Age-Related Changes in Fibronectin Expression in Spontaneously Hypertensive, Wistar-Kyoto, and Wistar Rat Hearts

Wilfred Mamuya, Aram Chobanian, and Peter Brecher

The effects of age and blood pressure on fibronectin expression in the rat heart were studied in the normotensive Wistar and Wistar-Kyoto (WKY) strains and in the spontaneously hypertensive rat (SHR). Fibronectin mRNA expression decreased threefold between 10 and 40 weeks of age in Wistar hearts, with changes of similar magnitude occurring between 6 and 24 weeks in WKY rats. In contrast, no decrease in fibronectin mRNA was observed in SHR hearts during this time span. These results are in contrast to changes observed previously in the aorta, where an increase in fibronectin mRNA occurred with age in all three rat strains. Ribonuclease protection analysis showed a small age-specific increase in the relative content of EIIIA + fibronectin mRNA isoforms in hearts from Wistar rats, whereas no change was found in the relative amount of either EIIIA or EIIIB isoforms in SHR hearts. Changes similar to those observed for fibronectin mRNA, although of different magnitudes, were observed in mRNA levels for collagen α1(III) and β1 integrin. In Wistar hearts, collagen α1(III) mRNA levels decreased fivefold to sixfold between 10 and 40 weeks of age, whereas a twofold to threefold decrease in β1 integrin was observed in WKY hearts between 6 and 24 weeks of age. Western blot analysis revealed a positive correlation between fibronectin mRNA and protein for age-dependent changes in ventricular tissue but not in the atria, suggesting that the regulation of fibronectin expression during the changes common to both aging and hypertension could involve both transcriptional and posttranscriptional mechanisms. (Circulation Research 1992;71:1341–1350)

**KEY WORDS** • myocardium • age-related changes • fibronectin • Wistar rats • spontaneously hypertensive rats

Fibronectin is a dimeric glycoprotein found in the extracellular matrix of most tissues and has been shown to influence diverse processes, including cell growth, adhesion, migration, and wound repair. The single gene is alternatively spliced, leading to isoforms whose biological functions are yet to be elucidated. Fibronectin binds to other components of the extracellular matrix and also binds to the integrin family, a class of cell-membrane receptors. The interactions between fibronectin and associated cells can alter the phenotype of the adhering cells because of mediated changes in gene expression and are presumed to influence the mechanical properties of the heart.

Most of the studies involving age-related changes occurring in the myocardium have used the rat model. Age-related losses in cardiac myocyte number have been documented in the ventricles of rats and humans. An 18–20% loss in myocytes has been observed in both Fischer and Sprague-Dawley rat strains between 12 and 40 weeks of age, suggesting that an age-related decrease in the number of myocytes due to cell death is a common occurrence in the aging rat heart. The continued loss of myocytes in the ventricles is thought to generate a greater work load on the remaining myocytes, which may serve as a mechanical stimulus for both myocyte hypertrophy and hyperplasia of nuclei and myocytes.

Little information is currently known about the in vivo or in vitro changes in the relative expression of cardiac fibronectin with age. Even less is known about age-related changes in the relative amounts of alternate spliced fibronectin mRNA isoforms. In a recent study, we have shown that during experimentally induced cardiac hypertrophy in the rat using either triiodothyronine administration or mineralocorticoid/salt–induced hypertension, the amount of steady-state fibronectin mRNA increased differentially among the various chambers of the heart. These changes were accompanied by selective changes in the expression of alternatively spliced forms of fibronectin mRNA and a temporally dissociated increase in immunodetectable fibronectin in the myocardium. In the present study, we examined fibronectin expression in both normotensive and genetically hypertensive rat strains of different ages to determine if changes in fibronectin expression correlate with known myocardial structural changes that may be common to both aging and hypertension.

