Role of Microtubules in Contractile Dysfunction of Hypertrophied Cardiocytes

Hiroyuki Tsutsui, MD; Hirofumi Tagawa, MD; Robert L. Kent, PhD; Patrick L. McCollam, PharmD; Kazuaki Ishihara, MD; Masayoshi Nagatsu, MD; George Cooper IV, MD

Abstract Cardiac hypertrophy in response to systolic pressure overloading frequently results in contractile dysfunction, the cause for which has been unknown. Since, in contrast, the same degree and duration of hypertrophy in response to systolic volume overloading does not result in contractile dysfunction, we postulated that the contractile dysfunction of pressure hypertrophied myocardium might result from a direct effect of stress as opposed to strain loading on an intracellular structure of the hypertrophied cardiocyte. The specific hypothesis tested here is that the microtubule component of the cytoskeleton is such an intracellular structure, which, forming in excess, impedes sarcomere motion. The feline right ventricle was either pressure overloaded by pulmonary artery banding or volume overloaded by atrial septotomy. The quantity of microtubules was estimated from immunoblots and immunofluorescent micrographs, and their mechanical effects were assessed by measuring sarcomere motion during microtubule depolymerization. We show here that stress loading increases the microtubule component of the cardiac muscle cell cytoskeleton; this apparently is responsible for the entirety of the cellular contractile dysfunction seen in our model of pressure-hypertrophied myocardium. No such effects were seen in right ventricular cardiocytes from normal or volume-overloaded cats or in left ventricular cardiocytes from any group of cats. Importantly, the linked microtubule and contractile abnormalities are persistent and thus may be found to have significance for the deterioration of initially compensatory cardiac hypertrophy into the congestive heart failure state. (Circulation. 1994;90:533-555.)

Key Words • hypertrophy • cardiocytes • contractile dysfunction • cytoskeleton • microtubule

Cardiac hypertrophy is the generic response to a wide spectrum of physiological and pathological deviations from normal homeostasis that have as their common theme increased hemodynamic loading of the heart.1 This compensatory growth process proceeds until the load stimulus is abated by a renormalization of stress per unit of myocardial mass. Cardiac hypertrophy fails to be functionally compensatory, however, either when the load increase exceeds the inherent growth capacity of the terminally differentiated cardiac muscle cell or cardiocyte to renormalize stress or when the intrinsic contractile performance per unit mass of hypertrophied myocardium is less than that of normal myocardium. Thus, cardiac compensation for an increased load may be imperfect because of either quantitative and qualitative defects of hypertrophied myocardium.

This report focuses on the mechanisms for the qualitative defects of hypertrophied myocardium, which are thought to underlie the frequently observed deterioration of initially compensatory cardiac hypertrophy into the congestive heart failure state. Hemodynamic overloads causing cardiac hypertrophy consist of either volume overloading, wherein an increased blood volume is pumped during each cardiac cycle against a normal impedance, or pressure overloading, wherein a normal blood volume is pumped during each cardiac cycle against an increased impedance. Animal models of each type of cardiac overload have been created using the right ventricle, on which load alterations can be imposed without major effects on either coronary arterial or systemic hemodynamics.

On the level of isolated right ventricular tissue, we have found in such animal models that for an equivalent degree and duration of hypertrophy, volume overloading results in entirely normal cardiac contraction and energetics,2 whereas pressure overloading results in distinctly abnormal cardiac contraction and energetics,3 a result consonant with clinical experience with the intact right ventricle.1 On the level of isolated right ventricular cardiocytes, we have found for the same model of right ventricular pressure overload that the contractile defect seen in isolated tissue is duplicated when characterized as sarcomere shortening in the muscle cell.4 Thus, it is the nature of the inducing stress rather than hypertrophy itself that causes the qualitative defects of myocardium hypertrophying in response to a pressure overload, and the contractile defect, at least, resides in the cardiocyte.

We and others have advanced a number of potential causes for the functional abnormalities of hypertrophied myocardium,1 with a leading candidate being altered calcium metabolism,3,5 but none has accounted fully and convincingly either for the contractile abnormalities of hypertrophied myocardium when present on the tissue1 or the cellular4 level or, more particularly, for the dichotomy noted above between the pressure versus volume-overloaded right ventricle. The present study
had its genesis in considering this problem within the context of our general thesis that load is the primary variable responsible for regulating cardiocyte properties. That is, cardiocyte structure, composition, and function each respond dynamically to the full potential spectrum of imposed loads, with deviations either below or above normal loading causing rapid but reversible changes in each of these properties. Indeed, the cardiocyte itself is competent to respond directly to load in terms of RNA and protein synthesis rates. However, there are neither qualitative nor quantitative differences between hypertrophied cardiocytes from our models of pressure- versus volume-overloaded right ventricles when defined in terms of standard ultrastructure, yet the contractile defect is expressed quite clearly in the pressure-overloaded cell. We therefore thought it reasonable to seek the cause for this contractile defect in one or more intracellular structures that might (1) appear or increase in response to load, (2) discriminate between the stimuli of stress, or pressure loading versus strain, or volume loading, (3) not be obvious ultrastructurally, and (4) be plausibly thought to have the potential for interfering with sarcomere motion.

The hypothesis tested in the present study, which has been reported in part elsewhere, is that the microtubule component of the cytoskeleton is such an intracellular structure, which, in excess, is responsible for the contractile abnormalities of cardiocytes hypertrophying in response to a pressure overload. The basis for this hypothesis, in terms of the four criteria set forth above, is as follows. First, there is evidence in taxa as diverse as plans and both invertebrate and vertebrate animals that microtubules form along stress axes and bear intracellular and transcellular loads, suggesting selective polymerization or organization in response to their mechanical environment. Second, for a linear steady-state polymer such as the α-β tubulin heterodimer-microtubule system, thermodynamics dictate that the critical subunit concentration for assembly is lowered and polymer stability is enhanced by an extending, or stress force; and there is experimental evidence that this obtains for microtubules in growing axons. Third, microtubules are not obvious ultrastructurally in mature striated muscle absent special efforts. Fourth, given the intimate cardiac myofibrillar investment by microtubules, there is a clear opportunity for an excess of this polymer to interfere with sarcomere motion.

**Methods**

**Experimental Models**

Pressure-overload right ventricular (RV) hypertrophy was induced by partially occluding the pulmonary artery with a 3.5-mm internal diameter band, just as we have described before. These cats, identified as the PAB group, were allowed to recover for 2 to 26 weeks.

Volume-overload RV hypertrophy was induced by partially resecting the interatrial septum during venous inflow occlusion, just as we have described before. These cats, identified as the ASD group, were allowed to recover for 2 to 4 weeks.

Controls consisted of either normal cats or sham-operated cats submitted to thoracotomy and pericardiotomy without hemodynamic intervention. Since sham operation was without effect on any experimental variable, all control cats are considered throughout as a single group.

