Time course of right ventricular pressure-overload induced myocardial fibrosis: relationship to changes in fibroblast postsynthetic procollagen processing

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Baicu CF, Li J, Zhang Y, Kasiganesan H, Cooper GI, Zile MR, Bradshaw AD. Time course of right ventricular pressure-overload induced myocardial fibrosis: relationship to changes in fibroblast postsynthetic procollagen processing. Am J Physiol Heart Circ Physiol 303: H1128–H1134, 2012. First published August 31, 2012; doi:10.1152/ajpheart.00482.2012.—Myocardial fibrillar collagen is considered an important determinant of increased ventricular stiffness in pressure-overload (PO)-induced cardiac hypertrophy. Chronic PO was created in feline right ventricles (RV) by pulmonary artery banding (PAB) to define the time course of changes in fibrillar collagen content after PO using a nonrodent model and to determine whether this time course was dependent on changes in fibroblast function. Total, soluble, and insoluble collagen (hydroxyproline), collagen volume fraction (CVF), and RV end-diastolic pressure were assessed 2 days and 1, 2, 4, and 10 wk following PAB. Fibroblast function was assessed by quantitating the product of postsynthetic procollagen processing, insoluble collagen, and levels of SPARC (secreted protein acidic and rich in cysteine), a protein that affects procollagen processing. RV hypertrophic growth was complete 2 wk after PAB. Changes in RV collagen content did not follow the same time course. Two weeks after PAB, there were elevations in total collagen (control RV: 8.84 ± 1.03 mg/g vs. 2-wk PAB: 11.50 ± 0.78 mg/g); however, increased insoluble fibrillar collagen, as measured by CVF, was not detected until 4 wk after PAB (control RV CVF: 1.39 ± 0.25% vs. 4-wk PAB: 4.18 ± 0.87%). RV end-diastolic pressure was unaltered until after the hypertrophic process is essentially complete (12, 18).

Defining these temporal differences in the hypertrophic and fibrotic response to PO in a nonrodent relevant model is important in understanding the progression from compensated hypertropy to decompensated heart failure. In addition, it is important to define the cellular and molecular mechanisms responsible for the differences in these temporal responses, particularly those responsible for the time course of myocardial fibrosis. Collagen homeostasis can be altered by a number of mechanisms that include changes in procollagen synthesis, postsynthetic procollagen processing, posttranslational modification of collagen, and collagen degradation (25). Based on our laboratory’s previous studies defining the importance of fibroblast-dependent postsynthetic procollagen processing, we chose to focus the present study specifically on the role that fibroblast-dependent postsynthetic procollagen processing plays in the temporal course of PO-induced myocardial fibrosis (3).

Accordingly, the purposes of the present study were to define the time course of changes in myocardial fibrillar collagen content after the imposition of PO using a large nonrodent model, and to determine whether this time course was dependent on changes in cardiac fibroblast function. Fibroblast function was assessed by quantitating the product of postsynthetic processing, insoluble fibrillar collagen, and by examining the abundance of SPARC (secreted protein acidic and rich in cysteine), a protein shown to affect postsynthetic procollagen processing (23). SPARC is a collagen-binding matricellular protein implicated in collagen deposition in PO myocardium (3). Mice lacking SPARC expression exhibit significantly less collagen accumulation in response to PO compared with wild-type mice. In these experiments, we used the pulmonary artery band (PAB) model in cats (10). This model has been shown to result in significant right ventricular (RV) hypertrophy, myocardial fibrosis, and cardiac dysfunction (16, 20). However, the time course of changes in remodeling and fibrosis, the mechanisms that underlie these changes, and changes in fibroblast function have not been previously examined (17). This model provides a PO-induced hypertrophied RV and a same-animal normally loaded nonhypertrophied left ventricular (LV) control. Primary fibroblast cultures can be separately generated from the RVs and LVs, allowing analysis of PO RV fibroblasts along side same-animal control LV fibroblasts.

