A coarctation hypertensive rat model was used to examine the effects of elevated blood pressure on basement membrane component synthesis by cardiac myocytes and aorta using immunohistochemistry and Northern blot analysis. Carotid arterial pressure increased immediately on coarctation, and left ventricular hypertrophy was maximal within 5 days. In immunohistochemical studies, fibronectin and laminin were increased and the basement membrane chondroitin sulfate proteoglycan decreased in both the subendothelial space and smooth muscle cell basement membranes of the aorta above the clip compared with controls, whereas only fibronectin was elevated in the aorta below the clip. No change in basement membrane staining intensity for the cardiac myocytes was observed. Alterations in steady-state mRNA levels for fibronectin and laminin in the aorta paralleled those observed by immunohistochemical analysis with regard to protein and tissue type affected as well as intensity of the changes. However, changes in mRNA levels (but not protein deposition) for perlecan and type IV collagen were also observed in aortas from hypertensive rats compared with controls. Increases in steady-state mRNA levels for all basement membrane components in the heart and vasculature peaked before maximal cardiac hypertrophy (5 days). These studies indicate that alterations in basement membrane component deposition in the hypertrophied vasculature occur at both transcriptional and translational levels and suggest that the cell attachment glycoproteins fibronectin and laminin may be important factors in the vascular response to elevated transmural pressure. (Hypertension. 1993;22:743-753.)

KEY WORDS • extracellular matrix • fibronectins • laminin • collagen • proteoglycans • hypertension, coarctation

Basement membranes (BMs) are complex, highly organized meshworks of proteins, glycoproteins, and proteoglycans in close association with the cellular components of tissues. The proteins that comprise the BM, including type IV collagen, fibronectin, laminin, entactin, perlecan (the BM heparan sulfate proteoglycan), and the BM chondroitin sulfate proteoglycan (BM-CSPG), perform diverse functions. The principal component of all BMS is the nonfiber-forming collagen, type IV collagen, molecules of which are thought to associate to form a meshwork to which many other components, such as fibronectin, laminin, and perlecan, can attach to produce the mature BM structure. In addition, many BM components, particularly fibronectin, laminin, type IV collagen, and entactin, play an essential structural role by anchoring cells to the interstitial matrix. Because of its high net negative charge density, the BM heparan sulfate proteoglycan perlecan allows the BM to act as a selectively permeable barrier. Perlecan has also been demonstrated to bind and sequester growth factors. The cell attachment glycoproteins fibronectin and laminin are believed to modulate such important functions as cell proliferation, migration, and differentiation. Alterations in the amounts, distribution, and associations of components that comprise the BMS have been demonstrated to dramatically affect cell, tissue, and organ function.

The regulation of extracellular matrix (ECM) synthesis by the heart and blood vessels is of particular interest, because the heart and blood vessels undergo cyclic alterations in transmural pressure, stretch, and shear force. One of the most important functions of the ECM produced by cardiac and vascular tissue is to moderate the intensity of these physical forces in both normal and pathological conditions.

Recently, investigators have begun to examine alterations in BM component synthesis in vascular endothelial and smooth muscle cells and cardiac tissue in response to hypertension. However, a comprehensive examination of the effects of hypertension on the alterations in the synthesis/deposition of many BM components by vascular and cardiac tissues has not been performed. In the present study, we used a rat coarctation model to examine the effects of systemic hypertension on the synthesis of several BM components in the heart and aorta with Northern blot analysis and immunohistochemical techniques.