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Materials and Methods

Materials

Gene Screen membrane, [α-32P]dCTP, [α-32P]CTP, and [γ-32P]dCTP were obtained from DuPont/New England Nuclear Corp., Boston. A multiprime labeling kit was purchased from Amersham Corp., Arlington Heights, Ill. RNase A, RNase T1, protein K, and in vitro transcription kits were purchased from Ambion, Inc., Austin, Tex. Calf intestinal alkaline phosphatase was purchased from Clonetech, Palo Alto, Calif., and T4 polynucleotide kinase was obtained from IBI, New Haven, Conn. Bicinechnic acid reagent was purchased from Pierce Chemical Co., Rockford, Ill. Nitrocellulose paper and protein standards for sodium dodecyl sulfate–polyacrylamide gel electrophoresis were purchased from Bio-Rad Laboratories, Richmond, Calif. Polyclonal anti-rabbit plasma fibronectin and anti-sheep horse-radish peroxidase–linked second antibody were obtained from The Binding Site, Inc., San Diego, Calif. An enhanced chemiluminescence detection kit was obtained from Amersham, United Kingdom.

Animals

Male Wistar rats, Wistar-Kyoto (WKY) rats, and spontaneously hypertensive rats (SHRs) were purchased from Charles River Breeding Laboratories, Wilmington, Mass., and from Taconic Farms, Inc., Germantown, N.Y. The animals were killed after a 1–2 week adjustment period to their animal quarters. Systolic blood pressure in all the experiments was measured by tail-cuff plethysmography and a photoelectric cell detector as described previously.19 Animals were killed with an overdose of sodium pentobarbital. Hearts were rapidly excised, the pericardium was removed, and atria and ventricles were separated at the atrioventricular sulcus, while ensuring that the tissue was always kept moist with cold phosphate-buffered saline. The ventricular tissue used in this study was dissected from the free wall of the left ventricle. All the tissues were rinsed free of blood before extraction for either RNA or protein analysis.

RNA Isolation and Northern Blot Analysis

Total RNA from rat tissues was extracted using the guanidinium thiocyanate/cesium chloride centrifugation method,19 with minor modifications.17 RNA concentration was determined by ultraviolet spectrophotometry, and 5–20 μg total RNA was separated using 0.9–1.2% agarose/1.4 M formaldehyde gel electrophoresis. Equivalent amounts of RNA in each lane were confirmed by visual examination of ribosomal RNA after ethidium bromide staining. Northern blot analysis was performed as previously described.17 The membranes were exposed to preflashed x-ray films (Kodak X-Omat) by use of intensifying screens (Cronex lightning plus) for 12–36 hours at −70°C. Laser densitometry (300A computing densitometer, Molecular Dynamics, Sunnyvale, Calif.) was used to quantitate the relative signal intensity of the bands obtained.

Western Blot Analysis

Tissue samples were homogenized in the cold by use of a Polytron apparatus in 1:15 (wt/vol) phosphate-buffered saline containing aprotenin (50 trypsin inhibitory units/ml), leupeptin (10 mM), pepstatin A (1 mM), and phenylmethylsulfonyl fluoride (1 mM) at a 10:1 ratio of buffer volume to tissue wet weight. The homogenate was centrifuged at 25,000g for 20 minutes at 4°C, and the supernatant was removed, designated as the S1 fraction, and saved. The pellet was resuspended in a 1:15 (wt/vol) volume of 4% sodium dodecyl sulfate (SDS) and 2 M urea, and the resuspension was heated at 100°C for 4 minutes and then centrifuged at 12,000g for 5 minutes at room temperature. The supernatant, designated as the SDS extract (S2), was removed and saved. A further extraction of the remaining pellet was carried out by using 4% SDS, 2 M urea, and 10 mM dithiothreitol, and this fraction was called the S3 fraction. All protein samples were stored at −70°C to minimize degradation. Western blot analysis using chemiluminescence detection was performed as described previously.17

Ribonuclease Protection Assay

The procedure followed was identical to that described previously by us.17 After electrophoresis, the gel was fixed, dried, and exposed to x-ray film. The autoradiogram was scanned by laser densitometry to quantitate the amount of protected labeled cRNA. The relative amounts of fibronectin containing a particular isoform were calculated as follows: Protected mRNA fragments that contained EIIIA (280 nt) and EIIIB (350 nt) exons were larger than protected fragments that lacked EIIIA (109 nt) and EIIIB (100 nt). The calculated amounts of protected fragments lacking the alternatively spliced exons were corrected to take into account the size difference, with the assumption that an equal rate and extent of label incorporation occurred for all protected fragments. The amount of total detectable fibronectin mRNA was the sum of the amounts of both protected fragments, and the relative amounts were computed by dividing the amount of the exo-positive moiety by the total amount of fibronectin mRNA.