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**METHODS**

*Feline model of chronic PO.* Chronic PO of the RV was created by banding of the pulmonary artery (PAB), as previously described (21). This animal model has been used by our laboratories for more than 20 yr with consistent results in terms of hemodynamic response and appropriate hypertrophic growth of the RV (29). The LV of PAB cats were used as same-animal non-PO controls. To confirm that structural changes did not occur in the LV of PAB animals, serial echocardiograms were analyzed from four cats before PAB (baseline) and 2 and 4 wk after PAB. Echocardiography measurements showed that there were no significant changes in the LV end-diastolic dimension (EDD), LV mass, and LV fractional shortening (FS) after 2 or 4 wk of PAB compared with the baseline pre-PAB values. For example, 4 wk after PAB, LV EDD was 13 ± 1 vs. 14 ± 1 mm at baseline, LV FS was 39 ± 3 vs. 40 ± 7% at baseline, and LV mass was 8.2 ± 3.4 vs. 7.8 ± 1.7 g at baseline (means ± SE, all comparisons between 4 wk after PAB and pre-PAB baseline were not statistically significant).

Male cats that had not undergone PAB were compared with male cats that had undergone PAB for 2 days and 1, 2, 4, and 10 wk. For Fig. 1, RV mass-to-tibia length ratios for 24 h and additional animals at later time points were generated as a result of additional studies performed by the authors of this study and were included for completeness and robustness of the time course (7, 8, 19). All procedures were in accordance with Institutional Animal Care and Use Committee (IACUC) guidelines and approved by the IACUC at the Ralph H. Johnson Veteran’s Administration Medical Center, Charleston, SC.

**Hydroxyproline analysis.** Total, soluble, and insoluble myocardial collagen was quantified in the RV and LV free walls using hydroxyproline analysis, as previously described (3). Briefly, frozen LV and RV tissue was lyophilized, weighed (dry weight), pulverized, resuspended in 1 M NaCl with protease inhibitors, tumbled overnight at 4°C, and centrifuged. The supernatant then contained the “NaCl soluble” collagen (i.e., largely non-cross-linked collagen); the pellet contained the “NaCl insoluble” collagen (fully mature cross-linked fibrillar collagen). Each collagen fraction was processed separately. Collagen fractions underwent complete acid hydrolysis with 6 N HCl for 18 h at 120°C, and then each was neutralized to pH 7 with 4 N NaOH. One milliliter of chloramine T was added to 2-ml volumes of collagen sample and incubated at room temperature for 20 min. One milliliter of Ehrlich’s reagent (60% perchloric acid, 15 ml 1-propanol, 3.75 g p-dimethyl-amino-benzaldehyde in 25 ml) was added, and samples were incubated at 60°C for 20 min. Absorbance at 558-nm wavelength was read on a spectrophotometer. Collagen was quantified as milligrams hydroxyproline per gram dry weight myocardium.

**Histology.** Sections of the free wall of RVs and LVs were fixed in 10% formalin and stained with picrosiris red (PSR), as previously described (3). PSR is a fibrillar collagen-specific stain when viewed under polarized light. Images generated from polarized light microscopy were captured using a Leica microscope equipped with an isocap camera. Each section was evaluated for total area of stained collagen fibers [collagen volume fraction (CVF)] from at least five fields from three separate animals per time point using Sigmascan software, as described in Bradshaw et al. (3). Sections containing epicardium and/or large vessels were excluded from the analysis.

**Fibroblast isolation and function.** RV and LV fibroblasts were cultured from normal, 2-wk PAB, and 4-wk PAB cats using previously published methods (24). RV and LV tissue from each animal was excised and incubated with Blendzyme 3 (Roche) for 2-3 h to liberate cells from myocardium (24). Digested tissue was triturated, washed three times in growth media [Dulbecco’s modified Eagle’s media (Gibco), 10% fetal calf serum (Cascade Biologics), and antibiotics/antimycotic (Gibco)], and plated onto tissue culture dishes. Following 1 h at 37°C, nonadherent cells were removed, and adherent cells were cultured in growth media. These cultures are routinely found to be >95% fibroblastic in composition, as assessed by immunofluorescence of cell type-specific markers [vimentin (+), desmin (−), vascular endothelial growth factor (VEGF) receptor (−)]. Fibroblast cultures were used for the studies described below at passages 2–4.