All operative procedures were carried out under full surgical anesthesia with meperidine (2.2 mg/kg IM), acepromazine maleate (0.25 mg/kg IM), and ketamine HCl (50 mg/kg IM). All procedures and the care of the cats were in accordance with institutional guidelines.

**Hemodynamic Status**

At the time of terminal study, the cats were anesthetized as above. Right heart pressures were obtained using a stiff, fluid-filled catheter attached to a strain gauge, which was inserted through the right external jugular vein and advanced into the right atrium and ventricle. Arterial pressure was monitored by a fluid-filled catheter attached to a strain gauge, which was positioned in the proximal left common carotid artery. The mid chest position was taken as a zero reference point for pressure measurements. The arteriovenous difference in oxygen content was used as a measure of the adequacy of systemic perfusion and was determined in duplicate by measuring simultaneous blood samples obtained from the carotid artery and the right ventricle. The difference in percent oxygen saturation of hemoglobin between duplicate samples drawn from the right atrium and the superior vena cava was used as the indication of a left-to-right shunt at the atrial level.

**Cardiocyte Isolation**

With the exceptions noted below, the methods used to obtain reproducible yields of calcium-tolerant, quiescent cardiocytes have been described previously. After completion of the hemodynamic studies, the cats were heparinized (1000 U IV) and placed on oxygen. A left thoracotomy was performed, the pericardium was opened, and the heart was rapidly removed, placed in cold buffer solution, and weighed. The aorta was then cannulated, and the coronary arteries were perfused retrograde for 10 minutes first with a recirculating buffer solution of the following composition (mmol/L): NaCl 130.0, KCl 4.8, MgSO4 1.2, NaH2PO4 1.2, NaHCO3 4.0, CaCl2 0.5, HEPES 10.0, and glucose 12.5, second with a nonrecirculating buffer of the same composition but without supplemental calcium, and third with a recirculating calcium-free buffer supplemented with type II collagenase (155 U/mL). Buffer perfusion, which we have shown to be equal in terms of flow/unit cardiac mass for both ventricles in feline RV hypertrophy, was terminated when the heart was flaccid. The heart was removed from the cannula, the right ventricle was carefully dissected from the heart, and the right ventricle and remaining myocardium were weighed separately. The left ventricular (LV) free wall then was dissected from the heart and weighed; the remaining myocardium was discarded. The weight of each ventricle was multiplied by the ratio of preperfusion/postperfusion heart weight to correct for intervening edema. Cardiocytes from the RV and LV free walls then were isolated as described previously; they were maintained for 1 hour at 37°C and pH 7.4 in collagenase-free 2.5 mmol/L Ca2+ buffer before defining contractile function.

**Cardiocyte Morphology**

Cardiocyte length, width, and surface area were obtained from digitized photographs both of each living cell in which contractile function was characterized and of a random sampling of fixed cells from the right ventricle and left ventricle of each cat. For cardiocyte fixation, a portion of the cells to be used for studies of sarcomere dynamics was immersion-fixed in buffered 300 mOsM 1.5% glutaraldehyde. Cardiocyte surface area was determined by digitizing the lateral edges of the cell images obtained from a ×7 magnification of the resulting 35-mm negatives (final magnification, 1400×). Cell length and width were determined directly from these cell images when digitized by using a computer program that automatically found the maximum value for each of these two parameters after the cellular long-axis orientation was selected by the operator.
Cardiocyte Mechanics

The use of laser diffraction techniques for measuring sarcomere motion in isolated cardiocytes is well established. A brief outline of our method is as follows. An aliquot of isolated cardiocytes was added to 4 mL of the 2.5 mmol/L Ca\(^{2+}\) buffer in a well affixed to a glass slide. The cardiocytes came to rest on the bottom of this chamber, which was placed on the stage of an inverted microscope. The buffer was kept at 37±0.1°C by a thermostated heating stage. Only cardiocytes with the following characteristics were analyzed: single, rod-shaped cells unattached to adjacent cells that contracted with each stimulus and were quiescent between stimuli. The cardiocytes were stimulated to contract between platinum wire electrodes by 0.25-Hz, 100-\(\mu\)A DC pulses of alternating polarity. When after 10 to 15 contractions the extent of shortening was stable, 10 contractions were sampled and averaged to yield a final profile of sarcomere length and velocity versus time during contraction. Changes in sarcomere length were measured from movement of the first-order diffraction pattern cast by a substage laser light passing through the sarcomeres of a given cardiocyte onto diametrically opposed optical sensors situated above the microscope stage. Each sensor was composed of a linear array of 256 photodiodes interrogated at a frequency of 1 kHz. The distance between the first-order diffraction patterns at every millisecond was calculated by and stored in a computer.

Cardiocyte Loading

In experiments in which we wished to impose an external load on cells during contraction, methylcellulose was included in the superfusate, as we have described before. Briefly, a 2% solution of methylcellulose with a molecular mass of 26 kDa was prepared in the 2.5 mmol/L Ca\(^{2+}\) buffer. The viscosity of the methylcellulose solution was 160 centipoise (cP) as measured at 37°C by both a Brookfield viscometer and by falling ball viscometry; the two techniques produced identical results. To ensure that potential osmotic changes in the viscous solution did not affect contractile function and morphology, the osmolarity of the methylcellulose solution was measured by both equilibrium vapor pressure and freezing point depression. The osmolarity of the standard buffer was 290±5 mOsm/kg, and as would be expected for 2% solutions of very large molecules, this value did not increase significantly in the methylcellulose solution.
Sarcomere versus Cardiocyte Mechanics

In experiments in which we wished to compare sarcomere with cardiocyte mechanics, a video frame grabber running at a rate of 30 frames per second was attached to the microscope. The cell images were stored on standard half-inch videotape, with simultaneous recording of the stimulus signals for a frame-by-frame analysis of cardiocyte length by computerized edge detection. Sarcomere and cardiocyte mechanics were measured during the same series of contractions: 0.25 Hz stimulation was begun, and after the extent of cardiocyte shortening became stable, sarcomere motion was recorded during an initial 10 contractions and cell motion recorded during the next 10 contractions; cardiocyte and sarcomere shortening did not change during these contractions. Sarcomere shortening, obtained as the average for 10 contractions, was evaluated by laser diffraction as described above. Cardiocyte shortening, measured as resting length minus the length at peak shortening, was also obtained as the average for 10 contractions.

Experimental Interventions

The strategy used was first to determine whether microtubule depolymerization restored normal contractile function in pressure hypertrophied cardiocytes and then to determine whether in normal cardiocytes microtubule hyperpolymerization recreated the contractile abnormalities characteristic of pressure-overload hypertrophy. The efficacy of the experimental interventions described below is documented in Fig 1.

Microtubule depolymerization: colchicine. To characterize the effects of microtubule depolymerization, colchicine at a final concentration of $10^{-6}$ mol/L was added to the chamber containing the cardiocytes, and contractile function of the same single cell was followed over the ensuing hour.

