed to return to a normal sinus rhythm. After a 30-minute stabilization period, the pigs were anesthetized with isoflurane (0.5%, 1.5 l/min), placed in a supine position, and allowed to breathe spontaneously through a nonrecirculating anesthesia circuit. An externally calibrated micromanometer-tipped transducer (PPG Biomedical Systems, Pleasantville, N.Y.) was positioned in the LV through an exposed carotid artery. LV pressures were recorded using a pressure amplifier (model 78394A, Hewlett-Packard Co., Andover, Mass.) and a multichannel recorder (model FWR3701, Western Graphtec, Inc., Irvine, Calif.).

A two-dimensional echocardiograph (Irex System III, Ramsey, N.J.) with a 2.5-mHz transducer was used to image the LV from a right parasternal approach. Minor-axis dimensions and wall thickness measurements were performed by established methods using two-dimensional directed M-mode in the short-axis view just below the mitral valve leaflets. End diastole was defined as the onset of the QRS complex, and end systole was defined as the minimal dimension. Fractional shortening was computed as the difference between the LV end-diastolic and end-systolic dimensions divided by the end-diastolic dimension and expressed as a percent. Circumferential, global average wall stress was computed at end systole and end diastole by 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.

After completion of hemodynamic and ECG recordings, a median sternotomy was performed. The heart was extirpated, immediately flushed, and immersed in a calcium-free, oxygenated Krebs' solution. A 5×5-cm section of the LV free wall was excised with the left circumflex coronary artery in place, quickly weighed, and prepared for myocyte isolation. Three 2×2-mm full-thickness sections of the LV free wall were removed, and the endocardial and epicardial layers were trimmed away and prepared for electron microscopy. In addition, a 2×4-cm section was also taken from the LV for the measurement of water content. These sections were weighed, placed in a 37°C oven and dried for 48 hours, and reweighed. Water content was determined as (wet weight−dry weight)/wet weight and was expressed as a percent.

Preparation of Isolated Myocytes

Cardiac myocytes were isolated using a modified version of a previously described method. Briefly, the left circumflex coronary artery was cannulated, and the tissue was rinsed free of blood with 35 ml modified Kraft-Bruhe (KB) solution containing (mM) KCl 80, K2HPO4 30, MgSO4 5, glucose 10, Na2ATP 5, taurine 20, creatine 5, succinate 5, and HEPES 5, supplemented with 5 mM nitrilotriacetic acid and 0.1% salt-free bovine serum albumin (BSA). Collagenase (146 units/mg) (0.5 mg/ml, type II, 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. The tissue was then minced into
2-mm sections and added to an oxygenated trituration solution of fresh KB solution containing 2% BSA, deoxyribonuclease II (DNase, 51 Kunitz units/ml, type IV, Sigma Chemical Co., St. Louis, Mo.), 300 μM CaCl₂, and collagenase (0.5 mg/ml).

The tissue and trituration solution were transferred to a centrifuge tube and gently agitation. 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 (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), and DNase (71.4 Kunitz units/ml). The number of cells was counted at ×100 magnification using a hemocytometer (Reichert-Jung, Cambridge Instruments Inc., Buffalo, N.Y.).

Myocyte Morphology

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. Morphometric measurements were then performed with an automatic image analysis system (model IBAS 2000, Zeiss/Kontron, Oberkochen/Munich, FRG). The myocytes were imaged with an inverted microscope (model IM-35, Zeiss, Oberkochen, FRG) with a phase-contrast objective (×10, PlanAchromat). The image was entered into the image analysis system through a high-resolution monochrome video camera (series 68, Dage-MTI, Inc., Michigan City, Ind.). The images were digitized at 512x512 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 maximum and minimum diameters. A minimum of 50 randomly selected rod-shaped myocytes from each coverslip were analyzed.

