Remodeling of the Rat Right and Left Ventricles in Experimental Hypertension


Pathological left ventricular hypertrophy in renovascular hypertension is associated with the accumulation of fibrillar collagen within the extracellular space and around intramyocardial coronary arteries. Even though the angiotensin converting enzyme inhibitor captopril was previously found to attenuate this interstitial and perivascular fibrosis, the relative importance of arterial and ventricular systolic pressures versus circulating angiotensin II (AII) and aldosterone (AL) in promoting hypertrophy and collagen accumulation in renovascular hypertension is uncertain. By drawing on the in-parallel arrangement of the right and left ventricles, with respect to their coronary circulation, and the in-series mechanical alignment of the ventricles, with a pressure-overloaded left and a normotensive right ventricle, this study sought to address this uncertainty. Three models of experimental hypertension, each having a different circulating AII and AL profile, were examined and compared with their controls: renovascular hypertension, where both AII and AL are increased; infrarenal aorta banding, where AII and AL are normal; and a chronic infusion of AL, where AII is suppressed or normal and AL is increased. In renovascular hypertension, as well as with AL, we found a significant rise in the interstitial collagen volume fraction and perivascular collagen area of the pressure-overloaded, hypertrophied left ventricle as well as the normotensive, nonhypertrophied right ventricle. This remodeling was not seen in either ventricle with infrarenal aorta banding despite comparable systemic hypertension and left ventricular hypertrophy. Thus, in experimental arterial hypertension in the rat, myocyte and nonmyocyte compartments of the myocardium are under separate controls: myocyte hypertrophy is most closely related to ventricular loading while circulating AII and AL, acting alone or in concert with other humoral factors, regulate the accumulation of collagen within the right and left ventricles. (Circulation Research 1990;67:1355–1364)

Previous studies from this1–4 and other5–9 laboratories have described the accumulation of fibrillar collagen that occurs within the extracellular matrix and around intramyocardial coronary arteries of the hypertrophied rat left ventricle in experimental and genetic hypertension. This reactive interstitial and perivascular fibrosis, which is not seen with the hypertrophy that accompanies anemia,5 an arteriovenous fistula,9 or an atrial septal defect,10 is held responsible for abnormal myocardial stiffness1–3,8 and impaired pumping capacity of the heart.7 As a result, it is this remodeling of nonmyocyte compartments that represents a determinant of pathological hypertrophy in hypertension.11 These findings further suggest that the growth and remodeling of the muscular, vascular, and interstitial compartments of the myocardium may be under separate controls.

The relative importance of hemodynamic factors versus circulating hormones in promoting myocyte hypertrophy or fibrosis in hypertension is therefore of interest. In this regard, the relative contribution of the elevation in arterial and systolic ventricular pressures and circulating angiotensin II (AII) and aldosterone (AL) in renovascular hypertension (RHT) needs to be identified. Our recently completed trial,12 in which oral captopril, an angiotensin converting enzyme inhibitor, initiated before the induction of RHT was able to prevent hypertension, hypertrophy, and the interstitial and perivascular fibrosis, did not allow us to distinguish among these possibilities. Accordingly, this study was undertaken to distinguish the role of these hemodynamic factors and circulating AII and AL in promoting myocyte hypertrophy and a remodeling of the interstitial and vascular
compartmental. For this purpose, we took advantage of the in-parallel arrangement of the ventricles with respect to the coronary circulation (vis-à-vis their in-series mechanical arrangement) and examined the pressure-overloaded left and normotensive right ventricles and their intramyocardial coronary arteries in different models of arterial hypertension, for which the circulating renin-angiotensin system would either be elevated, normal, or partially suppressed. That is, abdominal aorta banding, with or without renal ischemia, or a chronic infusion of AL, respectively.