Microtubule hyperpolymerization: taxol. This complex diterpene lowers the critical concentration of $a\beta$-tubulin heterodimers required to form microtubules. Therefore, to characterize the effects of microtubule hyperpolymerization,

<table>
<thead>
<tr>
<th>TABLE 1. Characteristics of the Models</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=14)</td>
</tr>
<tr>
<td>RV systolic pressure, mm Hg</td>
</tr>
<tr>
<td>$\Delta O_2$% Saturation (SVC vs RA)</td>
</tr>
<tr>
<td>RV weight/body wt, g/kg</td>
</tr>
<tr>
<td>RV weight/tibial length, g/cm</td>
</tr>
<tr>
<td>LV weight/body wt, g/kg</td>
</tr>
<tr>
<td>Body wt, kg</td>
</tr>
<tr>
<td>Arteriovenous O$_2$ difference, m/L</td>
</tr>
<tr>
<td>RV end-diastolic pressure, mm Hg</td>
</tr>
<tr>
<td>Liver weight/body wt, g/kg</td>
</tr>
</tbody>
</table>

ASD indicates atrial septal defect; PAB, pulmonary artery band; SVC, superior vena cava; RA, right atrium; RV, right ventricular; and LV, left ventricular.

Values are mean±SEM. Statistical comparisons are by one-way ANOVA followed by Newman-Keuls test. For controls, there was no difference between sham-operated and normal cats with respect to any of these variables; data are therefore grouped together. For the ASD and PAB cats, there was no within-group difference for any of these variables at the various experimental time points, and these data are also grouped together.

*P<.05 for difference from control
†P<.05 for difference from ASD.

<table>
<thead>
<tr>
<th>TABLE 2. Characteristics of the Cardiocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV</td>
</tr>
<tr>
<td>All Groups (n=970)</td>
</tr>
<tr>
<td>Length, $\mu$m</td>
</tr>
<tr>
<td>Width, $\mu$m</td>
</tr>
<tr>
<td>Surface area, $\mu$m$^2$</td>
</tr>
</tbody>
</table>

LV indicates left ventricle; RV, right ventricle; ASD, atrial septal defect; and PAB, pulmonary artery band.

Values are mean±SEM. Statistical comparisons are by one-way ANOVA followed by Newman-Keuls test. For the LV, cardiocyte dimensions were the same in LVs from control and experimental groups; the data for all LV cells are therefore grouped together. For the control RV cells, cardiocyte dimensions were the same in sham-operated and normal cats, and these data are also grouped together. For the ASD and PAB RV cells, there was no within-group dimensional difference at the various experimental time points, and these data are grouped together as well.

*P<.05 for difference from control RV
†P<.05 for difference from LV.
normal cardiocytes were exposed to taxol at a final concentration of $10^{-5}$ mol/L, and contractile function was assessed by sequential sampling over 4 hours.

**Microtubule hyperpolymerization:** D$_2$O. Deuterium oxide (D$_2$O) is thought to reduce the critical concentration of $\alpha\beta$-tubulin heterodimers required to form microtubules by rapidly and reversibly strengthening hydrophobic interactions of tubulin molecules.$^{26}$ Therefore, to characterize the effects of microtubule hyperpolymerization by this additional technique, we first defined sarcomere mechanics in normal cardiocytes in the standard buffer, then 10 minutes after half of the H$_2$O in the superfusing buffer was replaced by D$_2$O, and finally after the 50% D$_2$O buffer was replaced once again by the standard buffer.

**Cytoskeletal Characterization: Tubulin and Microtubules**

Apart from demonstrating the effects on cardiocyte mechanics of interventions designed to have selective effects on the cardiocyte microtubules, we wished both to characterize the relative amounts of free and polymerized tubulin in the RV and LV myocardium of these experimental models and to visualize the appearance and density of the cardiocyte microtubule network in each instance. Furthermore, we wished to substantiate the putative effects on these variables of the experimental interventions used in the studies of cellular mechanics.

**Immunoblots**

For the immunoblot analysis, fresh 0.5-g specimens from the right ventricle and left ventricle of each cat were homogenized in 5 mL of microtubule stabilizing buffer$^{26}$ and centrifuged at 100,000g at 25°C for 15 minutes. The supernatants were saved as the free tubulin fractions, and the pellets were resuspended at 4°C in 4 mL of microtubule depolymerization buffer$^{29}$; after 1 hour at 0°C, they were centrifuged at 100,000g at 4°C for 15 minutes, and the supernatants were saved as the polymerized tubulin fractions. Protease inhibitors$^{30}$ were used throughout. For the subsequent 8% to 16% gradient SDS-PAGE, equal proportions of the free and polymerized samples were loaded onto the two lanes for each ventricle, and an equal amount of protein as determined by a bicinchoninic acid assay (BCA, Pierce) was loaded for the RV and LV samples. The samples then were transferred to polyvinylidene difluoride membranes (35V, 75 minutes) and probed with 1:500 dilution of a monoclonal antibody to $\beta$-tubulin (DM1B, Amersham). The bound antibody was visualized using a horseradish peroxidase–conjugated secondary antibody (Vector) and enhanced chemiluminescence (ECL, Amersham). No $\beta$-tubulin was detectable in the supernatant when the initial pellets were reextracted in stabilizing buffer. In all cases, a single band at 55 kDa having the same mobility as concurrently run bovine brain $\beta$-tubulin was detected. In addition, 0.5-g samples from the same RV and LV specimens were homogenized in depolymerization buffer$^{29}$ to isolate total tubulin in the same RV and LV fractions; these were run on the same gel as the free and polymerized fractions.

**Indirect Immunofluorescence Micrographs**

For visualization of the appearance and density of the cardiocyte microtubule network, freshly isolated$^{4,7}$ RV and LV cardiocytes were sedimented onto laminin-coated coverslips at 1g for 45 minutes, permeabilized for 1 minute by 1% Triton X-100 in stabilization buffer,$^{21}$ washed twice in the same buffer, and fixed for 10 minutes with 3.7% formaldehyde. After blocking with 10% horse serum in 0.1 mol/L glycine, the cells were incubated overnight at 4°C with a 1:100 dilution of the same antibody to $\beta$-tubulin used for the immunoblots followed by a fluorescein-conjugated secondary antibody (Vector). They were mounted with 1% triethylenediamine and 50% glycerol in phosphate-buffered saline, and 0.7-$\mu$m optical sections were acquired by confocal laser microscopy (LSM GB-200, Olympus). For evaluation of the cardiocyte intermediate filament network, the same protocol using a 1:100 dilution of a monoclonal primary antibody to desmin (DE-U-10; Sigma) was followed.