Extracellular Matrix Binding Study

Cell attachment studies were performed as described by Borg et al.²⁸ Multiwell dishes (16 mm, Costar Corp., Cambridge, Mass.) were coated with laminin (courtesy of Dr. T.K. Borg, University of South Carolina, Columbia, S.C.), fibronectin (Sigma Chemical), and collagen IV (GIBCO) at concentrations of 5, 10, 20, and 50 μg/ml. The isolated myocytes were then plated onto the coated multiwell dishes at a density of 7.5×10⁴ cells/well and allowed to attach to the substrates for 5, 15, and 60 minutes. At the indicated times, unattached cells were removed, and the wells were washed in triplicate with modified KB solution. As a control, myocytes were plated on collagen I at 50 μg/ml for 30 minutes. The number of attached rod-shaped cells was determined by counting five random fields at ×150 magnification (1 mm²/field) in each well.

Electron Microscopy

The LV sections were finely minced and immersed in a buffered 2% paraformaldehyde and 2% glutaraldehyde solution (750 mosm) for 2 hours, rinsed in 0.1 M phosphate buffer, postfixed for 1 hour in 1% osmium tetroxide, dehydrated, and embedded in Spurr’s resin (Ladd Research Industries, Inc., Burlington, Vt.). In addition, tannic acid was added to improve the preservation and electron density of the polyanionic collagen latticework as described by Robinson et al.¹⁶ Thin sections were stained with uranyl acetate and lead citrate and examined with an electron microscope (model 100S, JEOL U.S.A. Inc., Peabody, Mass.). The central portion of each section was examined thoroughly, and low- and high-magnification electron micrographs were taken of the extracellular space between adjacent myocytes. Six random electron micrographs of longitudinally oriented myocytes were taken from each section, printed at a calibrated magnification of ×5,000, and coded. This code was not broken until the end of the study. From these electron micrographs, the number of festoons occurring along the sarcolemma was determined. This computation was expressed as the number of festoons per 4 μm sarcolemma. In addition, LV sections were also prepared for examination using scanning electron microscopy. Samples were fixed as described above and postfixed in osmium tetroxide. The sections were then dehydrated in graded ethanol and critical point-dried (Ladd Research Industries). The samples were then freeze-fractured using liquid nitrogen, mounted on a stub, 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.

Statistical Analysis

Hemodynamic and volumetric comparisons between SVT and control groups were performed using an unpaired Student’s t test. Morphological data were averaged for each pig in the control and SVT groups, and comparisons were performed using Student’s t test. A repeated-measures analysis of variance was performed to determine the effect that different substrate concentrations and incubation times had on myocyte attachment. If the analysis of variance revealed significant differences, pairwise comparison of group means was performed using Tukey’s procedure.²⁹ Results are presented as mean±SEM. Values of p<0.05 were considered statistically significant.

Results

Two pigs from the SVT group died of congestive heart failure before completion of the 3-week tachycardia protocol. Therefore, data were obtained and myocytes were isolated from a total of six pigs in the chronic SVT group and eight pigs in the control
group. Autopsies performed on the chronic SVT pigs revealed a pathology consistent with severe congestive heart failure. Gross pathology of the SVT pigs revealed significant hepatomegaly, peripheral edema, and ascites (650±50 ml). Histopathologic examination of sections taken at autopsy revealed pulmonary edema within the alveolar space of the lung, and examination of the centrilobular zone revealed hemorrhagic necrosis within the liver.

**Ventricular Function**

Fractional shortening was significantly lower after 3 weeks in the SVT group as compared with the control group (p<0.05, Figure 1). Table 1 summarizes the LV pressure and dimension data obtained in this study. A significant reduction in LV wall thickness was noted in the pigs that were subjected to chronic SVT when compared with control pigs (p<0.05). LV end-diastolic dimension and pressure were significantly increased in the SVT group compared with the control group (p<0.05). These changes in LV geometry and pressure resulted in significantly increased circumferential wall stress (p<0.05). The LV weight/body weight ratio was not significantly different between the two groups (p>0.55).

**Myocyte Isolation and Morphometry**

Chronic SVT resulted in a significant increase in LV water content compared with LV water content in sham-operated controls (82±6% versus 75±4%, p<0.05, respectively). Cardiac myocytes were successfully isolated from all the control and chronic SVT ventricles, with an average of 55±5% quiescent rod-shaped myocytes obtained. Myocytes from the SVT hearts were significantly longer than those from control hearts (p<0.05, Figure 2 and Figure 3). No significant difference in myocyte width was observed between the SVT and control groups (32±4 versus 30±2 μm, respectively, p>0.50). These changes in myocyte dimensions in SVT hearts resulted in increased surface area compared with the surface area in control hearts (5,300±655 versus 4,400±565 μm², respectively, p=0.08).