Cardiac fibroblasts were plated at 5 × 10^4 per 100 mm^2 in growth media with ascorbic acid to promote collagen production. Conditioned media and cell layers were collected at appropriate time points. Cell layers were rinsed in PBS and then recovered in 1% deoxycholate containing 10 mM Tris pH 7.4 with protease inhibitors (Roche) and tumbled overnight at 4°C. Soluble cell layers were separated from insoluble cell layers by centrifugation at 15,000 × g for 15 min. Total protein in digested soluble cell layers was assayed with bicinchoninic acid assays to confirm equal loading of protein in insoluble fractions. Levels of collagen in soluble and insoluble cell layers were assessed by Western blot analysis, as previously described (24). Amounts of insoluble collagen from RV fibroblasts were normalized to that of LV fibroblasts from the same animals. Three separate primary fibroblast isolations were carried out for each time point.

**SPARC abundance.** Immunoblot analysis of myocardial proteins extracted from myocardial samples with 2.5% SDS was performed by transfer of separated proteins to nitrocellulose and detection with anti-SPARC antibodies [GSN4–2; Takara (Clonetech), Mountain View, CA]. Chemiluminescence was used to detect secondary antibodies conjugated to horseradish peroxidase. SPARC (osteonectin/ BM40) protein was similarly detected using anti-SPARC monoclonal antibodies in fibroblast primary cultures.

**Statistical analysis.** Temporal changes in myocardial structural and function, hydroxyproline, CVF, and fibroblast function were compared between the non-PO control and PO groups using a one-way ANOVA; pairwise comparisons were made using the Bonferroni test to adjust for multiple comparisons. Values of P < 0.05 were considered statistically significant. Results are presented as means ± SE in the figures. Results in Table 1 are presented as means ± SD. The authors had full access to the data and take full responsibility for its integrity.
### RESULTS

**PO-induced RV hypertrophy and diastolic dysfunction.** The placement of the PAB resulted in an immediate RV PO, as evidenced by the increase in RV systolic pressure shown in Table 1. This RV PO resulted in a time-dependent increase in RV mass. As shown in Fig. 1 and Table 1, RV mass-to-tibia length ratio increased rapidly over the initial 2 wk and reached a plateau at 2 wk that remained relatively constant through 10 wk. RV end-diastolic pressure was unchanged through 2 wk after PAB; however, RV end-diastolic pressure was increased at 4 and 10 wk after PAB.

PAB caused no change in the hemodynamic load on the LV, no change in arterial pressure, and no change in LV mass (Table 1). In addition, echocardiography measurements showed that there were no significant changes in the LV EDD and no changes in LV FS after 2 or 4 wk of PAB compared with the baseline pre-PAB values (see METHODS).

**PO-induced collagen content.** To determine the time course of changes in myocardial interstitial collagen content in the RV myocardium in response to PAB, myocardial collagen were quantified using two separate methods. Quantification of hydroxyproline was used to measure total collagen and soluble collagen (unprocessed procollagen or incompletely processed but non-cross-linked collagen) (Fig. 2). In addition, CVF was calculated from PSR-stained tissue sections (Figs. 3 and 4).

The increase in CVF represented increases in the interstitial insoluble fibrillar collagen content. Total RV hydroxyproline was not significantly changed in the RV of PAB cats after 2 days or 1 wk of PAB. By contrast, 2 and 4 wk after PAB, there were significant increases in total RV hydroxyproline compared with control cats or same-animal control LV of PAB cats (Fig. 2); total RV hydroxyproline was not significantly different between 2 and 4 wk.

The percentage of the total RV hydroxyproline that was soluble was 7.00 ± 1.86% in control and remained unchanged at 5.35 ± 1.16% after 2-wk PAB [P = nonsignificant (NS) vs. control RV]. However, 4 wk after PAB, the percentage of the total hydroxyproline that was soluble fell significantly to 3.27 ± 0.81% (P < 0.05 vs. control RV). Therefore, these data suggest that, while total collagen increased after 2 wk of PAB, the amount of insoluble collagen did not significantly change, whereas the amount of soluble collagen increased; by contrast, the increase in total collagen after 4 wk of PAB appeared to result from increases in insoluble collagen, with decreases in soluble collagen compared with both control and 2-wk PAB. Histological data presented below were concordant with these findings.

Representative PSR-stained histological examples of RV tissue from control cats, after 2 days, 2 wk, 4 wk, and 10 wk of PAB, are shown in Fig. 3. CVF was quantified in Fig. 4. CVF was not significantly altered in the RV 2 days or 2 wk after PAB compared with control cats (P = NS). However, after 4 and 10 wk of PAB, CVF increased significantly (P < 0.05), and collagen fibers appeared more robust in terms of numbers and thickness. Hence, CVF was unchanged at 2 wk, but increased at 4 and 10 wk after PAB.