Methods

Antibody Production and Characterization

Purified laminin and type IV collagen (provided by Drs Dale R. Abrahamson, University of Alabama,
Coarctation Model

Hypertension was produced in male Sprague-Dawley rats (approximately 190 g body weight) using the method of Liu et al. 30 Briefly, rats were anesthetized with ether, and a section of the abdominal aorta immediately caudal to the diaphragm was cleared of connective tissue. A silver clip (coarctation hypertensive, 0.81 mm internal diameter; sham, 1.70 mm internal diameter) was placed around the aorta above the bifurcation of the right renal artery. The incision was closed, and pericardium and sternum were secured with sutures. The aorta from the arch or 0.5 cm posterior to the coarctation site was ligated using a 7-0 silk ligature. The abdominal aorta was then ligated with a 6-0 silk ligature. The area was then closed using 4-0 silk sutures. The rats were allowed to recover and were maintained on Purina 5001 pellets and tap water ad libitum.

Preliminary studies were performed to examine temporal changes in carotid and femoral arterial pressures and right and left ventricular plus septal masses from 0 to 30 days after coarctation. Two animals were used for each time point. For immunohistochemical analysis of type IV collagen, fibronectin, laminin, perlecan, and the BM-CSPG, a second set of four, 14-day coarctation hypertensive rats and four shams was used. A third set of animals (approximately 190 g body weight) was used for the immunohistochemical analysis of alterations in steady-state levels of BM component mRNA (Northern analysis) including fibroblastic growth factor (FGF), and a monoclonal antibody (2D6) directed against the BM-CSPG were characterized previously.

Frozen vascular tissues pooled from all six animals (coarctation hypertensive and sham rats) were taken 1, 10, and 20 days after surgery. Isolation of total RNA and Northern analysis were performed according to the methods of Claycomb and Lanson. 32

Immunohistochemistry

For immunohistochemistry, 14-day coarctation hypertensive and sham rats were anesthetized with pentobarbital (Nembutal, 50 mg/kg). To clear the vasculature of blood, an 18-gauge needle connected to a perfusion pump was inserted into the left ventricle (LV), and filtered (0.2 μm) phosphate-buffered saline (pH 7.4, room temperature) was perfused through the vasculature at pressures corresponding to the carotid arterial pressures of the animals. Tissues were then perfused with a filtered fixative solution (acid alcohol: 96:3:1, ethanol/glacial acetic acid/water, approximately 3 minutes). Tissues were removed and placed in acid alcohol for at least another 24 hours before embedding. Tissues were processed and embedded in paraffin using standard techniques. Immunohistochemical analysis was performed on hyaluronidase-treated sections (50,000 U/mL) according to the methods of McCarthy et al. 29 The aorta anterior to (AOR †) and posterior to (AOR ‡) the coarctation site and the right ventricle (RV) and LV were used for immunohistochemistry studies. Sections of aorta were taken at constant distances either 1.0 cm posterior to the top of the aortic arch or 0.5 cm posterior to the coarctation site.

Antisera to fibronectin, laminin, and type IV collagen were diluted 1:50; antisera to perlecan were used at a dilution of 1:30; and for the monoclonal antibody against the BM-CSPG, undiluted ascites fluid was used. Secondary antibodies [rhodamine-labeled goat anti-rabbit IgG F(ab)2, Jackson Immunoresearch Laboratories Inc, West Grove, Pa] or fluorescein isothiocyanate–labeled goat anti-mouse IgG (Cappel)] were applied using a dilution of 1:100 (antibody/phosphate-buffered saline) to visualize the primary antibodies. Control staining with preimmune sera or secondary antibodies only was routinely performed to verify the specificity of the primary antibodies.

Sections were examined with a Nikon Optiphot microscope equipped with appropriate epifluorescence filters. Photomicrographs of control and experimental tissues were taken using the same exposure times for each pair of slides (four pairs in all for each tissue) so that direct comparisons of staining intensities could be made. Care was taken to maintain the same exposure and development times during all subsequent photographic work. For the immunohistochemical studies, only those changes observed in at least three of four comparisons between the coarctation hypertensive and sham rats are discussed below, except where noted.