**Myocyte Attachment Studies**

Representative myocyte attachment studies for laminin are presented in Figure 4. Myocyte attachment was significantly reduced in the SVT group as compared with the control group (p<0.05). A summary of the myocyte attachment data for all laminin concentrations and incubation times is presented in Table 2. Figure 5 presents representative myocyte binding data for the substrate fibronectin. A significant reduction in myocyte attachment was observed in the chronic SVT group compared with the control group (p<0.05). A summary of the myocyte attachment data for all concentrations and incubation times for fibronectin is shown in Table 3. Similarly, myocyte attachment to collagen IV was observed to be significantly reduced in the chronic SVT pigs compared with control pigs (Table 4). There was no significant difference in myocyte attachment to collagen I between SVT and control hearts (2.7±1.5 and 4.2±1.2 myocytes/mm², respectively; p>0.5).

There was a significant substrate concentration-dependent and incubation time-dependent effect on myocyte attachment for control cells (F=3.3, p<0.001). However, a cooperative binding effect between substrate concentration and incubation time...
was not observed for myocytes isolated from chronic SVT pigs \((F=1.0, p=0.43)\).

Electron Microscopy

Preembedding fixation with tannic acid revealed a well-formed basement membrane–sarcolemma interface with scalloping of the sarcolemma (Figure 6A). In these control sections, the extracellular matrix surrounding the sarcolemma was very electron dense, and the collagen fibrils spiraling into the extracellular space were quite evident. Despite the same fixation protocol, focal disruptions in the continuity of the basement membrane were observed in myocardial sections taken from SVT hearts (Figure 6B). In addition, the extracellular matrix surrounding the sarcolemma of SVT myocytes was not as electron dense, and the collagen fibrils could not be clearly seen. In control sections, the sarcolemmal festooning pattern was clearly evident along the basement membrane–sarcolemma interface; however, the degree of

**FIGURE 3.** Contrast photomicrograph of isolated myocytes attached to Matrigel (Collaborative Research Inc., Bedford, Mass.). Panel A: Isolated myocytes from control hearts are rod-shaped. Panel B: Isolated myocytes from hearts after 3 weeks of supraventricular tachycardia were significantly longer than control myocytes. Magnification for panels A and B, \(\times 300\).

**FIGURE 4.** Graph showing attachment of myocytes to wells coated with various concentrations of laminin and two incubation times (5 and 60 minutes). Attachment of myocytes from hearts subjected to chronic supraventricular tachycardia (SVT) at all concentrations of laminin was substantially reduced compared with attachment of myocytes from control hearts. Complete results for isolated myocyte attachment studies performed using laminin as an attachment substrate are found in Table 2.
Table 2. Isolated Myocyte Attachment to Laminin in Pigs

<table>
<thead>
<tr>
<th>Incubation time (min)</th>
<th>Laminin concentration (µg/ml)</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>50</th>
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<tbody>
<tr>
<td>Control (myocytes/mm²)</td>
<td>Control (myocytes/mm²)</td>
<td>5</td>
<td>10</td>
<td>20</td>
<td>50</td>
</tr>
<tr>
<td>5 min</td>
<td>4.6±1.1</td>
<td>10.8±4.1</td>
<td>23.7±4.2</td>
<td>32.2±4.8</td>
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<tr>
<td>15 min</td>
<td>7.3±2.2</td>
<td>13.7±3.5</td>
<td>30.6±4.7</td>
<td>51.2±4.0</td>
<td></td>
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<tr>
<td>60 min</td>
<td>8.5±2.0</td>
<td>15.7±4.2</td>
<td>37.2±7.1</td>
<td>60.9±4.5</td>
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</tbody>
</table>

Values are mean±SEM. Control, sham-operated pigs; chronic SVT, pigs subjected to 3 weeks of pacing-induced supraventricular tachycardia (240 beats/min).