cDNA Probes

Rat fibronectin (p-rlf-1), prFN-A, prFN-B, and prFN-V were obtained from R.O. Hynes,20,21 Human β1 integrin and glyceraldehyde 3-phosphate dehydrogenase (GAPD) were purchased from the American Type Culture Collection, Rockville, Md. A 330-bp HincII/EcoRV fragment (pDT1505) for mouse type III collagen was a gift from Benoit de Crombrugghe, M.D. Anderson Cancer Center, Houston, Tex.

Statistical Analysis

The grouped results are expressed as mean±SEM. A one-way analysis of variance (ANOVA) showed significant differences among groups. The groups were then compared using a two-tailed unpaired t test. Statistical significance was set at p<0.05.

Results

Wistar rats, WKY rats, and SHRs were killed between 6 and 40 weeks of age. Table 1 summarizes the differences in heart weight and blood pressure for the 50 rats used in this study. Heart weights for all strains of rats increased between 6 and 40 weeks of age, as did the body weight (data not shown). The heart weight/body
TABLE 1. Heart Weight, Blood Pressure, and Heart Weight/Body Weight Ratio in Spontaneously Hypertensive and Normotensive Rats as a Function of Age

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Blood pressure (mm Hg)</th>
<th>Heart weight (g)</th>
<th>Heart weight/body weight (×10³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY 6 weeks</td>
<td></td>
<td>6 . . .</td>
<td>0.50±0.00</td>
<td>4.29±0.08</td>
</tr>
<tr>
<td>SHR 6 weeks</td>
<td></td>
<td>6 . . .</td>
<td>0.47±0.01</td>
<td>4.48±0.08</td>
</tr>
<tr>
<td>WKY 10 weeks</td>
<td>10</td>
<td>108±4</td>
<td>0.86±0.03</td>
<td>2.99±0.06</td>
</tr>
<tr>
<td>SHR 10 weeks</td>
<td>6</td>
<td>165±5</td>
<td>1.02±0.00</td>
<td>4.03±0.11*</td>
</tr>
<tr>
<td>WKY 24 weeks</td>
<td>5</td>
<td>113±6</td>
<td>1.07±0.02</td>
<td>3.62±0.08</td>
</tr>
<tr>
<td>SHR 24 weeks</td>
<td>5</td>
<td>177±9</td>
<td>1.51±0.05</td>
<td>3.86±0.06†</td>
</tr>
<tr>
<td>WKY 40 weeks</td>
<td>2</td>
<td>136±12</td>
<td>1.42±0.04</td>
<td>3.42±0.13‡</td>
</tr>
<tr>
<td>SHR 40 weeks</td>
<td>5</td>
<td>185±12</td>
<td>1.80±0.07§</td>
<td>4.32±0.12§</td>
</tr>
</tbody>
</table>

n, Number of rats; WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats. Values are mean±SEM. Comparisons at 40 weeks were performed between Wistar and WKY rats and between WKY rats and SHRs.

*<p<0.001, †p<0.05, and ‡p<0.01 vs. age-matched control (WKY or Wistar) rats; §p<0.001 vs. Wistar rats (data from four rats only).