Northern Analysis

For Northern blot analysis, tissues (AOR †, AOR ‡, RV, and LV) were removed from freshly killed (ether overdose) sham and coarctation hypertensive rats. After loose connective tissue was removed, tissues were snap-frozen by immersion in liquid nitrogen and maintained at −80°C until use. AOR † and AOR ‡ of six control and coarctation hypertensive rats were taken 3, 10, and 20 days after placement of the clip; RV and LV tissues were taken 1, 10, and 20 days after surgery. Isolation of total RNA and Northern analysis were performed according to the methods of Claycomb and Lanson. 32

Frozen vascular tissues pooled from all six animals
within a group, three RVs, or portions (approximately 200 mg) from three LVs were used for Northern analyses. Because RNA was extracted from control and coarctation hypertensive rats over a span of 20 days, during which the rats were growing, comparisons of mRNA isolated from control or hypertensive tissues at different times are not valid. Thus, in interpreting the data, only comparisons of steady-state levels of mRNA between control and coarctation hypertensive rats within a given day were used.

The cDNA probe for rat fibronectin is from a region encoding the 10th and 11th type I repeat (a gift from Dr R. Hynes) and recognizes the 7.9-kb band corresponding to fibronectin on Northern blot analysis. The cDNA probes for laminin-B2, perlecan, and a1-type IV collagen were produced from a library of Englebreth, Holm, Swarm tumor RNA and were received from Sasaki and Yamada,33 Noonan et al,34 and Nath et al,35 respectively. These probes specifically hybridize to 8.0 kb (laminin-B2), 6.7 kb (type IV collagen), and 12 kb (perlecan) bands on Northern analysis. cDNA probes for the BM-CSPG are currently unavailable.

Statistics
All statistical analyses were performed using Student's t test for paired or unpaired data. The level of statistical significance was at a value of P ≤ 0.05.

Results

Antibody Characterization

Antibodies prepared against laminin and type IV collagen did not cross-react with other purified BM components as determined by ELISA (data not shown). Western blot analysis and ELISA demonstrated that antibodies to laminin recognized subunits corresponding to the A and B chains of laminin, whereas antisera directed against type IV collagen recognized purified type IV collagen. Both laminin and type IV collagen antisera specifically recognized profiles of BMs in cryostat sections from several rat tissues.

Time Course of Coarctation Hypertension

Coarctation hypertension had no apparent effect on weight gain compared with the sham hypertensive group (Fig 1A). Carotid arterial pressure increased immediately after placement of the constricting clip (Fig 1B), and although the pressure differential between the carotid artery and AOR↓ in coarcted animals varied considerably throughout the course of the study, it was always higher than in sham coarcted animals. RV weights (normalized to body weight) in the hypertensive and sham groups did not appear to be different (Fig 1C); however, normalized LV mass was twice control values in coarcted rats by 5 days after placement of the
Immunohistochemistry, Myocardium

LV myocardial capillaries stained positively for laminin and type IV collagen (Fig 4a through 4d) as well as for fibronectin and the BM-CSPG (not shown), and there appeared to be no difference in the staining intensities between sham and hypertensive tissues. Myocytic BMs from the LV also exhibited prominent staining for laminin and type IV collagen (Fig 4a through 4d), but these structures stained only minimally for fibronectin and the BM-CSPG. There did not appear to be differences in staining intensities between control and hypertensive tissues despite overt hypertrophy of the LV myocytes in the latter group. Similar staining patterns were observed for fibronectin, laminin, type IV collagen, and BM-CSPG in the nonhypertrophied RV, where again there was no difference between hypertensive and sham coarcted tissues (not shown). All cardiac myocytes were negative for the BM-associated heparan sulfate proteoglycan, perlecan, as reported previously.28

Table 2 summarizes the immunohistochemical alterations in BM components in vascular and cardiac tissues of coarcted compared with sham rats.