**Intracellular Calcium**

To ascertain whether any inotropic changes in response to colchicine might result from changes in intracellular Ca$^{2+}$, we measured as before$^{25}$Ca$^{2+}$ in individual cardiocytes exposed for 1 hour to $10^{-6}$ mol/L colchicine using digital imaging fluorescence microscopy and the Ca$^{2+}$ fluorophore fluo-3. Freshly isolated cardiocytes were loaded with 20 umol/L fluo-3 acetylomethoxy ester for 45 minutes. To facilitate solubilization, 0.5 μL of 25% (wt/wt) Pluronic F-127 in anhydrous dimethyl sulfoxide was mixed with every 10 nmol/L of fluo-3, and the resulting solution was thoroughly dispersed in Krebs buffer. After loading, the cells were washed with Krebs buffer three times to remove extracellular fluo-3 and then equilibrated for an additional 30 minutes to permit complete hydrolysis of any remaining intracellular ester. Fluorescence brightness was measured by a microspectrofluorometer equipped with a xenon bulb. The filter set used delivered 490 nm excitation light, and emission was measured using a barrier filter that passed light at wavelengths longer than 520 nm. The resulting fluorescence image was analyzed by digital imaging microscopy (magnification, 400×), which integrated the intensity of fluorescence brightness (arbitrary units) over the area of an individual cardiocyte (μm$^2$). Thus, fluorescence was normalized in terms of cell surface area and expressed as arbitrary light units/μm$^2$ cell surface area. Nonspecific background fluorescence was eliminated by background subtraction, and autofluorescence was not detectable at the low visible light levels used.

**Data Analysis**

The mean value and the standard error of the mean are shown for each group of data. Differences in selected measures were evaluated by either a paired or unpaired Student’s t test, as appropriate, with a significant difference said to exist at the level specified for each set of data. Where stated, group means were first compared by a one-way or two-way ANOVA, and if a difference was found, each experimental mean was compared with that of the control group by the appropriate post hoc test as individually specified.

**Results**

**Characterization of the Experimental Models**

The experimental animals were studied at three operation–death intervals in order to provide a longitudinal sampling of the pathophysiology of interest. The first of these, 2 weeks, was chosen because this is the earliest time at which a step increase in RV load produces a stable increase in RV mass in the cat.$^{30}$ The second of these, 4 weeks, was chosen because this is the time at which this functional difference between RV myocardium from the ASD and PAB models, despite a comparable degree and duration of RV hypertrophy, was observed on the tissue level in our previous work.$^{2,3}$ The third of these, 6 months, was chosen both because our previous work had shown that the functional difference between RV myocardium from the ASD and PAB models is maintained at this time,$^{2,4}$ and, more importantly, because it was critical to determine whether any cytoskeletal effects identified in the present study at the earlier time points were persistent.

The major features of the animal models used in this study are summarized in Table 1. For the RV pressure–overloaded PAB group, RV systolic pressure was doubled. For the RV volume overloaded ASD group, there
was a substantial shunt at the atrial level. For both groups, the ratios of RV weight to body weight and tibial length were increased significantly and comparably. Preoperative versus postoperative weight was the same in each group, and the ratios of LV weight to body weight and tibial length did not differ among the groups, further precluding any effect of postoperative change in body weight. In neither group was there evidence for right heart failure in terms of either the presence of ascites and pleural effusion in any cat at the time of study or increases in AVO2 difference, RV end-diastolic pressure, or the ratio of liver weight to body weight.

The major features of the RV and LV cardiocytes from the PAB and ASD cats are summarized in Table 2. Each dimension of RV cells from the hypotrophy models was greater than that of both RV cells from control cats and LV cells from control and experimental cats, but these RV cellular dimensions did not differ in the PAB versus ASD groups. The cells used for mechanical studies excluded trypan blue and were quiescent in 2.5 mmol/L Ca2+. Their average resting sarcomere length, which did not differ among groups, was 1.93 μm; in no case was this length less than 1.85 μm. These values are the same as those both in explanted superfused myocardium at slack length and in perfusion-fixed unloaded diastolic myocardium when the fixative is isomotic and contracture is avoided. Furthermore, resting sarcomere length and sarcomere shortening behavior were observed as before to be uniform when obtained at any given point within a cell excluding nuclear regions.

Cardiocyte Mechanics: Cytoskeletal Effects on Sarcomere Motion

There were five goals for this series of experiments. First, we wished to determine whether RV and LV cardiocytes from control cats had comparable mechanical performance and whether this was also comparable to that of LV cardiocytes from the two experimental models. Second, we wished to determine whether our previous findings on the tissue level for the ASD and PAB models obtained on the cellular level, ie, is the normal contractile performance of RV papillary muscles from ASD cats based on normal contractile performance of RV cardiocytes, and is the abnormal contractile performance of RV papillary muscles from PAB cats based on abnormal contractile performance of RV cardiocytes? Third, we wished to determine whether microtubule depolymerization had a positive effect on the contractile performance of RV cardiocytes from PAB cats not seen in LV cardiocytes from the same PAB cats, in either RV or LV cardiocytes from control cats, or in either RV or LV cardiocytes from ASD cats having a similar degree and duration of RV hypertrophy. Fourth, we wished to determine whether the contractile abnormalities characteristic of RV pressure-overload hypertrophy could be reproduced in normal cardiocytes by causing microtubule hyperpolymerization. Fifth, we wished to determine whether any findings were specific to the microtubule component of the cytoskeleton and whether the experimental interventions used produced microtubule-specific as opposed to unintended secondary effects on cardiocyte mechanics.

Microtubule Depolymerization: Colchicine

The colchicine alkalds bind to the β-subunit of the αβ-tubulin heterodimer, preventing its assembly into microtubules. Because there is rapid interchange between free and polymerized tubulin heterodimers in interphase microtubules, the net effect of colchicine is a rapid loss of microtubules from interphase cells, reaching completion in about 1 hour at concentrations 10–6 mol/L in cultured fibroblasts. Fig 1 shows that this same effect was obtained in adult cardiocytes, and a dose-response assay of colchicine concentration versus cardiocyte mechanics, as defined by the sarcomere shortening behavior delineated in the figures immediately following, showed that the maximum effect of colchicine was achieved at a concentration of 10–6 mol/L.