**Materials and Methods**

**Animal Models**

Five experimental groups of 8-week-old male Sprague-Dawley rats weighing 160–180 g at the onset of the experiment were studied (Table 1) and are defined as follows. 1) RHT was created by abdominal aorta banding with constriction of the right renal artery for 8 weeks. This model of RHT, used in our laboratory, is associated with a progressive atrophy of the right kidney and an elevation in circulating AII according to the two-kidney, one-clip model. When the animals were killed, the presence of renal atrophy was confirmed. In three animals, not included in the RHT group, atrophy was not found. These animals were considered separately as having aorta banding without renal ischemia. 2) IRB was created by infrarenal aorta banding for 8 weeks. We have previously shown that the infrarenal band will raise arterial pressure to a level comparable to our model of RHT without an elevation in circulating AII. 3) AL was created by unilateral nephrectomy followed by the sequential subcutaneous implantation of three osmotic minipumps with each pump continuously delivering AL for 20 days to raise plasma AL to within the range seen with RHT and to suppress AII. Animals were placed on a 10 g/l sodium intake in their drinking water throughout. The first pump contained AL administered at 0.25 μg/hr for 20 days. This provided for a modest elevation in arterial pressure. Subsequently, a second and then third pump were implanted with a larger dose of AL (0.75 μg/hr) to create a greater elevation in arterial pressure for the remaining 5 weeks. 4) ALC was created by unilateral nephrectomy and a similar sequential implantation of pumps, none of which contained AL. These animals were maintained on a daily sodium intake of 10 g/l drinking water for 8 weeks and served as controls to the AL group. 5) C was unoperated rats that were studied after 8 weeks and used as controls for the RHT and IRB groups.

**Experimental Protocol**

In animals without aorta banding, arterial pressure was measured on a weekly basis by the standard tail-cuff method. For all animals, arterial pressure was measured by carotid artery cannulation in the lightly anesthetized state before the chest was opened and the animals were killed. Previous studies in this laboratory had established that the banded animals develop arterial hypertension and left ventricular hypertrophy.

In five animals of each experimental group, circulating AII and AL levels were determined at baseline and after 1, 4, and 8 weeks (end of experiment). Blood samples were collected between 9 and 10 AM from barbiturate-anesthetized animals. To stop all angiotensin converting enzyme activity, the samples were immediately mixed with an inhibitor solution (50 μl/ml blood) containing EDTA 125 mM, o-phenanthroline 50 mM, ethanol 2%, and neomycin sulfate 2 g/l and kept on ice until centrifugation (6,000 g, 4°C, 3 minutes). The serum samples were then kept frozen at −80°C until AL and AII were measured. Serum AL was measured using a radioimmunoassay with sample AL competing with 125I-radiolabeled AL for antibody sites (Diagnostic Products Corp., Los Angeles). In similar fashion, circulating, immunoreactive AII was determined by a standardized radioimmunoassay after angiotensin peptides were extracted from plasma according to Nussberger et al. Both assays were performed in duplicate.

Before the animals were killed, they were anesthetized with methohexital (50 mg/kg i.p.), intubated, and mechanically ventilated. The chest was opened by median sternotomy, and the heart and lungs were removed. The right and left (plus septum) ventricles were weighed, and coronal sections of each ventricle, taken at the equator of the heart, were immediately fixed in 10% formalin.
Collagen Morphology and Morphometry

The coronal sections were dehydrated and embedded in paraffin. Nine sequential, 5-μm-thick sections, containing a complete cross-sectional cut of both ventricles, were obtained from each heart: the first three were stained with hematoxylin and eosin, the next three with Gomori’s trichrome, and the last three with the collagen-specific stain Sirius Red F3BA (Pfalz and Bauer, Inc., Stamford, Conn.), as reported elsewhere.1-3 The interstitial collagen volume fraction of the trichrome-stained tissue was determined by planimetry as previously reported.1-3 In brief, each coronal section was divided into four quadrants by using the center of the section as the origin. Four fields from each quadrant were randomly selected. Connective tissue and muscle areas were identified and manually traced using a projecting microscope connected to a digitizing pad. The digitized profiles were transferred to a computer that calculated collagen volume fraction as the sum of all connective tissue areas in the 16 fields divided by the sum of all connective tissue and muscle areas in all fields. Perivascular collagen was excluded from this analysis and was measured separately. We have previously shown that total collagen volume fraction (including perivascular collagen), as determined by this morphometric approach, is closely related to hydroxyproline concentration of the left ventricle.18 The perivascular collagen area normalized to vessel luminal area of intramural coronary arteries was determined in the Sirius Red–stained tissue by using an automated image analyzer (Quantimet 520, Cambridge Instruments, Inc., Deerfield, Ill.). Only those intramyocardial vessels that appeared circular on cross section were analyzed; on the average, there were 15 such vessels found in the left ventricle and five in the right ventricle. The investigator responsible for the morphometrical analysis (R.F.) was blinded as to each experimental group.

Statistical Analysis

All grouped data are expressed as mean±SEM and compared by one-way analysis of variance. For a significant F value, pairwise group comparisons were performed using t statistics. Levels of significance were taken at p<0.05 and were corrected for multiple comparisons according to Bonferroni bounds.