Fibronectin mRNA and protein have recently been shown to be differentially distributed among the chambers of the heart, with the atria having significantly higher levels of mRNA and protein compared with the ventricles.17 Northern blot analysis was used to determine whether localized changes in cardiac fibronectin mRNA occurred with age. Figure 2 shows a representative Northern analysis of total RNA extracted from atria of SHR, Wistar, and WKY rats. GAPD mRNA expression did not show any major age-related change in the three rat groups and could be used to normalize the amounts of total RNA in each lane. No change in the steady-state mRNA levels of fibronectin was observed in the atria of SHRs between 6 and 40 weeks of age (Figure 2), whereas a threecold decrease in the fibronectin mRNA occurred in the atria of Wistar rats between 10 and 40 weeks of age (Figure 2, top panel). Collagen α1(III) mRNA levels increased threefold in the atria of SHRs, whereas a fivefold decrease was observed in the atria of Wistar rats between 10 and 40 weeks of age. The bottom panel of Figure 2 extended the analysis to atria of WKY rats, in which a threefold decrease in fibronectin mRNA occurred between 6 and 24 weeks of age, similar to that found in the Wistar strain. Analysis of atrial mRNA levels for β1 integrin, a component of several heterodimeric integrins known to interact with fibronectin, showed a similar trend in changes in expression, with integrin levels remaining unchanged in the SHR with age but showing a twofold to threefold decrease in the WKY rat.

Further studies were carried out to determine whether similar changes in the amounts of fibronectin mRNA also occurred in an age-related manner in the ventricles of SHR and Wistar rats. Differences in the effects of age on the left and right ventricular tissue have been documented,22,23 and in this study only tissue from the free left ventricular wall was analyzed. In agreement with data obtained from whole hearts and from atria, no decrease in steady-state fibronectin mRNA was observed in the ventricles of SHRs between 10 and 40 weeks of age. If the amount of fibronectin mRNA was expressed as a ratio to GAPD mRNA, an increase was noted that was mainly due to the small but consistent decrease in GAPD expression in the older group of rats (Figure 3). In age-matched Wistar rats, the amount of ventricular fibronectin mRNA decreased threefold between 10 and 40 weeks of age. The expression of β1 integrin in ventricles from SHR and Wistar rats showed no major age-related changes. Collagen α1(III) mRNA levels showed only a slight decrease with age in ventricles from SHRs, whereas a sixfold decrease was observed in ventricles from Wistar rats. The changes in collagen α1(III) mRNA in ventricles from Wistar rats were similar in magnitude to those observed in whole hearts, a finding not surprising since the ventricles constitute the bulk of the heart.

Ribonuclease protection assays were performed on hearts from Wistar rats to determine whether changes in the relative expression of fibronectin mRNA isoforms also occurred with age. The cRNA probes that were used spanned both the alternatively spliced exon and the adjacent exon, thus making possible an estimation of the relative amount of specific mRNA isoform expressed. We had previously shown that cardiac tissue from Wistar rats contains relatively high levels of fibro-
nectin EIIIA+ mRNA isoforms (44%) and relatively low levels of detectable EIIIB+ mRNA isoforms (5%). Although the total amount of fibronectin mRNA was observed to decrease between 10 and 40 weeks of age in hearts from Wistar rats (Figure 4, top panel), densitometric analysis revealed a small but significant increase in the relative amount of EIIIA-containing fibronectin mRNA isoforms from 40% to 54%. No significant change in the relative content of EIIIB-containing mRNA isoforms occurred between 10 and 40 weeks (Figure 4, top panel), suggesting that the increase in EIIIA+ isoforms was relatively specific. When ventricular tissue from both SHR and Wistar rats was analyzed by ribonuclease protection (Figure 4, bottom panel), there was no significant change in the relative content of fibronectin EIIIA mRNA isoforms between 10 and 40 weeks of age in the SHR.

Changes in steady-state mRNA amounts do not necessarily correlate with measurable changes in the amounts of protein expressed. Conflicting results have been described in the basal and maximum rates of protein synthesis in hearts of senescent rats. Western blot analysis was used to determine whether the age-related changes in cardiac fibronectin mRNA in SHR and Wistar rats paralleled amounts of immunodetectable protein. By use of procedures previously described, protein was extracted from atria and ventricles of SHR and Wistar rats and subjected to Western analysis by the enhanced chemiluminescence procedure. Atria from Wistar rats showed no change in the amounts of immunodetectable fibronectin between 10 and 40 weeks of age (Figure 5), in marked contrast to the decrease in steady-state mRNA levels shown above. However, extracts from ventricles from the same rats showed a sixfold decrease in the amount of detectable protein in the older rats. In the SHR, there was an increase in immunoreactive protein in both the atria