Table 3. Isolated Myocyte Attachment to Fibronectin in Pigs

<table>
<thead>
<tr>
<th>Incubation time (min)</th>
<th>Fibronectin concentration (µg/ml)</th>
<th>5</th>
<th>10</th>
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<th>50</th>
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<td>Control (myocytes/mm²)</td>
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<td>50</td>
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<tr>
<td>5 min</td>
<td>6.8±1.8</td>
<td>6.5±1.6</td>
<td>6.2±1.9</td>
<td>7.4±2.1</td>
<td></td>
</tr>
<tr>
<td>15 min</td>
<td>6.1±1.9</td>
<td>10.4±3.6</td>
<td>10.4±3.4</td>
<td>8.3±2.0</td>
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</tr>
<tr>
<td>60 min</td>
<td>8.0±3.7</td>
<td>13.7±6.2</td>
<td>12.8±4.9</td>
<td>12.6±3.0</td>
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</table>

Chronic SVT (myocytes/mm²)

<table>
<thead>
<tr>
<th>Incubation time (min)</th>
<th>Fibronectin concentration (µg/ml)</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>50</th>
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</thead>
<tbody>
<tr>
<td>5 min</td>
<td>0.4±0.2*</td>
<td>0.6±0.3*</td>
<td>0.7±0.3*</td>
<td>2.6±1.0*</td>
<td></td>
</tr>
<tr>
<td>15 min</td>
<td>1.2±0.3*</td>
<td>1.4±0.4*</td>
<td>1.5±0.3*</td>
<td>1.8±0.5*</td>
<td></td>
</tr>
<tr>
<td>60 min</td>
<td>0.7±0.2*</td>
<td>1.2±0.5*</td>
<td>1.9±0.8*</td>
<td>2.5±0.8*</td>
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Values are mean±SEM. Control, sham-operated pigs; chronic SVT, pigs subjected to 3 weeks of pacing-induced supraventricular tachycardia (240 beats/min).

Figure 5. Graph showing attachment of myocytes to wells coated with various concentrations of fibronectin and two incubation times (5 and 60 minutes). Attachment of myocytes from hearts subjected to chronic supraventricular tachycardia (SVT) at all concentrations of fibronectin was substantially reduced compared with attachment of myocytes from control hearts. Complete results for isolated myocyte attachment studies performed using fibronectin as an attachment substrate are found in Table 3.

Discussion

This study examined LV functional and cellular changes that occurred with chronic SVT. The most important findings of this investigation were as follows: 1) Chronic SVT caused significant LV dysfunction with chamber dilatation and wall thinning. 2) Myocyte length was significantly increased after SVT. 3) Chronic SVT resulted in a significant reduction in myocyte attachment to components of the basement

Table 4. Isolated Myocyte Attachment to Collagen Type IV in Pigs

<table>
<thead>
<tr>
<th>Incubation time (min)</th>
<th>Collagen type IV concentration (µg/ml)</th>
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<th>10</th>
<th>20</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (myocytes/mm²)</td>
<td>Control (myocytes/mm²)</td>
<td>5</td>
<td>10</td>
<td>20</td>
<td>50</td>
</tr>
<tr>
<td>5 min</td>
<td>5.1±1.6</td>
<td>3.8±1.5</td>
<td>7.0±2.2</td>
<td>6.4±0.3</td>
<td></td>
</tr>
<tr>
<td>15 min</td>
<td>9.6±3.1</td>
<td>10.4±2.7</td>
<td>14.9±4.4</td>
<td>16.1±4.0</td>
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</tr>
<tr>
<td>60 min</td>
<td>9.0±3.2</td>
<td>8.5±3.0</td>
<td>9.2±2.8</td>
<td>10.0±2.5</td>
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</tbody>
</table>

Chronic SVT (myocytes/mm²)

<table>
<thead>
<tr>
<th>Incubation time (min)</th>
<th>Collagen type IV concentration (µg/ml)</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 min</td>
<td>0.7±0.2*</td>
<td>0.7±0.3*</td>
<td>1.0±0.3*</td>
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<tr>
<td>15 min</td>
<td>2.0±1.2*</td>
<td>1.3±0.5*</td>
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<tr>
<td>60 min</td>
<td>1.5±0.7*</td>
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<td>2.0±0.9*</td>
<td>2.6±1.4*</td>
<td></td>
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</tbody>
</table>

Values are mean±SEM. Control, sham-operated pigs; chronic SVT, pigs subjected to 3 weeks of pacing-induced supraventricular tachycardia (240 beats/min).