Results

Hypertension and Hypertrophy

Arterial systolic and mean pressures were significantly elevated above control and to a comparable degree in each model of experimental hypertension (Table 2). No significant difference in systolic and mean pressures was found between the different hypertensive groups. In the three animals with suprarenal aorta banding without renal ischemia and atrophy, systolic and mean pressures ranged between 185 and 245 and between 170 and 205 mm Hg, respectively. Systolic and mean pressures in each of the two control groups did not differ from one another.

Right ventricular weight in each of the hypertensive groups was comparable to the two control groups. The ratio of left to right ventricular weights, as well as left ventricular weight normalized to body weight, was significantly elevated in each model of arterial hypertension; the degree of hypertrophy was comparable in all hypertensive groups; that is, no significant difference in the ratios of left to right ventricular weight or left ventricular weight to body weight was found between these groups. The ventricular weight ratio in the three animals without renal atrophy was 4.6, 5.0, and 5.1 and is in keeping with the presence of left ventricular hypertrophy; right ventricular weight was not different between these three animals and the other experimental groups. In the unilaterally nephrectomized/high-salt diet control group, the ratio of ventricular weights was greater than in unoperated controls, in keeping with a volume overload state.

Circulating Angiotensin II and Plasma Aldosterone Concentrations

One week after intervention, AII was significantly elevated in RHT (p<0.02) and suppressed in the AL group (p<0.05) compared with their controls (RHT versus C, AL versus ALC) (Figure 1). During the 8-week period, AII increased further in RHT and returned to normal in AL-treated animals, while it remained unaffected in the IRB group and in control

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Values are mean±SEM. RHT, renovascular hypertension; IRB, infrarenal band; AL, aldosterone infusion; ALC, aldosterone control; C, unoperated control; SBP, systolic arterial pressure; MAP, mean arterial pressure; LV/RV, the ratio of left to right ventricular weights; LV/BW, left ventricular weight normalized to body weight; RVW, right ventricular weight.

*p<0.002: RHT or IRB vs. C; AL vs. ALC.
†p<0.01: IRB vs. C; AL vs. ALC.
‡p<0.05: ALC vs. C.
Figure 1. Plasma angiotensin II profile for the different experimental groups over 8 weeks. In renovascular hypertension (RHT), circulating angiotensin II was increased throughout the whole period, while in the aldosterone (AL) administration group angiotensin II was suppressed after 1 week and returned to normal after 8 weeks. No change in angiotensin II occurred with infrarenal aorta banding (IRB) or after unilateral nephrectomy (ALC) compared with control (C).

Interstitial Collagen Volume Fraction

The interstitial collagen volume fraction of the pressure-overloaded, hypertrophied left and normotensive, nonhypertrophied right ventricles was significantly elevated in the experimental group with RHT (left ventricle: 8.8±1.6%, *p<0.002; right ventricle: 4.1±0.8%). In the three rats of the subgroup with aorta banding and no renal atrophy, interstitial collagen volume fraction of the left ventricle was 3.2%, 3.3%, and 4.1%, appeared to be no different from control, and was clearly less than that seen with RHT or AL administration.

Perivascular Collagen Area

Perivascular fibrosis of intramural coronary arteries was present in both the pressure-overloaded, hypertrophied left and normotensive, nonhypertrophied right ventricles of animals with RHT and those receiving AL (Figures 5 and 6). Perivascular collagen area normalized to vessel luminal area of the left and right ventricles was significantly increased in each of these hypertensive groups compared with their normotensive controls. We found that perivascular collagen area was increased in RHT and after AL irrespective of vessel size with a range of luminal vessel diameter between 19 and 316 μm. The average vessel luminal area, including all analyzed vessels in

Figure 2. Plasma aldosterone profile over 8 weeks. See Figure 1 for abbreviations. In RHT and with continuous AL infusion, circulating AL was significantly increased to comparable values throughout the experimental period, while in IRB no change occurred compared with C.

Figure 3. Interstitial collagen volume fraction (CVF) for the rat left ventricle in the five experimental groups. See Figure 1 for abbreviations. A significant interstitial fibrosis was seen only in RHT and AL relative to their respective controls.

Figure 4. Right ventricular interstitial fibrosis was also seen in RHT and AL groups, but not the other groups. See Figure 1 for abbreviations. CVF, collagen volume fraction.
of intramural arteries in the RHT and AL groups. From their perivascular location, septa of collagen were seen to extend outward into intermuscular spaces. This perivascular fibrosis was present in both ventricles. This was not the case for the IRB or ALC groups.