**Northern Analyses**

Fig 5 shows the time course for alterations in steady-state mRNA levels for fibronectin, laminin-B1, perlecan, and α1-type IV collagen in aortic tissues from coarctation hypertensive and control rats by Northern analysis. For this study, comparisons were made only between sham and hypertensive rats within a given day and not over time. In the AOR ↑ of hypertensive rats, fibronectin and the B2 chain of laminin were elevated by day 3 and remained elevated through day 10 of coarctation compared with the AOR ↑ from day 10 shams. Temporal changes in steady-state levels of the core protein of perlecan and α1-type IV collagen were similar to fibronectin and laminin, although the magnitude of the differences between sham and hypertensive tissues was smaller. At day 20 after coarctation, steady-state mRNA levels for all BM components examined were higher in sham aortas than in aortas from coarcted rats (Fig 5). In general, steady-state levels of BM component mRNAs in AOR ↓ were increased in coarctation hypertensive rats compared with shams. The magnitude of the differences between sham and hypertensive tissues was smaller in the AOR ↓ from hypertensive rats compared with time-matched shams than seen in the AOR ↑, however. In addition, maximal increases in steady-state levels of mRNA for the BM components in the AOR ↓ occurred at a later time (10 days) after coarctation compared with those in the AOR ↑. Fibronectin-specific mRNA was virtually undetectable in LVs of both hypertensive and control rats throughout the study (Fig 6). In contrast, steady-state mRNA levels for both laminin and type IV collagen were detectable in mRNA from LVs of sham and coarctation hypertensive rats. Steady-state levels of mRNA for laminin and type IV collagen were significantly reduced in LVs taken from coarctation hypertensive rats compared with shams at 10 days of hyperten-
Membranes, Immunohistochemistry

from SHR compared with normotensive Wistar-Kyoto and those of other investigators suggests that there are fundamental differences in the factors influencing the production of some ECM components between genetic models of hypertension, such as the SHR, and models in which hypertension is induced by surgical (coarctation of the aorta) intervention.

Immunohistochemical alterations observed in BM components were generally confined to the pressure-overloaded portion of the aorta, indicating that physical forces, such as pressure, stretch, or both, are primary stimuli for changes in the production and distribution of laminin, BM-CSPG, and fibronectin in coarctation hypertension. Fibronectin was also observed in the AOR. In vitro, physical forces, such as pressure, stretch, and shear force, have been demonstrated to stimulate ECM synthesis in vascular endothelial and smooth muscle cells; however, the mechanisms through which these physical forces operate have yet to be determined.

Increases in fibronectin were observed in the AOR of two of four coarctation hypertensive animals, suggesting that the production of fibronectin may be under the influence of additional factors expressed during hypertension. This is consistent with our previous demonstration of elevated proteoglycan production in the normotensive, nonhypertrophied RV and kidneys of coarctation hypertensive rats. We have observed increases in the expression of steady-state levels of mRNA for the potent ECM synthesis stimulating factor, transforming growth factor-β1, in the aorta both above and below the coarctation site of 3-day coarctation hypertensive rats compared with shams (unpublished observations). Thus, the observed increases in BM component synthesis in the aorta below the coarctation site may be mediated by alterations in the synthesis of this growth factor.

Whereas the distributions of type IV collagen, laminin, perlecan, and the BM-CSPG are limited to BMs, fibronectin is also found in both plasma and the interstitium. Thus, by immunofluorescence microscopy, the exact structural location of the fibronectin within the heart and aorta cannot be determined. However, the fact that laminin, a BM cell attachment glycoprotein with functions similar to fibronectin, is increased in the subendothelial space and media of aortas from coarctation hypertensive rats suggests that at least some of the increased fibronectin deposited in the aorta is localized to the BMs. It is also possible that the source of the fibronectin may actually be the plasma; however, the increase in the steady-state level of mRNA for fibronectin in the coarctation hypertensive aorta indicates that the synthetic capacity of this vessel for fibronectin is increased by coarctation as well.