Control. Fig 2A shows immunoblots of free (lanes 1 and 3) and polymerized (lanes 2 and 4) β-tubulin in myocardium and immunofluorescence micrographs of microtubules in isolated cardiocytes from control cats. Lanes 1 and 2 and the corresponding micrograph are from the right ventricle; lanes 3 and 4 and the corresponding micrograph are from the left ventricle. For both the right ventricle and the left ventricle, the majority of the β-tubulin is unpolymerized, and the quantities of free and polymerized β-tubulin are comparable for RV and LV myocardium. Similarly, the micrographic density of the microtubule network is alike for the RV and LV cardiocytes. Sequential contractions for a single LV cardiocyte from a control cat are shown in Fig 2B, and sequential contractions for a single RV cardiocyte from the same control cat are shown in Fig 2C. For each contraction, sarcomere length versus time is given above, and the rate of length change versus time is given below. The time in minutes after the addition of colchicine is indicated. Sarcomere motion and its first derivative did not change noticeably during sequential sampling of either the LV or the RV cardiocyte, and the sarcomere motion of the two cells is entirely comparable throughout the period of study. Fig 2D gives summary data for the maximum
extent of sarcomere shortening defined as initial sarcomere length minus minimum sarcomere length at the indicated times after the addition of colchicine to RV and LV cardiocytes from the same control cats. All cells were sampled sequentially at the indicated times after drug exposure. Fig 2E gives summary data for the maximum velocity of sarcomere shortening defined as the maximum positive rate of length change for the same contractions summarized in Fig 2D. As shown, the contractile function of cardiocytes from the two ventricles was identical. Furthermore, colchicine caused only a small, statistically insignificant improvement in contractile function for both RV and LV cardiocytes during the first 30 minutes of drug exposure.

**Two-week atrial septal defect.** The format for the figure giving these data, Fig 3, is identical to that of Fig 2. Of interest, the data as well are in all respects indistinguishable from those of the control cats in Fig 2 despite the fact that there was substantial RV volume-overload hypertrophy in these ASD cats. Thus, the data for the right ventricle and left ventricle of the 2-week ASD cats are both equivalent and duplicate those of the control cats.

**Two-week pulmonary artery band.** The format for the figure giving these data, Fig 4, is also identical to that of Fig 2. Here, however, the data for these 2-week PAB cats show several conspicuous differences from those of the control and 2-week ASD cats. First, the immunoblot shows a clear increase in both free and polymerized β-tubulin in RV myocardium. Second, the micrographic density of the microtubule network is greater in the RV than in the LV cardiocyte. Third, comparison of Fig 4B with Fig 4C shows that the initially depressed sarcomere shortening and shortening velocity of the RV cardiocyte when compared with that of the LV cardiocyte from the same cat returns toward normal after colchicine exposure; the sarcomere mechanics for the LV cardiocyte are the same as those for control RV and LV cardiocytes. This is confirmed in the summary data of Fig 4D and Fig 4E, which show that the initial differences between the RV and LV cardiocytes are no longer statistically significant 30 minutes after the addition of 10⁻⁶ mol/L colchicine, whereas for the RV cardiocytes there is after 30 minutes a significant difference from their initial values for both sarcomere shortening and sarcomere shortening velocity. Thus, there is biochemical and structural evidence of increased microtubules in RV myocardium from 2-week PAB cats, and exposure of hypertrophied RV cardiocytes from these animals to colchicine largely normalized the initially quite abnormal contractile function.

**Four-week atrial septal defect.** The format for the figure giving these data, Fig 5, differs from that of the preceding three figures in that only sarcomere mechanics are shown; the format is otherwise identical. Of note, 4 weeks is the time after volume overloading at which normal myocardial contractile function was defined previously in this model. Here, as was the case for the 2-week ASD cats, the mechanical data for the RV and LV cardiocytes of the 4-week ASD cats are both equivalent and duplicate those of the control cats.

**Four-week pulmonary artery band.** The format for the figure giving these data, Fig 6, is identical to that of Fig 5. Of note, 4 weeks is the time after pressure overloading at which abnormal myocardial contractile function was defined previously in this model. The findings here for the 4-week PAB cats replicate those shown in Fig 4 for the 2-week PAB cats, with the single exception that contractile function of the hypertrophied RV cardiocytes became both statistically and numerically equivalent to that of the normal LV cardiocytes by 30 minutes after colchicine exposure.

To summarize the data to this point, a central element of the hypothesis underlying this study is that the intracellular alteration responsible for the contractile dysfunction of myocardium hypertrophying in response to a pressure overload must not be present in myocardium hypertrophying for a comparable time and to a comparable extent in response to a volume overload; that is, the alteration should appear only in response to stress overloading. It was further suggested that the specific intracellular alteration causing contractile dysfunction would be an increase in microtubules. The data shown in Figs 3 through 6, using similar durations and degrees of RV hypertrophy in response to volume versus pressure loading, support the hypothesis. Furthermore, the etiologic assignment of the contractile defect to increased microtubules is apparently correct.

**Six-month pulmonary artery band.** If any abnormality of contractile function is to have eventual significance for the transition from initially compensatory cardiac hypertrophy to decompensated cardiac failure, it must be a persistent feature of hypertrophied myocardium rather than a transient feature of the hypertrophic growth process. Therefore, this same study was repeated on a further group of PAB cats at a time remote from the induction of RV pressure overload. The format for the figure giving these data for the 6-month PAB cats, Fig 7, is identical to that of Figs 5 and 6. Cardiocyte contractile function as characterized by sarcomere motion was again quite abnormal at baseline in the hypertrophied RV cells, but both the extent and the velocity of sarcomere shortening became identical to those of LV cardiocytes from the same cats and duplicated those of the RV and LV cardiocytes from control cats after 30 minutes of exposure to colchicine.

**Microtubule Depolymerization: Cold**

Colchicine affects the frequency but not the intensity of neonatal cardiocyte contractions but its effects on the mechanics of adult cells had not been defined previously. Both for this reason, and more importantly to exclude the possibility that the effects observed in pressure-overload hypertrophy were unrelated to microtubule depolymerization, we examined sarcomere mechanics after depolymerizing cardiocyte microtubules by a quite different means. These data for 2-week PAB cats are given in Fig 8. The left half of the figure, comprising the contractions above and the summary data below, are for RV and LV cardiocytes from 2-week PAB cats that were kept at 37°C

---

Fig 3. Facing page. Colchicine, 2-week ASD cats: The format for this figure is identical to that of Fig 2. Resting sarcomere length was 1.93 ± 0.03 μm for left ventricular (LV) cells versus 1.94 ± 0.03 μm for right ventricular (RV) cells (P = NS). For D and E, statistical comparisons were by two-way ANOVA and a means comparison contrast,* where n = number of cells; no significant differences were found.

---

**Contractile Performance of Hypertrophied Cardiocytes**

Tsutsui et al

Downloaded from http://circ.ahajournals.org/ by guest on July 16, 2017
**A**

Circulation Vol 90, No 1 July 1994

**B**

[Graph showing time (min) vs. velocity (um/sec) for LV and RV.

**C**

[Graph showing time (min) vs. extent of shortening (um) for LV and RV.

**D**

[Graph showing time (min) vs. velocity of shortening (um/sec) for LV and RV.

**E**

[Graph showing time (min) vs. extent of shortening (um) for LV and RV.
Fig 4. Facing page. Colchicine, 2-week PAB cats: The format for this figure is identical to that of Fig 2. Resting sarcomere length was 1.93±0.02 μm for left ventricular (LV) cells versus 1.93±0.02 μm for right ventricular (RV) cells (P=NS). For D and E, statistical comparisons were by two-way ANOVA and a means comparison contrast, where n=number of cells. Reprinted, in part, with permission. *P<.001 for difference from the initial time 0 value within a group.