**Fibroblast function.** Deposition of insoluble collagen by primary fibroblast cultures was monitored to detect changes in postsynthetic procollagen processing by PAB vs. control fibroblasts. Changes in fibroblast function were measured by quantifying insoluble collagen deposition by RV fibroblasts normalized to same animal control LV fibroblasts (RV/LV, Fig. 5). Insoluble collagen content was not significantly altered in RV

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**Table 1. Structural and hemodynamic assessment**

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>2 days</th>
<th>1 wk</th>
<th>2 wk</th>
<th>4 wk</th>
<th>10 wk</th>
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<tbody>
<tr>
<td>n</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>10</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>3.7 ± 0.4</td>
<td>3.6 ± 0.3</td>
<td>3.3 ± 0.6</td>
<td>3.4 ± 0.2</td>
<td>3.8 ± 0.3</td>
<td>4.2 ± 0.1</td>
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<tr>
<td>LV weight, g</td>
<td>7.6 ± 1.2</td>
<td>9.8 ± 1.3</td>
<td>8.4 ± 1.6</td>
<td>7.8 ± 0.7</td>
<td>8.5 ± 0.9</td>
<td>9.3 ± 0.6</td>
</tr>
<tr>
<td>RV weight, g</td>
<td>2.3 ± 0.3</td>
<td>2.7 ± 0.3</td>
<td>2.8 ± 0.3</td>
<td>3.2 ± 0.3*</td>
<td>3.7 ± 0.4*</td>
<td>3.7 ± 0.2*</td>
</tr>
<tr>
<td>LV/BW, g/kg</td>
<td>2.1 ± 0.2</td>
<td>2.7 ± 0.3</td>
<td>2.6 ± 0.4</td>
<td>2.4 ± 0.2</td>
<td>2.5 ± 0.1</td>
<td>2.2 ± 0.1</td>
</tr>
<tr>
<td>RV/BW, g/kg</td>
<td>0.62 ± 0.08</td>
<td>0.76 ± 0.09</td>
<td>0.88 ± 0.10</td>
<td>0.97 ± 0.11*</td>
<td>0.98 ± 0.08*</td>
<td>0.89 ± 0.05*</td>
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<td>RV/TL, g/cm</td>
<td>0.20 ± 0.02</td>
<td>0.23 ± 0.02</td>
<td>0.25 ± 0.02</td>
<td>0.29 ± 0.02*</td>
<td>0.31 ± 0.02*</td>
<td>0.35 ± 0.02*</td>
</tr>
<tr>
<td>Aortic SP, mmHg</td>
<td>124 ± 27</td>
<td>127 ± 30</td>
<td>124 ± 32</td>
<td>123 ± 25</td>
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<td>128 ± 26</td>
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<tr>
<td>RV SP, mmHg</td>
<td>30 ± 2</td>
<td>44 ± 4*</td>
<td>56 ± 9*</td>
<td>56 ± 3*</td>
<td>78 ± 7*</td>
<td>80 ± 6*</td>
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<tr>
<td>RV EDP, mmHg</td>
<td>5 ± 2</td>
<td>8 ± 2</td>
<td>7 ± 2</td>
<td>7 ± 3</td>
<td>14 ± 3#</td>
<td>18 ± 3#</td>
</tr>
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</table>

*Values are averages ± SD; n, no. of animals. LV, left ventricle; RV, right ventricle; BW, body weight; TL, tibia length; SP, systolic pressure; EDP, end-diastolic pressure; PAB, pulmonary artery band. *P < 0.05 vs. normal. #P < 0.05 vs. 2 wk after PAB.

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![Fig. 2.](http://ajpheart.physiology.org/)

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fibroblasts from 2-wk PAB [RV/LV collagen incorporation 1.00 ± 0.11 arbitrary units (AU)] compared with fibroblasts from normal cats (RV/LV = 0.80 ± 0.06 AU, P = NS). By contrast, insoluble collagen was significantly increased in cardiac fibroblasts isolated from 4-wk PAB vs. 2 wk and control cells (RV/LV = 1.40 ± 0.14 AU, P < 0.05 vs. control).