* p<0.05 vs. corresponding control value.
membrane. 4) Disruptions of the sarcolemma–basement membrane interface were observed in chronic SVT hearts.

Contractile forces generated by individual myocytes depend on sarcolemmal to basement membrane attachments for the transduction of mechanical en-

FIGURE 6. Panel A: Transmission electron microscopy revealing a well-formed sarcolemmal–basement membrane interface (arrow) with scalloping of the sarcolemma just lateral to the Z band in control sections. An electron-dense extracellular matrix was clearly visible. Panel B: Focal disruptions of the sarcolemmal–basement membrane interface and a loss of festooning of the sarcolemma (arrows) evident after chronic supraventricular tachycardia. In addition, the electron density of the extracellular matrix surrounding the myocytes was diminished in the sections subjected to supraventricular tachycardia. Magnification for panels A and B, $\times 13,500$.

FIGURE 7. Panel A: Photomicrograph showing ultrastructure of the myocytes from control myocardial sections and revealing densely packed mitochondria surrounding well-formed myofibrils. Panel B: Photomicrograph showing myocytes from hearts subjected to chronic supraventricular tachycardia and revealing a reduction in mitochondria and reduced myofibrils. The cytoplasm surrounding the perinuclear space was increased and devoid of organelles. Magnification for panels A and B, $\times 15,000$. 
ergy throughout the connective tissue framework of the heart. We are unaware of any investigations that have specifically examined myocyte–basement membrane attachments in any form of dilated cardiomyopathy. The chronic SVT group showed a significant decrease in the attachment of isolated myocytes to components of the basement membrane (laminin, fibronectin, and collagen IV) compared with the control group. This finding suggests that anchoring of the myocyte to the extracellular matrix of the heart is significantly reduced as a result of chronic SVT and provides a potential structural basis for at least some of the functional abnormalities observed in this model of a dilated cardiomyopathy. Specifically, by separating the myocyte from the basement membrane, chronic SVT may result in a loss of force transmission throughout the ventricular wall. In addition, interruption of basement membrane attachments with chronic SVT may effectively uncouple the myocyte from the extracellular matrix. In a recent review, Weber postulated that disruption of the anchoring between the myocyte and connective tissue framework would permit muscle fiber slippage, fiber realignment, and ventricular wall thinning.

Myocyte attachment to laminin, fibronectin, and collagen IV was significantly greater in the control group compared with the chronic SVT group. Myocytes isolated from control hearts exhibited the highest affinity for laminin. Fibronectin was a less effective cell attachment substrate. Borg et al and Lundgren et al have observed that adult cardiac myocytes do not attach as well to fibronectin as they do to laminin. Borg et al have shown that the adhesive interaction of myocytes with components of the basement membrane are developmentally regulated and that myocyte attachment to fibronectin diminishes during the maturation of neonatal rats. In our present study, collagen IV was also less effective than laminin as a cell binding substrate. This finding contrasts studies by Borg et al and Lundgren et al in which adult rat myocytes were found to adhere equally well to collagen IV and laminin. This discrepancy may be related to the heterogeneity of extracellular matrix receptors and their expression between different species. Additionally, cell surface–bound laminin has been shown to mediate the attachment of isolated adult myocytes to collagen type IV. However, we did not examine the cooperative binding effects between components of the basement