**FIGURE 1.** Northern blot analysis of cardiac RNA from Wistar rats, spontaneously hypertensive rats (SHRs), and Wistar-Kyoto (WKY) rats. GAPD, glyceraldehyde 3-phosphate dehydrogenase. Top panel: Each lane contains 20 μg total RNA obtained from Wistar rats at the designated age. The same nylon membrane was rehybridized with the different probes used. Bottom panel: Each lane contains 20 μg total RNA obtained from SHR and WKY rat hearts.
and ventricles between 10 and 40 weeks of age, with the atria showing a greater change (Figure 5). Since the relative amount of fibronectin mRNA in atria from SHRs was not observed to change between 10 and 40 weeks of age (Figure 2), there appears to be a dissociation between levels of fibronectin mRNA and protein in the atria of both SHR and Wistar rats as they mature and age.

**Discussion**

During maturation and aging, a progressive compensatory hypertrophy has been observed to occur throughout the myocardium of rats and humans, leading to an increase in myocyte cell volume. The documented changes in myocyte volume are greater in magnitude in the left side of the heart; thus, this study focused on the left free ventricular wall, to the exclusion of the right side of the heart. We have recently shown that myocardial fibronectin gene expression increases during hypertension-induced cardiac hypertrophy in the Wistar rat, suggesting the possibility that an increase in fibronectin expression may also be observed during the compensatory hypertrophy that occurs with age. We used SHRs between 6 and 40 weeks of age as the model for chronic hypertension and compared them with WKY rats. However, WKY rats are genetically heterogeneous and because of these concerns, we also used Wistar rats as normotensive age-matched controls. Forty-week-old rats are considered mature adults; thus, the changes observed in this study, although age related, may reflect the maturational adaptations of the myocardium instead of aging per se. Age-related changes in fibronectin gene expression were observed in all control rats, and these changes differed substantially from those observed in SHRs.

The age-related decrease in fibronectin mRNA and protein that was found in the ventricles of control rats...
between 10 and 40 weeks of age was in marked contrast to our findings in the rat aorta. In the aorta, an age-related increase in fibronectin mRNA was observed between 10 and 40 weeks of age in the Wistar, SHR, and WKY rats, with the greatest increase observed in aortas from SHRs. In the present study, a decrease in fibronectin gene expression was observed in hearts from Wistar and WKY rats, whereas the steady-state mRNA levels remained essentially unchanged in SHR hearts during this period. A decrease in the amount of β1 integrin mRNA was also observed in hearts from Wistar and WKY rats in the same period. Coordinate changes in the expression of fibronectin and its receptors have also been observed during the transition from the fetal to the adult heart. A recent study used in situ hybridization techniques to show that fibronectin mRNA was localized predominantly in the nonmyocytic cells of young rat hearts, with higher concentrations found in arteries and arterioles, implicating vascular smooth muscle as a major source of fibronectin mRNA in cardiac tissue. Our own studies in the rabbit aorta and those of Glukhova et al indicate that vascular smooth muscle cells are the major source of fibronectin in aortic tissue; thus, the opposing changes in fibronectin mRNA occurring in the heart and aorta from normotensive rats suggest that tissue-specific regulatory factors affect fibronectin expression.