Levels of SPARC increased in 4-wk PAB RV fibroblast cultures compared with both LV fibroblasts from PAB cats and RV fibroblasts from control cats (Fig. 6, A and B). Similarly, in myocardial tissue samples, SPARC was increased in the RV
Temporal patterns of collagen expression and deposition following hypertrophy have also been characterized in rodent models of PO. Generally, and in agreement with the present findings, hypertrophic growth was found to increase, and in most cases, level off, before accumulation of elevated levels of tissue collagen. For example, PO in rabbits generated a significant temporal difference in the development of the hypertrophic vs. fibrotic response to chronic PO. The hypertrophic response is rapid and complete within ~2 wk of creation of the PO. By contrast, the fibrotic response is delayed, does not begin until the hypertrophic response is nearly complete, and is progressive after ~4 wk of PO. Second, the mechanisms underlying this temporal difference include a change in fibroblast function, specifically, a change in fibroblast-dependent postsynthetic procollagen processing. Early in the course of PO (after 2 wk), total collagen was increased, soluble collagen was increased, but insoluble collagen, as measured by CVF, was not changed. These data suggested that procollagen synthesis was increased, but procollagen processing to mature insoluble fibrillar collagen was not. In addition, one matricellular protein critical to procollagen processing, SPARC, was also not increased after 2 wk of PO. Finally, in vitro fibroblast studies showed that insoluble collagen was unchanged in primary fibroblast cultures taken from RV after 2 wk of PO. By contrast, after 4 wk of PO, insoluble collagen and SPARC abundance were increased, but soluble collagen was decreased. Moreover, insoluble collagen in primary fibroblast cultures taken from the RV after 4 wk of PO was increased. These data support the hypothesis that the delayed fibrotic response to PO was caused, at least in part, by a temporal change in fibroblast-dependent postsynthetic procollagen processing.

PO induced by PAB results in a RV hypertrophy. The majority of animal and clinical studies carried out to date have focused on LV PO hypertrophy. PO whether RV or LV, results in chamber hypertrophy, hypertrophy of cardiomyocytes, increased CVFs, and abnormal systolic and diastolic function (9, 10). However, there are notable differences between RV and LV, including myocyte cellular origin during development, disparate geometries, and differences in pressures under normal conditions (15). Thus differences in cellular response to LV and RV, including myocyte cellular origin during development, disparate geometries, and differences in pressures under normal conditions (15). Thus differences in cellular response to PO might differ between ventricles. Urashima, et al. (30) recently compared responses in gene expression between RV and LV PO ventricles in a murine model. Although, in general, many common pathways of gene expression were activated in response to PO in each ventricle, some changes in gene expression unique to RV PO were found. Notably, similar changes in gene expression associated with extracellular matrix (ECM) remodeling, the focus of the present study, were found in both RV and LV PO hypertrophy (30).

Temporal patterns of collagen expression and deposition following hypertrophy have also been characterized in rodent models of PO. Generally, and in agreement with the present findings, hypertrophic growth was found to increase, and in most cases, level off, before accumulation of elevated levels of tissue collagen. For example, PO in rabbits generated a signif-
icant hypertrophic response at 2 days following PO, whereas increases in tissue collagen were not found until 2 wk of PO (18). Kuwahara et al. also reported hypertrophic growth in response to suprarenal aortic banding in rats that reached significant differences at 1 wk and continued to increase at 2 and 4 wk. However, the percentage of myocardial fibrosis was not significantly elevated until 2 wk and further elevated at 4 wk (17).

Increases in expression of mRNA encoding fibrillar collagens have been found shortly after induction of PO. In rats subjected to abdominal aortic banding, a sixfold increase in mRNA encoding collagen α1(I) was detected at 3 days PO that declined to control at 1 wk and remained constant through 8 wk of PO. However, no increases in levels of tissue collagen were evident at 1 wk and were first apparent at 4 wk of PO (6). Similarly, Villarreal and Dillmann (31) found, in thoracic banded rats, a heart weight-to-body weight ratio that was significantly increased at 3 days following PO that coincided with peak expression of mRNA encoding collagen I and III. Tissue levels of collagen were not measured in the latter study (31). Thus hypertrophic growth and increased levels of mRNA encoding fibrillar collagens appear to precede the accumulation of fibrillar collagen in hypertrophic hearts and suggested that mechanisms other than increases in transcription might contribute to increased CVF in PO hearts.