Fig 5. Colchicine, 4-week ASD cats: The format for this figure is identical to that of Fig 2, with the exception that only the effects of colchicine on sarcomere mechanics are shown. Resting sarcomere length was 1.89±0.04 μm for left ventricular (LV) cells versus 1.91±0.02 μm for right ventricular (RV) cells (P=NS). For C and D, statistical comparisons were by two-way ANOVA and a means comparison contrast, where n=number of cells; no significant differences were found.

Throughout the study period. The initially normal contractile function of LV cardiocytes and abnormal contractile function of RV cardiocytes were maintained unaltered for the entire 2 hours. The right half of the figure, comprising the contractions above and the summary data below, are for RV and LV cardiocytes from these same cats that were first studied at 37°C (T0), studied again at 37°C after an intervening hour at 0°C (T1), and then studied again after a further hour at 37°C (T2). The initially abnormal contractile function of RV cardiocytes at T0 was normalized at T1 and remained the same as that for LV cardiocytes at T2; both RV and LV cardiocytes showed a modest decre-
Colchicine, 4-week PAB cats: The format for this figure is identical to that of Fig 2, with the exception that only the effects of colchicine on sarcomere mechanics are shown. Resting sarcomere length was 1.92±0.02 μm for left ventricular (LV) cells versus 1.94±0.03 μm for right ventricular (RV) cells (P=NS). For C and D, statistical comparisons were by two-way ANOVA and a means comparison contrast, where n=number of cells. *P<.001 for difference from the LV value at matched time points. tP<.001 for difference from the initial time 0 value within a group.

Fig 6. Colchicine, 4-week PAB cats: The format for this figure is identical to that of Fig 2, with the exception that only the effects of colchicine on sarcomere mechanics are shown. Resting sarcomere length was 1.92±0.02 μm for left ventricular (LV) cells versus 1.94±0.03 μm for right ventricular (RV) cells (P=NS). For C and D, statistical comparisons were by two-way ANOVA and a means comparison contrast, where n=number of cells. *P<.001 for difference from the LV value at matched time points. tP<.001 for difference from the initial time 0 value within a group.

ment in contractile function during microtubule repolymerization between T1 and T2 similar in degree to the increment in contractile function seen for normal cardiocytes during microtubule depolymerization in Figs 2 through 7. Of particular interest in the context of our hypothesis is the fact that when cardiocyte microtubules which polymerized under a stress load in vivo polymerized under zero load in vitro, the initial contractile abnormality was not recapitulated (Fig 8C and 8D, RV T0 versus RV T2).

Microtubule Hyperpolymerization: Taxol

If an excess of microtubules is responsible for the contractile dysfunction seen in cardiocytes hypertrophying in response to a pressure overload, the same contractile dysfunction should appear in normal cardiocytes if excessive microtubule polymerization is caused to occur in these cells by a different means. Taxol, by causing hyperpolymerization of microtubules, acts in a manner closely analogous to the hypothesized effect of
Colchicine, 6-month PAB cats: The format for this figure is identical to that of Fig 2, with the exception that only the effects of colchicine on sarcomere mechanics are shown. Resting sarcomere length was 1.93±0.03 μm for left ventricular (LV) cells versus 1.95±0.05 μm for right ventricular (RV) cells (P=NS). For C and D, statistical comparisons were by two-way ANOVA and a means comparison contrast,40 where n=number of cells. Reprinted with permission.11 *P<.001 for difference from the LV value at matched time points. †P<.001 for difference from the initial time 0 value within a group.

an extending stress load on the αβ-tubulin heterodimer–microtubule system of cardiocytes exposed to a pressure overload. After a dose-response assay of taxol concentration versus cardiocyte mechanics showing that 10^{-5} mol/L taxol had a maximum effect on the extent and velocity of sarcomere shortening in normal cardiocytes, the data shown in Fig 9 were obtained. Fig 9A shows that sarcomere motion and its first derivative did not change appreciably during the sequential sampling of aliquots of normal cardiocytes exposed to the drug vehicle; however, Fig 9B shows that the initially normal extent and velocity of sarcomere shortening seen in normal cardiocytes decreased markedly during 3 hours of exposure to taxol and remained at this diminished level at 4 hours. The summary data in Fig 9C and Fig 9D show that the contractile function of normal cardiocytes exposed to taxol became statistically different from the baseline function for these same cells as well as the time-matched contractile function of normal cardiocytes exposed to vehicle by 2 hours after taxol exposure.
Circulation Vol 90, No 1 July 1994

**A**

Extent of Shortening (µm) vs Time (T0, T1, T2)

- **NORMOTHERMIA**
  - LV: (n=21), (n=11), (n=7)
  - RV: (n=20), (n=9), (n=7)

- **HYPOTHERMIA**
  - LV: (n=20), (n=11), (n=7)
  - RV: (n=20), (n=11), (n=7)

**B**

Velocity of Shortening (µm/sec) vs Time (T0, T1, T2)

- **NORMOTHERMIA**
  - LV: (n=21), (n=11), (n=7)
  - RV: (n=20), (n=9), (n=7)

- **HYPOTHERMIA**
  - LV: (n=20), (n=11), (n=7)
  - RV: (n=20), (n=11), (n=7)

**C**

**D**

Downloaded from http://circ.ahajournals.org/ by guest on July 16, 2017
and remained different thereafter. Thus, taxol causes the sarcomere motion of normal cardiocytes to mimic the abnormal sarcomere motion of cardiocytes hypertrophying in response to a stress load.

**Microtubule Hyperpolymerization: D₂O**

For reasons analogous to those that led us to use cold as well as colchicine in examining the mechanical effects of microtubule depolymerization, we thought it important to use an additional means quite different from taxol in examining the mechanical effects of microtubule hyperpolymerization. For this purpose we chose D₂O, which causes rapid and readily reversible microtubule hyperpolymerization. As shown by the contractions and summary data in Fig 10, when 50% D₂O/50% H₂O was substituted for 100% H₂O in the superfusing buffer of normal cardiocytes and the same cells were followed sequentially, there was a decrease in the extent and velocity of sarcomere shortening within 10 minutes; these effects were then fully reversed 10 minutes after resubstitution of 100% H₂O in the superfusing buffer. Thus, D₂O also causes the sarcomere motion of normal cardiocytes to mimic the abnormal sarcomere motion of cardiocytes hypertrophying in response to a stress load.

**Microtubule Specificity**

The goal here, as noted before, was to determine whether the findings to this point, especially those demonstrating improved contractile function in pressure hypertrophied cardiocytes coincident with interventions designed to cause microtubule depolymerization, resulted from microtubule-specific as opposed to unintended effects of the experimental interventions.