Endocrine and paracrine regulatory mechanisms influence gene expression in the heart. Significant alterations in isoform distribution of myosin and creatine kinase mRNA and protein have been observed in the rat heart during aging. Circulating thyroid hormone (T3 and T4) is significantly lower at 32 weeks than at 8 weeks of age. This decrease was associated with isoform shifts in myosin and creatine kinase isoforms in the rat ventricles, and it was shown that the administration of thyroid hormone could modulate the myosin isoform shifts that occur with age. Myocardial fibronectin gene expression also has been shown to be modulated by thyroid hormones in vivo. Since older rats have lower circulating levels of thyroid hormone, the decrease in fibronectin mRNA and protein observed in ventricles of Wistar and WKY rat hearts may be due in part to the lowered levels of circulating thyroid hormone. However, studies conducted in a variety of cell types under different experimental conditions have shown fibronectin expression to be controlled by many hormonal and growth factors, including transforming growth factor-β (TGF-β), epidermal growth factor, platelet-derived growth factor (PDGF), glucocorticoids, and cAMP. We have previously shown that hypertension increases PDGF, β mRNA in the rat aorta, whereas no changes were observed in the heart. Changes in TGF-β, mRNA have also been observed between 5- and 40-week-old rats. Such local growth factor changes could play a role in determining the age-related levels of expression of fibronectin in the heart. The changes in the local expression of growth factors might account for the tissue differences observed between the aorta and the heart, since it has been shown that TGF-β mRNA is upregulated with age in the aorta, whereas it is downregulated with age in hearts from the same animals. The relative changes in the level of expression of fibronectin mRNA in the atria were similar to those observed in ventricles. However, a clear dissociation between amounts of mRNA and protein was observed in the atria as a function of age. Whereas fibronectin mRNA levels decreased or showed no significant change in Wistar and SHR atria, respectively, the amount of immunoreactive protein was unchanged in Wistar and increased in SHR atria between 10 and 40 weeks of age. These results contrasted sharply with those obtained from ventricular tissue, in which the changes in immunodetectable protein paralleled mRNA changes in both SHR and Wistar rats. At the present time, no functional correlates can be drawn from these results, but this in vivo observation might be useful in the study of post-translational mechanisms controlling fibronectin protein biosynthesis in the rat atria.

The myocardium contains two major types of collagen, types I and III, which make up more than 90% of all cardiac collagen. Hypertension and age are conditions in which various types of collagen have been

**FIGURE 3.** Northern blot analysis of total ventricular RNA obtained from hearts of 10- and 40-week-old spontaneously hypertensive rats (SHRs) and Wistar rats. LV, left ventricle; GAPD, glyceraldehyde 3-phosphate dehydrogenase. Each lane contains 10 μg total RNA, and the membrane was successively rehybridized with all four probes used.
shown to be differentially regulated. Both are known to increase the expression of collagen type I mRNA, although coordinate changes in amounts of protein have not been observed. Collagen type III mRNA has also been shown to be upregulated during the early stages of acute hypertension-induced cardiac hypertrophy, although changes in immunodetectable protein were not observed. Acute changes in collagen type I mRNA have also been induced in the myocardium by norepinephrine, and these changes have been shown to be not entirely due to blood pressure alterations. Thus, a dissociation between mRNA and protein for collagen type I has been observed to occur in the aging heart, analogous to the effects we noted for fibronectin in the atria. Steady-state collagen type I mRNA levels have been shown to decrease between 4 and 88 weeks of age in rat ventricular tissue, whereas the amount of protein was shown to increase in a linear fashion between 4 and 44 weeks of age. Furthermore, a shift in the collagen ratios has been observed with age, with a progressive increase in the collagen type I/type III ratio between 10 and 34 weeks of age in Wistar rat hearts. This study shows for the first time an age-related decrease in the steady-state levels of collagen type III mRNA, in contrast to documented increases in collagen type I mRNA with age and hypertension-induced cardiac hypertrophy. Northern blot analysis of ventricular RNA indicated that a significant decrease in
the amount of collagen III mRNA occurred between 10 and 40 weeks in Wistar rats. Our results suggest, but do not directly show, that the amount of collagen type III synthesized in the myocardium may decrease with age. An alternative explanation would be that collagen type III also exhibits an age-associated dissociation between mRNA and protein levels.