**Figure 8.** Scanning electron microscopy. Panel A: Freeze-fractured sections taken from control hearts revealing a well-defined pattern of invaginations along the outside surface of the myocyte. Collagen fibrils could be clearly seen spanning the extracellular space between adjacent myocytes. Magnification, ×3,400. Panel B: Myocardial sections taken from hearts subjected to supraventricular tachycardia revealing a reduced festooning pattern and flattening of the sarcolemma. The regular pattern collagen fibril lattice-work surrounding the myocytes could no longer be seen. The collagen latticework appeared disrupted and disorganized within the extracellular space. Magnification, ×3,740.
membrane in this study. Collagen I served as a control for nonspecific myocyte binding. Few myocytes bound to collagen I from either the control or SVT hearts, and no significant difference in myocyte attachment was observed between the two groups. Collagen I is not a component of the basement membrane, and other attachment studies have indicated that adult myocytes attach only to collagen type IV.28,32

Possible mechanisms responsible for reduced basement membrane affinity in this model of dilated cardiomyopathy include 1) structural injury to the basement membrane–sarclemma interface, 2) alterations in the number or expression of basement membrane receptors on the myocyte, and 3) reduced concentration of basement membrane components. Terracio et al22 have localized basement membrane components and their receptors to specific regions, just lateral to the Z band, on the surface of myocytes. Using cationic dyes, Robinson et al16,17 further characterized this region of interface between the basement membrane and sarclemma and demonstrated that scalloping of the sarclemma and basement membrane occurred between Z bands. We observed a similar pattern of festooning of the sarclemma–basement membrane interface in sections taken from control hearts. Chronic SVT resulted in disruptions of this interface and an apparent loss of the festooning pattern of the sarclemma. Other studies35–37 have demonstrated similar disruptions of the sarclemma–basement membrane interface during severe ischemia in vivo and during total ischemia in vitro. In addition to direct structural injury of the sarclemma–basement membrane interface, alterations in the number and/or affinity of binding proteins to basement membrane components may occur with chronic SVT. In pressure-overload hypertrophy, it has been suggested that an increase in the expression of basement membrane binding proteins occurs.22 In contrast, chronic SVT–induced dilated cardiomyopathy resulted in a significant reduction of myocyte affinity to components of the basement membrane. Concentrations of laminin, fibronectin, and collagen IV in vivo were not measured in this study. However, increasing the concentration of these basement membrane components in vitro had little effect on myocyte attachment in the chronic SVT group. These results suggest that reduced basement membrane affinity of binding proteins on the surface of the myocyte may be responsible for disruptions of the basement membrane and diminished basement membrane component attachment observed in this model.

Increasing ventricular wall stress secondary to chronic pressure overload has been shown to increase ventricular wall thickness and mass, accompanied by an increase in myocyte volume and diameter (hypertrophy).38,39 Grossman et al40 described two distinct patterns of ventricular hypertrophy induced by pressure or volume overload. In pressure-overload hypertrophy, an increase in wall thickness occurs with a reduction in radial chamber dimension.40,41 In volume-overload hypertrophy, ventricular wall thickness increases slightly or remains unchanged with a significant increase in chamber radius.40,41 In both forms of hypertrophy, adaptive changes in chamber morphology occur in response to the hemodynamic burden. These changes in ventricular architecture result in a normalization of wall stress and maintenance of pump performance.40 The present study demonstrated that chronic SVT resulted in a significant reduction in wall thickness with a significant increase in chamber dimensions. These changes in ventricular geometry with chronic SVT resulted in a significant increase in wall stress with a reduction in pump performance.

Although LV mass increased with chronic SVT, there was a significant reduction in LV wall thickness and no change in LV mass/body weight as compared with control values. The increase in absolute LV mass was due, in part, to the significant increase in myocardial water content that occurred with SVT. At the cellular level, there was a significant increase in myocyte length with no change in cell width as compared with control values. At the ultrastructural level, there was a qualitative reduction in the density of mitochondria and myofibrils observed in SVT sections compared with control sections. In pressure- and volume-overload hypertrophy, one of the early cellular events is an increase in mitochondrial density.42 This increase in mitochondria will result in increased capacity for ATP synthesis and contractile protein production.42 Increased synthesis results in new sarcomeres added in parallel in pressure overload and new sarcomeres added in series in volume overload.43 In both forms of hypertrophy, an increase in mitochondria and myofibrils occurs in conjunction with increased myocyte and chamber dimensions.41,43 In the present study, absolute LV mass and isolated myocyte length increased with chronic SVT, suggestive of hypertrophy. However, the ultrastructural composition of the myocytes with SVT cardiomyopathy appeared dissimilar to the intracellular changes observed during the development of hypertrophy.41,43