**Intermediate filaments.** Microtubule dissolution by colchicum alkaloids also may alter the normal architecture of intermediate filaments in fibroblasts, but hypothermic microtubule dissolution does not have this secondary effect. Nonetheless, to be certain that intermediate filament alterations were not responsible for the ameliorative effects on cardiocyte contractile function described above, cells from the T0, T1, and T2 groups in Fig 8 were prepared for laser scanning confocal microscopy using a monoclonal antibody against desmin. The results are shown in Fig 11. The clearly defined intermediate filament network present in control cells (T0) was not altered either by cold exposure (T1), which completely removed the microtubules, or by rewarming (T2), which restored the microtubule appearance to normal.

**Microfilaments.** To assess the possible role of microfilament alterations in cardiocyte contractile dysfunction, cytochalasin D, which disrupts the cortical microfilaments of mature myotubes at a concentration of 2 μg/mL within 45 minutes, was applied at this concentration to RV and LV cardiocytes from control and 2-week PAB cats. No significant improvement in contractile function was observed over a period of 1 hour in any cell, i.e., the change in the extent and velocity of sarcomere shortening, whether positive or negative, was <8% in all cases. Thus, there is presumptive evidence that of the three major classes of cytoskeletal filaments, only the microtubules are causally involved in the contractile dysfunction of cardiocytes hypertrophying in response to a pressure overload.

**Calcium metabolism.** The concern here was to evaluate the possibility that the improved sarcomere mechanics in pressure-hypertrophied RV cardiocytes after microtubule depolymerization by either colchicine or hypothermia was due to a change in pCa²⁺, attendant on these interventions. For colchicine, there is evidence in mast cells that this agent at concentrations ≥10⁻⁶ mol/L causes a decrease rather than an increase in pCa²⁺, and the data in Fig 12 show that this applies, at least qualitatively, to cardiocytes as well. Fig 12A shows that in normal cardiocytes there is the expected increase in pCa²⁺ in resting cells with increasing superfusate calcium and a further increase in pCa²⁺ with contraction. Fig 12B shows that for cardiocytes from 2-week PAB cats there is a minor, statistically insignificant decrease in pCa²⁺ in both resting and contracting cells upon exposure to 10⁻⁴ mol/L colchicine. This finding obtains for both the normally loaded LV cardiocytes and the pressure hypertrophied RV cardiocytes, and the calcium-related fluorescence is equivalent in the two types of cells as well. For hypothermia, there is internal evidence in Fig 8C and Fig 8D that this intervention does not cause a physiologically significant increase in pCa²⁺. That is, at T1, immediately after rewarming, there is not a significant increase either in the extent or in the velocity of sarcomere shortening in the normally loaded LV cardiocytes.

**Lumicolchicine.** Finally, we wished to address the possibility that colchicine might have an effect on sarcomere mechanics unrelated to its tubulin binding properties. For this purpose we used lumicolchicine, the inactive stereoisomer of colchicine, which at 10⁻⁵ mol/L for 1 hour was without effect on any aspect of sarcomere mechanics in RV or LV cardiocytes from 2- or 4-week PAB cats.
Fig 9. Taxol, control cats: The effects of taxol on sarcomere motion in cardiocytes isolated from normal cats; the time in hours after the addition of vehicle or taxol is indicated. As is the case for the contractions shown in Fig 8, each contraction shown here represents an average for the number of cardiocytes given in parentheses at each time point. A, Contractions of normal cardiocytes at the specified times after the addition of $10^{-6}$ mol/L dimethyl sulfoxide vehicle to the superfusate; sarcomere length versus time is given above and the rate of length change versus time is given below. B, Contractions of normal cardiocytes at the specified times after the addition of $10^{-5}$ mol/L taxol to the superfusate; sarcomere length versus time is given above and the rate of length change versus time is given below. C, Summary data for the maximum extent of sarcomere shortening at the indicated times after adding vehicle or taxol to normal cardiocytes. D, Summary data for the maximum velocity of sarcomere shortening for the same cells shown in C. Resting sarcomere length was 1.94±0.02 μm. For C and D, statistical comparisons are by two-way ANOVA and a means comparison contrast,40 where n=number of cells. Reprinted, in part, with permission.11 *P<.001 for difference from the vehicle value at matched time points. †P<.001 for difference from the initial time 0 value within a group.

Cardiocyte Mechanics: Sarcomere versus Cardiocyte

The data in Figs 1 through 12 demonstrate that microtubules are selectively increased in myocardium hypertrophying in response to pressure as opposed to volume overloading, that this alteration is apparently responsible for the entirety of the contractile defect observed in RV cardiocytes from this PAB model, that the contractile defect can be reproduced in normal cardiocytes when microtubule hyperpolymerization is induced by chemical as opposed to pathophysiological
interventions, and that the contractile defect is specific to the microtubule component of the cytoskeleton. As yet undetermined was the functional significance to cellular mechanics of these changes. That is, the mechanical data presented heretofore have been based on measurements of sarcomere motion. If the microtubules have a significant role in tethering the Z-lines to the sarcolemma, it is entirely possible that the favorable effects of microtubule depolymerization documented on the level of sarcomere mechanics might have little importance to cellular mechanics, especially when, as is the case in vivo, the cell is externally loaded. While there is evidence that in striated muscle intermediate filaments rather than microtubules play the predominant role in physically attaching myofilaments to the sarcolemma,47 we nonetheless believed that it was critical to explore this point under the particular pathophysiological circumstances of interest here.

The data shown in Fig 13 were obtained from normally loaded LV cardiocytes and pressure-hypertrophied RV cardiocytes from the 2-week PAB model. The intervention used to depolymerize microtubules in these cells was hypothermia. That is, they were studied under conditions of normothermia corresponding to the T0 point in Fig 8C and 8D or immediately after rewarming from 1 hour of hypothermia, corresponding to the T1 point. The cells were either externally unloaded during contraction, ie, they contracted in a superfusate having a viscosity of 1 cP, or they were externally loaded during contraction, ie, they contracted in a methylcellulose-containing superfusate having a viscosity of 160 cP. Fig 13A and 13B give data for sarcomere shortening and cardiocyte shortening, respectively, in alternating series of contractions of the same cardiocytes. The data in Fig 13A gathered at an external load of 1 cP essentially replicate those of Fig 8C. That is, for the LV cardiocytes there is only a minor, statistically insignificant increase in the extent of sarcomere shortening after microtubule depolymerization by hypothermia. In contrast, for the RV cardiocytes the initially quite abnormal extent of sarcomere shortening becomes statistically indistinguishable from that of the LV cells after cold-induced microtubule depolymerization. The data in Fig 13A gathered at an external load of 160 cP show the same basic interrelation between sarcomere shortening versus experimental interventions as the data gathered at an external load of 1 cP. Notably, there are parallel decrements in the extent of sarcomere shortening for all