Using ribonuclease protection analysis, we have documented an age-related change in the relative amounts of EIIIA+ mRNA, an alternatively spliced fibronectin mRNA isoform, in the hearts of male Wistar rats between 10 and 40 weeks of age. An increase in the relative amount of EIIIA-containing isoforms was observed in Wistar hearts, whereas no changes occurred in ventricular tissue from SHR hearts from the same period. A transient induction of EIIIA-containing fibronectin mRNA has previously been shown to occur during the initial stages of cardiac hypertrophy; the magnitude of increase in the relative amount of EIIIA+ isoforms in Wistar rat hearts with age was similar to that observed during experimentally induced cardiac hypertrophy.17 We did not observe any age-related changes in EIIIA content in ventricles from SHRs. This could be attributed to the early induction of myocardial hypertrophy in the SHR, which occurs as early as 2 weeks of age,26,46,47 or before the initiation of our studies. The biological function of EIIIA-containing fibronectin isoforms remains unclear, although it has been shown that EIIIA- and EIIIB-containing isoforms have an enhanced matrix-forming ability.3 A 5–10-kd increase in fibronectin molecular weight has been observed to occur at the protein level in senescent E11 cells48 and might reflect the inclusion of alternatively spliced exons in the protein.

A recent study using the polymerase chain reaction as an analytical technique showed a slight decrease in the relative amount of EIIIA+ fibronectin mRNA isoforms in hearts from aging 60- and 120-week-old male Fischer rats49 and suggested that posttranslational modifications, such as glycosylation, may explain the reported increase in protein size. The study also documented low levels of the relative amount of EIIIA+ mRNA isoforms in hearts and the absence of the isoform from liver, lung, and spleen, which is in contrast to other studies that have documented the presence of relatively high amounts of EIIIA+ isoforms in the adult rat lung.17,50 The differing results may be a reflection of the techniques used or could be due to the significant differences in the age of animals used in the studies.

Both hypertension and aging lead to cardiac hypertrophy in experimental animal models and humans.13 The similarities between the morphological, biochemical, and functional changes in the heart that are common to both acute pressure overload and aging have led some investigators to propose that hypertension represents an accelerated form of aging51 and that common molecular events occur in both situations. Similar vascular changes occur both in experimental hypertension and aging, and the attenuation of such changes by blood pressure lowering further suggests common mechanisms in both processes.52 However, the data in this study clearly show that the pattern of cardiac fibronectin expression differs between aging in normotensive rats, in which a decrease is apparent, and SHRs, which show no change with age, as compared with a temporal upregulation observed in acute states of experimental hypertension. The increased cardiac fibronectin observed in SHRs may reflect responses of microvessels and macrovessels and may be localized largely to vascular cells within the myocardium.29

The functional implications of the changes observed in this study are unclear at present. Previously observed increases in the expression of fibronectin and collagen with age in aortic tissue might play a role in the increasing stiffness and thickening of the vasculature with age. The decreased elasticity of the vasculature is
thought to contribute to the increases in systolic blood pressures that occur with age.24,51,52 Changes in cardiac function with age include a decrease in early diastolic filling, an increased atrial contribution to ventricular filling, prolonged isometric relaxation, increased end-diastolic ventricular pressure, decreased cardiac index, and decreased coronary blood flow.24,52,53 Data have been presented showing that age-related changes in cardiac collagen content do not correlate with left ventricular stiffness.54–56 This is because the changes in collagen content presumably do not affect changes in the collagen weave. Similarly, one would need to study the fibronectin–collagen structural interactions and their effects on coronary vasculature patency before making conclusions about the fibroelastic and functional properties of the aging myocardium. Age-related myocardial function changes are also known to be affected by changes in myosin isozyms, α-actin iso- genes, diminished sarcoplasmic reticulum Ca2+ pumping rates, reduced mitochondria/myofibril ratios, and changes in the transmembrane potential.31–33,52,53,55 The result of these changes is an altered excitation–contraction coupling mechanism, which may also account for some of the documented age-related changes in myocardial functional parameters.

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**References**


38. Dean DC, Newby RF, Bourgeois S: Regulation of fibronectin biosynthesis by dexamethasone, transforming growth factor β, and cAMP in human cell lines. *J Cell Biol* 1988;106:2159–2170

44. Eghbali ME, Eghbali M, Robinson TF, Seifert S, Blumenfeld O: Role of fibroblasts in accumulation of collagens in growing and adult rat hearts. (abstract) *J Mol Cell Cardiol* 1987;19:S54
54. Lakatta EG: Do hypertension and aging have a similar effect on the myocardium? *Circulation* 1987;75(suppl 1):I-69–I-77
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