Changes in Vascular Basement Membranes, Northern Analysis

The largest increases in steady-state levels of BM component-specific mRNA in coarctation hypertensive rats compared with time-matched shams were observed for fibronectin and laminin in the AOR. These increases occurred before maximal cardiac hypertrophy, suggesting that alterations in BM components are a constituent of aortic hypertrophy and the adaptation of conducting vessels to increased pressure. Increases in the steady-state levels of fibronectin mRNA in the AOR are also consistent with the immunohistochem-

Discussion

To our knowledge these data represent the most comprehensive examination of alterations in the expression and deposition of BM components produced by cells of aortic and ventricular tissues in response to pressure-overload–induced hypertension. The molecules examined typify the proteins, glycoproteins, and proteoglycans present in the BM and represent proteins with distinct and important functions within the heart and blood vessels. Examinations of cardiac and aortic tissues in both pressure-overloaded (LV, AOR) and normotensive (RV, AOR) regions revealed that although directly applied pressure/stretch is an important stimulus for alterations in BM component deposition, it is apparent that other possibly neural/humoral factors induced by hypertension influence BM component synthesis in areas of the cardiovascular system not directly exposed to increased pressure. Although speculative, the marked increases in the cell attachment glycoproteins fibronectin and laminin in the AOR suggest that enhanced attachment of smooth muscle and endothelial cells to the surrounding matrix is an important response to hypertension, and this may have relevant pathophysiological consequences. The decrease in immunolocalizable BM-CSPG suggests a regression of the mature vascular BMs to a more undifferentiated state.

Changes in Vascular Basement Membranes, Immunohistochemistry

The results of the immunohistochemical analyses are in general agreement with previous findings of alterations in ECM component amount, type, or both in the cardiovascular system in several models of hypertension. Although most previous studies have focused on changes in interstitial ECM components, studies that have examined BMs in hypertensive blood vessels and cardiac tissue corroborate the results presented here. Guyton et al observed that increased subendothelial BM width in aortas from coarctation hypertensive rats was primarily due to increased deposition of BM-like material. McGuire et al observed increases in fibronectin and laminin in the subendothelial space of conducting vessels from spontaneously hypertensive rats (SHR) using both immunohistochemistry and Northern blot analysis. However, unlike the present study, several other BM components, including type IV collagen and perlecan, were also found by immunohistochemistry to be increased in large vessels from SHR compared with normotensive Wistar-Kyoto rats. Similarly, we have previously described differences in proteoglycan synthesis between SHR and coarctation hypertensive rats. The dissimilarity between our results and those of other investigators suggests that there are fundamental differences in the factors influencing the production of some ECM components between genetic models of hypertension, such as the SHR, and models in which hypertension is induced by surgical (coarctation of the aorta) intervention.

Increases in fibronectin were observed in the AOR of two of four coarctation hypertensive animals, suggesting that the production of fibronectin may be under the influence of additional factors expressed during hypertension. This is consistent with our previous demonstration of elevated proteoglycan production in the normotensive, nonhypertrophied RV and kidneys of coarctation hypertensive rats. We have observed increases in the expression of steady-state levels of mRNA for the potent ECM synthesis stimulating factor, transforming growth factor-β1, in the aorta both above and below the coarctation site of 3-day coarctation hypertensive rats compared with shams (unpublished observations). Thus, the observed increases in BM component synthesis in the aorta below the coarctation site may be mediated by alterations in the synthesis of this growth factor.