---

**Fig 10.** Deuterium oxide (D₂O), control cats: The effects of D₂O on sarcomere motion in cardiocytes isolated from normal cats. Sarcomere motion was defined initially (T0), 10 minutes after replacing 50% of the H₂O in the superfusate with D₂O (T1), and a further 10 minutes after returning to a normal superfusate (T2). Each contraction shown here represents an average for the number of cardiocytes given in parentheses at each time point. A, Contractions of normal cardiocytes at the time points specified above; sarcomere length versus time is given above and the rate of length change versus time is given below. B, Summary data for the maximum extent of sarcomere shortening at the indicated experimental time points. C, Summary data for the maximum velocity of sarcomere shortening for the same cells shown in B. Resting sarcomere length was 1.90±0.05 μm. For B and C, statistical comparisons are by two-way ANOVA and a means comparison contrast,46 where n=number of cells. †P<.001 for difference from the initial time 0 value within a group.
four groups of cardiocytes. This is the result that would be expected if, despite microtubule depolymerization, the normal attachment of the myofibrils to the sarcolemma is maintained. The data in Fig 13B, which describe cardiocyte shortening for the same groups of cells under the same experimental conditions during a series of contractions successive in each case to that used to quantitate sarcomere shortening in Fig 13A, show essentially the same result. The fact that sarcomere and cardiocyte mechanics are entirely in parallel demonstrates not only that the microtubules are mechanically unimportant as structural attachments of myofibrils to the sarcolemma but also that the normalization of contractile function for the pressure hypertrophied RV cardiocytes after microtubule depolymerization obtains equally on both the subcellular and cellular levels.

Fig 13C and 13D summarize the relation for external loads of 1 cP and 160 cP between sarcomere and cardiocyte shortening for LV and RV cardiocytes, respectively. The data in Fig 13C show first during normothermia for the normal LV cardiocytes that under an increased external load there is the expected decrement in the extent of cardiocyte and sarcomere shortening; however, there is only a modest increase in the extent of cardiocyte and sarcomere shortening at either load after microtubule depolymerization. The data in Fig 13D show first during normothermia for the hypertrophied RV cardiocytes that the initially abnormal cardiocyte and sarcomere shortening in the 1-cP superfusate decreases still further when a 160-cP external load is imposed. However, the normalization of both sarcomere and cardiocyte shortening at either load after microtubule depolymerization is quite apparent in comparing the RV hypothermia data in Fig 13D with the LV data in Fig 13C. Again, the implication here is that the role of the microtubules in the contractile dysfunction of pressure-hypertrophied RV cardiocytes is equally prominent on the subcellular and cellular levels.
Cytoskeletal Characterization: Tubulin and Microtubules

The immunoblots in Fig 2A and Fig 3A demonstrate comparable amounts of free and polymerized β-tubulin in right and left ventricular myocardium from a control and an ASD cat. The immunoblot in Fig 4A demonstrates a selective increase in both free and polymerized β-tubulin in RV myocardium from a PAB cat. The data in Fig 14 represent a more systematic, quantitative analysis of β-tubulin in immunoblots of RV and LV myocardium from the three experimental models. The illustrative immunoblot from normal myocardium shows free β-tubulin in lanes 1 and 3, polymerized β-tubulin in lanes 2 and 4, total myocardial β-tubulin in lanes 8 and 9, and bovine brain β-tubulin standards in lanes 5 through 7. Quantification was obtained as the integrated optical density increase over background optical density in a rectang-
Previously, increased cardiac myofibrillar density has been observed only transiently during the onset of cardiac hypertrophy in rodents or as a feature of a subset of degenerated cardiomyocytes from failing hearts in humans. In contrast, decreased cardiac myofibrillar density has been observed in myopathic cardiac muscle. Here, a sustained increase in pCa, may well be responsible for microtubule depolymerization. Indeed, in our canine model of mitral regurgitation, where there are myopathic changes in the most severely affected animals, we have found no effect of microtubule depolymerizing agents on the abnormal contractile function of hypertrophied cardiocytes (data not shown). In contradistinction to these earlier findings, the present study shows that, as predicted by the initial hypothesis, there is a persistent increase in polymerized tubulin in cardiocytes that hypertrophy in response to a stress but not a strain overload. Our major finding is that this cytoskeletal alteration apparently accounts for the entirety of the contractile abnormality observed on the cellular level in the pressure-overloaded feline right ventricle.

Given the association of excessive microtubules with contractile dysfunction in pressure-overload cardiac hypertrophy and the evidently full reversibility of this dysfunction after microtubule depolymerization, several questions are of immediate interest. First, are the increased microtubules in stress-hypertrophied cardiocytes the direct result of mechanical load as our hypothesis would suggest, or are additional or alternative mechanisms such as posttranslational modifications of tubulin or increased microtubule-associated proteins?
each of which enhances microtubule stability, operative? Second, while it should not be of consequence for cardiocyte mechanics, the increased free RV β-tubulin seen in the immunoblots of Fig 4 and Fig 14 for the PAB cats is perhaps a bit surprising in view of the negative feedback control which tubulin is thought to exert on its own rate of synthesis. Thus, how is tubulin synthesis and its assembly into microtubules controlled in the pressure-hypertrophied cardiocyte? Third, is the contractile defect described here at the cellular level also observed at the tissue level, and is this defect found equally in the pressure-overloaded left ventricle where the great preponderance of clinically significant human pathophysiology is observed?

Irrespective of the answers to these questions, the present data may be of central importance to a unified understanding of the quantitative and qualitative defects responsible for the transition from initially compensatory cardiac hypertrophy into the congestive heart failure state. That is, in the face of a sustained hemodynamic overload, cardiac hypertrophy fails to be fully compensatory either quantitatively, when the increased stress cannot be renormalized by growth, or qualitatively, when the contractile function per unit mass of hypertrophied myocardium is diminished. The selective increase in microtubules in response to stress loading and their contribution to contractile dysfunction in this situation suggest that when the hypertrophic growth process does not renormalize myocardial stress, this inadequate quantitative response is compounded by a qualitative defect that further jeopardizes the ability of the heart to compensate functionally.

Acknowledgments

This study was supported by grants HL-37196 and HL-48788 to Dr Cooper from the National Heart, Lung, and Blood Institute and by research funds to Dr Cooper from the Department of Veterans Affairs. This work was done during the tenure of a Clinical Investigator Award to Dr Cooper from the Department of Veterans Affairs. We thank Dr Douglas...
Thrower, Department of Biological Sciences, University of California, Santa Barbara, for generously providing the bovine brain tubulin. The authors also thank Stacy Prutting, Heather Downs, Valerie Young, and Mary Barnes for technical assistance.

References


Role of microtubules in contractile dysfunction of hypertrophied cardiocytes.
H Tsutsui, H Tagawa, R L Kent, P L McCollam, K Ishihara, M Nagatsu and G Cooper, 4th

Circulation. 1994;90:533-555
doi: 10.1161/01.CIR.90.1.533
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/90/1/533

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/