Chronic SVT resulted in a significant reduction in LV fractional shortening. This decrease in LV systolic function was accompanied by significant chamber dilatation, an increase in end-diastolic pressure, and ventricular wall thinning. In addition, LV mass was not increased after 3 weeks of SVT. Damiano et al47 and others47,48 have reported that chronic tachycardia in dogs resulted in reduced ejection fraction, significant LV dilatation, and no change in mass. Wilson et al48 demonstrated that dogs subjected to ventricular tachycardia showed increased left atrial pressures and LV volumes with no change in mass when compared with control dogs. Our present study builds on these previous studies by demonstrating that chronic SVT not only resulted in LV dilatation and dysfunction but also was accompanied by changes in myocyte morphology and reduced basement membrane attachment.
Using embedded tissue sections and stereological principles, we reported that the volume percent of the ventricular free wall occupied by myocytes decreased with chronic SVT. In addition, we reported that in these paraffin-embedded sections, a reduction in myocyte diameter was observed. In the present study, we observed no change in diameter of myocytes isolated from SVT hearts compared with control hearts. These different results are probably due to the following differences in methodology: 1) The ventricular sections used in the morphological study were formalin-fixed and embedded in paraffin; therefore, a certain degree of cell shrinkage occurred. 2) The ventricular sections were examined in situ where chamber stress and geometry were in force as opposed to the isolated unloaded myocytes. 3) Morphological examination of the ventricular sections was performed within the subendocardium, whereas the isolated myocytes were harvested from the entire ventricular free wall. Despite these differences, both this past report and the present study demonstrated that chronic SVT resulted in a significant reduction in ventricular wall thickness with no increase in myocyte width.

Several limitations to the present study must be recognized. Myocytes isolated and examined may have not been representative of the entire myocyte population contained within the control and SVT hearts. However, previous studies examining isolated myocytes in pressure and volume overload using techniques similar to those used in the present study have reported changes in isolated myocyte structure that corresponded to changes in ventricular architecture. Further, the receptors of the SVT myocytes may be more susceptible to the methods used for cell isolation. Therefore, the reduced affinity for basement membrane components may have been due, in part, to preferential sarcolemmal injury of SVT myocytes. A future study examining receptor density and affinity in both myocardial homogenates and isolated myocytes may help address this possibility. The festooning index used in the present study was a semiquantitative approach and subject to methodological limitations. The number of sarcolemmal invaginations determined from LV sections can be affected by section orientation, fixation methods, and distension pressure. Although in the present study the number of festoons were determined from longitudinally oriented sections, the LV sections were immersion-fixed. Immersion fixation prevented fixation of the myocytes at a constant distension pressure.

Chronic SVT has long been associated with ventricular dysfunction in humans. The present study and others have demonstrated that chronic SVT results in severe LV chamber dilatation and reduced pump performance. Recent clinical reports have shown that this tachyarrhythmia can be successfully ablated using surgical methods. Results from the present study revealed that chronic SVT results in significant LV chamber dilatation, cellular remodeling, and a reduction in the anchoring of the myocyte to the basement membrane and, consequently, the extracellular matrix. Experimental studies have suggested that a disrupted or destroyed extracellular matrix favors healing by scarring. Therefore, structural alterations of the sarcolemma–basement membrane interface in chronic SVT hearts may promote the process of scar formation and have significant impact on the degree of recovery after termination of the tachycardia.

In summary, chronic SVT in swine resulted in a reduction of LV pump performance associated with significant chamber dilatation and cellular remodeling, manifested by a reduction in wall thickness and a significant increase in myocyte length. These functional and morphological changes were accompanied by a significant decrease in myocyte attachment to major components of the basement membrane and disruptions of the sarcolemma–basement membrane interface. These changes in cellular morphology and structural organization of the myocardium may play a significant role in the ventricular dysfunction and altered chamber architecture that are due to chronic tachycardia.

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