Discussion

Myocardial hypertrophy is an expected adaptation to the elevation in ventricular systolic pressure that accompanies isometric exercise training. However, and quite unlike the adaptive hypertrophy seen with exercise, the hypertrophic growth of the myocardium in systemic hypertension is likely to become pathological with left ventricular dysfunction. It is now recognized that left ventricular hypertrophy is associated with all major cardiovascular complications, especially the appearance of symptomatic heart failure. Hence the origins of ventricular dysfunction, on which the appearance of symptomatic failure is based, are likely to be found in the hypertrophic remodeling of the muscular, vascular, and interstitial compartments of the myocardium.

Morphological studies indicate that the increment in myocardial mass in left ventricular hypertrophy is primarily related to an increase in myocyte size. However, a structural remodeling and accumulation of fibrillar collagen within the interstitium and surrounding intramyocardial coronary arteries may also be involved depending on the nature of the hypertrophic stimulus. For example, in humans, as well as in various experimental and genetic models of arterial hypertension, an interstitial and perivascular fibrosis of these vessels has been observed. On the other hand, myocardial fibrosis is not seen in the hypertrophy that accompanies anemia, arteriovenous fistula, or atrial septal defect. These findings raise the intriguing possibility that the growth of cellular constituents of these compartments, namely, cardiac myocytes and cardiac fibroblasts, which are known to contain the mRNAs for types I and III collagens, may each have different regulatory mechanisms. Differences in the growth of fibroblasts and collagen synthesis, relative to myocyte hypertrophy, may lead to an intercompartmental disequilibrium and thereby pathological hypertrophy.

This study sought to address the issues surrounding myocyte hypertrophy and the remodeling of the interstitial and vascular compartments by fibrous tissue in the rat having experimental arterial hypertension. It further sought to distinguish the relative importance of hemodynamic factors, such as the elevation in ventricular chamber systolic pressure, from circulating hormones (e.g., Ang II) in mediating these responses. Because the coronary circulation subtends both the right and left heart, circulating Ang II and AL would reach each ventricle based on this in-parallel arrangement, while the importance of the ventricular systolic pressure could be distinguished in these models of arterial hypertension by the in-series arrangement of the ventricles. The importance of arterial hypertension, which represents an elevation...
Figure 7. Right ventricle. Picrosirius technique and direct light (microscope magnification, ×40). Unoperated control (upper left panel), renovascular hypertension (upper right panel), infrarenal banding (lower left panel), and aldosterone administration (lower right panel). Collagen fibers appear black, whereas the media of intramural coronary arteries and myocytes are gray. The normal interstitial collagen and meshwork of collagen surrounding an intramyocardial coronary artery are shown in the upper left panel. With renovascular hypertension, the interstitial and perivascular accumulation of fibrillar collagen, or fibrosis, is evident. Such a remodeling of the myocardium was not found with infrarenal banding. After chronic aldosterone administration, an interstitial and perivascular fibrosis was seen.
FIGURE 8. Left ventricle. Periradial technique and direct light (microscope magnification, ×40). Untreated control (upper left panel), renovascular hypertension (upper right panel), infarct (lower left panel), and aldosterone administration (lower right panel). The remodeling of interstitial and perivascular collagen in the left ventricle for the various models of experimental hypertension was similar to that found in the right ventricle.
in coronary perfusion pressure,\textsuperscript{29} could not be distinguished in this study, even though it is frequently presumed that intramyocardial coronary arteries are protected from such variations in arterial pressure. Toward this end, we examined the remodeling of intramural coronary arteries and interstitium in the nonoverloaded right ventricle and the pressure-overloaded left ventricle in three models of systemic hypertension, for which circulating AII and AL are either increased (i.e., RHT) or normal (i.e., infrarenal band), or circulating AII is reduced while AL is increased (i.e., AL infusion).

Our results indicate the heterogeneity that can exist in the hypertrophic remodeling of the myocardium. Myocyte growth and myocardial hypertrophy were most closely related to elevations in myocyte loading created by the rise in ventricular systolic pressure. Left ventricular hypertrophy was present in all models of arterial hypertension and with the circulatory overload associated with unilateral nephrectomy and a high-salt diet. Myocardial fibrosis, however, was not seen with infrarenal aorta banding, suprarenal aorta banding without renal ischemia and atrophy, or unilateral nephrectomy with high-salt intake. Cooper et al\textsuperscript{30} have previously reported that myocyte loading is the major determinant of hypertrophy in the intact animal. In the in vitro heart, Schreiber et al\textsuperscript{30} found protein synthesis to be related to ventricular systolic pressure. Additionally, it has been reported that an elevation in arterial pressure will enhance myocardial protein synthesis in the isolated heart.\textsuperscript{31} For a comparable elevation in left ventricular systolic pressure, created by RHT, AL administration, or infrarenal aorta banding, we found a comparable increment in left ventricular mass.