In support of this, Eleftheriades, et al. (12) reported that PO in juvenile rats induced a gradual hypertrophic response, but no increases in mRNAs encoding collagen α1(I) at 1 and 4 wk following PO. However, significant differences in tissue incorporation of collagen were detected in 4-wk hypertrophic ventricles (12). Previous studies showed that a majority of newly synthesized collagen protein was degraded before incorporation into the insoluble ECM in the heart (22). Hence, fibrillar collagen accumulation in the studies by Eleftheriades et al. (12) was attributed to significant decreases in rates of procollagen degradation that were shown in PO ventricles. Likewise, collagen content was not increased at 2 days following PO, but was increased at 2 wk in rabbits. Whereas mRNA encoding collagen I peaked at 2 days and decreased by 2 wk PO, newly synthesized procollagen that was degraded before ECM incorporation decreased from 50.7 to 26.8% in PO tissues (1). The conclusions from these studies were that mechanisms controlling mRNA synthesis, as well as postsynthetic processing of procollagen I, influence collagen deposition in response to PO hypertrophy. In support of this hypothesis, the results reported here demonstrated an increased capacity of 4-wk PAB fibroblasts to efficiently deposit collagen.

Recently, Stewart, et al. (28) analyzed differences in fibroblast function in cells from PO ventricles. In these studies, significant hypertrophic growth in response to abdominal aortic constriction in rats was detected at 1 wk and reached a plateau at 2 wk. CVF was elevated following 1 wk of PO relative to sham control and was further increased at 2 wk of PO. Differences in fibroblast proliferation and gel contraction were found in cells isolated from ventricles subjected to 1 wk and greater lengths of PO. Collagen production and deposition were not assessed in these studies (28).

SPARC, a collagen-binding protein that is increased in PO hypertrophy, is one protein postulated to increase the efficiency of collagen deposition to insoluble ECM (3, 4, 26). SPARC expression is frequently associated with fibrotic deposition of collagen (5). The hearts of SPARC-null mice were shown to have decreased amounts of insoluble collagen and exhibited decreased insoluble interstitial collagen accumulation in response to PO hypertrophy (3). Results from the present study, in which increased levels of SPARC were found to be expressed by 4-wk PAB fibroblasts and in 4-wk PAB ventricles, were consistent with a key function of SPARC in fibrillar collagen deposition in hypertrophic hearts. Increases in total collagen, as measured by hydroxyproline analysis in 2-wk PAB RV, were not reflected in increased CVF at 2 wk. Thus we hypothesize that the lack of significant increases in SPARC expression at 2 wk following banding resulted in no change in insoluble collagen incorporation, despite elevated levels of total collagen.

Whereas factors that regulate synthesis of collagen I mRNA in response to PO have been the subject of a number of studies, the mechanisms that control postsynthetic processing of procollagen to collagen in PO hypertrophic hearts are not well characterized (2, 6, 11, 12, 14, 31, 32). The results presented here demonstrate that collagen deposition by fibroblasts isolated from 4-wk PAB RV was increased compared with those from nonhypertrophic LV. Our interpretation of these results is that increased efficiency of collagen deposition into insoluble ECM is enhanced in hypertrophic vs. nonhypertrophic fibroblasts and parallels the increased collagen accumulation in vivo. Frequently, amounts of collagen in the conditioned media have been used to quantify collagen production by fibroblasts in vitro. Because the primary determinant of tissue properties mediated by collagen in vivo is governed by collagen incorporated into the insoluble ECM, our results would argue that an equally important, and often overlooked measurement, is the amount of collagen deposited by fibroblasts into insoluble cell layers.

One limitation of this study is that the focus was placed on fibroblast function in terms of postsynthetic procollagen processing, which represents one mechanism by which fibrillar collagen and diastolic dysfunction might be regulated. The present study did not examine changes in collagen synthesis, posttranslational modification, or degradation. Nonetheless, these data support postsynthetic processing as being at least one contributing mechanism to increased collagen content in PO hearts.

These studies support the hypothesis that an important regulatory component of collagen accumulation in the cardiac interstitium occurs at the level of processing of procollagen I. Thus ECM proteins that regulate procollagen processing might prove to be valid targets for the development of pharmaceutical agents to diminish cardiac collagen accumulation.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: C.F.B., G.C., M.R.Z., and A.D.B. conception and design of research; C.F.B., J.L., Y.Z., H.K., and A.D.B. performed experi-

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