Whereas the distributions of type IV collagen, laminin, perlecan, and the BM-CSPG are limited to BMs, fibronectin is also found in both plasma and the interstitium. Thus, by immunofluorescence microscopy, the exact structural location of the fibronectin within the heart and aorta cannot be determined. However, the fact that laminin, a BM cell attachment glycoprotein with functions similar to fibronectin, is increased in the subendothelial space and media of aortas from coarctation hypertensive rats suggests that at least some of the increased fibronectin deposited in the aorta is localized to the BMs. It is also possible that the source of the fibronectin may actually be the plasma; however, the increase in the steady-state level of mRNA for fibronectin in the coarctation hypertensive aorta indicates that the synthetic capacity of this vessel for fibronectin is increased by coarctation as well.
ical results. Similar increases in steady-state levels of mRNA for fibronectin and deposition of fibronectin have been observed in the aorta in other models of systemic hypertension, including deoxycorticosterone-salt, chronic infusion with angiotensin II, and the SHR.23,24

The Northern analysis and immunohistochemistry are apparently contradictory with regard to observed in-
FIG 2. (Facing page). Photomicrographs show immuno-

histochemical localization of fibronectin (a and b), laminin
(c and d), basement membrane chondroitin sulfate pro-

teoglycan (BM-CSPG, e and f), and perlecan (g and h) in

aortic tissue taken from above the coarctation site of

14-day coarctation hypertensive (a, c, e, and g) and sham

(b, d, f, and h) rats. Vascular lumen (L) is to the left of the

aortic wall in all micrographs. Note intense staining

pattern for BM-CSPG in the subendothelial space (ar-

rows) and basement membrane material surrounding the

smooth muscle cells (arrowheads) in control aortas (f)

and the reduction in staining in the subendothelial and

medial basement membranes of aortas from hypertensive

animals (e). Care was taken during all stages of micro-

graph preparation to keep exposure times constant so that

true comparison of staining intensities could be made

between experimental and control tissues. Thus, in d lami-
nin is present but there is comparatively much less than in

c. Micrographs are representative of comparisons between

four hypertensive and four sham rats. Bar=20 μm.

creases in the mRNA levels for perlecan and type IV

 collage in the AOR ↑, as well as these BM components

and laminin in the AOR ↓. These findings suggest that

posttranscriptional regulatory mechanisms differentially

control the deposition of select BM components in differ-

ent areas of the aorta of the coarctation hypertensive rat.

The finding that the expression of BM component-
specific mRNAs was greater in the aortas from sham
tissues than the coarctation hypertensive tissues at day

20 is paradoxical. It is possible that the sham clip may

produce some structural changes within the aorta as the

animals grow and the clip becomes progressively con-

strictive. It is also possible that the marked remodeling

of the aorta of the coarctation hypertensive rats has

actually reduced the pathological stimuli (such as in-

creased stretch) that originally induced the alterations

in mRNA in this group.

Alterations in Basement Membrane Components May

Mediate Pathophysiological Alterations in

Hypertensive Blood Vessels

Structural alterations in resistance vessels are thought
to be the primary mechanisms through which systemic

hypertension is mediated. Although this study focused

on changes occurring in the aorta, a conducting vessel, it

is anticipated that, because resistance vessels have a
greater capacity for ECM synthesis owing to their
greater relative amount of smooth muscle cell mass to
total weight, the response of resistance vessels would

also be similar to (or greater than) that observed in the

aorta in the present study.

Fibronectin and laminin are important cell attach-

ment molecules. Increased peripheral vascular resis-
tance observed in hypertension may be partly due to

increased adhesion of cells to the underlying ECM via

these cell attachment proteins, which would elevate the

series component of vascular resistance.52 Enhanced

adhesion of vascular smooth muscle to the interstitial

matrix may also contribute to the observed increases in

vascular reactivity of blood vessels from hypertensive

animals through an increase in the attachment of

contractile microfilaments within the cell to the sur-

rounding ECM via specific cell surface receptors.

The BM-CSPG, a component of BMs from many

tissues, was decreased in the hypertensive AOR ↑

compared with shams. Little is known about the func-
tion of this newly described proteoglycan, but studies

performed by Couchman et al indicate that this mol-
ecule is present in structurally mature and stable BMs

and absent from those of undifferentiated and imma-
ture cells. The marked reduction of this component in

endothelial and smooth muscle cell BMs of hypertensive

aortas, therefore, suggests that aortic BMs undergo a

fundamental structural reorganization in response to

the hypertensive state, a finding consistent with the

observed changes in fibronectin and laminin.

Changes in Myocardial Basement Membranes,

Immunohistochemistry and Northern Analysis

Other investigators have reported increases in fibro-
nectin mRNA and increased deposition of fibronectin in

the myocardium in response to cardiac injury55-56 as well

as cardiac hypertrophy associated with systemic hyper-
tension including the coarctation model.25-26 In contrast,
in the present study we observed no immunohistoche-

mical alterations in myocardial fibronectin or any other

BM component examined in either the RV or LV of

coarctation hypertensive rats, despite significant LV

FIG 3. Photomicrographs show fibronectin staining in aortic tissue taken from below the coarctation site of coarctation

hypertensive (a) and sham (b) rats. Micrographs were processed as in Fig 2 to ensure that direct comparisons could be

made. Increased staining intensity for fibronectin in experimental tissue was observed in two of four comparisons

between control and hypertensive rats. L, vascular lumen. Bar=20 μm.
hypertrophy. Alterations in mRNA specific for BM components in the hypertrophied LV were also minimal until 20 days after coarctation.

The finding that steady-state levels of fibronectin-specific mRNA were not changed in the hypertrophied LV in the present study supports the finding that, in our model of coarctation hypertension, minimal changes in fibronectin synthesis were observed in this tissue, despite overt hypertrophy. Differences between the present study and those of Contard et al., Mayuma and Brecher,26 and Villareal and Dillman37 may reflect differences in cardiac responses to different forms of hypertension, varying degrees of severity of the coarctation, different placement of the coarctation on the vascular tree, differences in the times at which cardiac tissue was examined, or differences in the use of tissues

<table>
<thead>
<tr>
<th>Table 2. Summary of Changes in Basement Membrane Components From 14-Day Coarctation Hypertensive Rats as Determined by Immunohistochemistry</th>
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<tbody>
<tr>
<td>Membrane Component</td>
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BM-CSPG indicates basement membrane chondroitin sulfate proteoglycan; LV, left ventricle; RV, right ventricle; AOR ↑, aorta above coarctation site; AOR ↓, aorta below coarctation site; 0, no changes; +, staining intensity increased compared with sham; -, staining intensity decreased compared with sham; and +/-0, change not observed in all comparisons between coarctation and sham tissues.
from individual ventricles (right and left) versus both ventricles in the analyses.

Our results suggest that pressure-overloaded cardiac and aortic tissues may respond and adapt differently to pressure-overload hypertension with regard to the synthesis of BM components. It is also possible that increases in BM component deposition in the hearts of coarctation hypertensive rats do not occur until a time point later than 2 weeks, as suggested by our Northern analysis data.

Northern analyses of changes in mRNA in ventricular tissues are somewhat limited because the ventricles are composed of several distinct tissue types, including endocardium, epicardium, nervous tissue, and the coronary vasculature, all of which contain BMs. Alterations in BM component mRNA observed in the RV and LV in the present study cannot preclude a contribution from these other cell types, particularly when the coronary vasculature is experiencing increased pressure. However, because the overwhelming majority of the mass of ventricular tissue is myocardium, it is expected that the majority of the mRNA would be derived from the myocytes.

This study demonstrates that hypertension produced by coarctation of the abdominal aorta leads to marked increases in the synthetic capacity and deposition of specific BM components; conducting vessels are particularly affected. These increases are observed in normotensive and hypertensive portions of the aorta, suggesting that both physical and chemical mediators are involved. A more thorough investigation of other vascular beds, particularly resistance vessels in pressure-
overloaded and normotensive portions of the vascular ture, is warranted. In particular, an examination of BM component alterations in the coronary circulations of the RV and LV would be interesting, because both groups of vessels encounter similar pressures, whereas only the LV is hypertrophied.

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