The structural remodeling of the interstitium and intramural vessels of the left ventricle in RHT and with AL, however, differed from that seen with infrarenal banding. With RHT and AL, an interstitial and perivascular fibrosis was observed. This remodeling was also found in the nonoverloaded, nonhypertrophied right ventricle. In contradistinction with infrarenal banding, we found neither interstitial nor perivascular fibrosis in either the right or left ventricle. The same was true for the small subgroup of animals with aorta banding and no gross evidence of renal ischemia. Hence, it would appear that circulating hormones are an essential element in the growth of nonmyocyte cells. Acting alone, or more likely in concert with other humoral factors (e.g., growth factors), they influence cardiac fibroblast growth and collagen synthesis and thereby are responsible for the remodeling of the interstitial and vascular compartments in each ventricle. In our prevention study with the angiotensin converting enzyme inhibitor captopril,\textsuperscript{32} myocardial fibrosis was prevented in the same model of RHT as was used in this study, where we found increased interstitial and perivascular fibrosis in both the high (RHT) and the low (AL) AII models. Therefore, elevations in circulating AII alone cannot be the decisive mediator of the fibrous tissue response. However, AL is elevated in both models and may be pivotal in the regulation of collagen synthesis within the interstitium and adventitia of intramyocardial coronary arteries. This hypothesis is supported by the findings of Meyer and Nichols,\textsuperscript{32} who detected AL receptors on fibroblasts isolated from the adventitia of rat aortic tissue. Further studies are needed to elucidate whether AL receptors are also present on cardiac fibroblasts and whether these receptors are activated during the fibrotic process within the myocardium.

In this study, we addressed myocardial fibrosis by quantitative morphometric analysis. Based on the objectives of this study, the morphometric approach was more rewarding than the hydroxyproline assay. This biochemical technique cannot be used to identify the location of collagen or its amount within the adventitia of intramural vessels and the cardiac interstitium. Furthermore, the morphological/morphometric approach permitted us to distinguish the interstitial and perivascular fibrosis from microscopic scars.\textsuperscript{33} We selected an entire coronal section at the equator of the left and right ventricles for these measurements based on the view that it was representative for the entire myocardium. We have previously shown that the connective tissue response of the myocardium is evenly distributed in systemic hypertension\textsuperscript{22} and that a good correlation exists between hydroxyproline and the collagen volume fraction.\textsuperscript{18}

At birth, the collagen concentration of the ventricles is similar; however, with the reduction in its workload and regression in myocyte size during the neonatal period, the collagen concentration of the adult right ventricle is 30% greater than that of the left ventricle.\textsuperscript{34} Given its smaller sized myocytes, the accumulation of collagen within the right ventricle in RHT and with AL administration was greater than that of the left ventricle. We would infer that the mechanical behavior of the right ventricle would more likely be compromised by this fibrous tissue reaction than would the left ventricle. It is important to note that right ventricular weight did not change despite a twofold to threefold increase in its collagen volume fraction. This is not unexpected, however, given that the weight of myocardial collagen is no more than 0.02 g/g wet wt heart tissue.\textsuperscript{35} Accordingly, a threefold increase in the collagen volume fraction would correspond to an increment in ventricular weight of only 0.01 g.

Other hormonal factors that may be operative in myocardial remodeling with chronic AL administration include those associated with the adrenergic nervous system. Norepinephrine is a known mitogenic stimulus for cultured fibroblasts,\textsuperscript{36} and its release into the cardiac interstitium is related to ventricular systolic pressure.\textsuperscript{37,38} In deoxycorticosterone hypertension, enhanced circulating or centrally mediated norepinephrine release has been observed.\textsuperscript{39,40} Regional myocardial denervation has been noted to prevent the expected fibrosis of the pressure-overloaded, hypertrophied right ventricle after pulmonary artery banding.\textsuperscript{26}
Ooshima et al have found that reserpine, an agent that depletes tissue norepinephrine, or hypophysectomy reduced collagen synthesis in both the myocardium and systemic vasculature of normotensive rats or rats with deoxycorticosterone acetate/salt hypertension. In rats with genetic hypertension, the expected early and late rise in myocardial collagen synthesis was prevented by α-methyldopa or reserpine, as well as the angiotensin converting enzyme inhibitor captopril. Additionally, reserpine prevents the increase in smooth muscle cell DNA synthesis that occurs in RHT. Similarly, sympathetic denervation of the ear artery was found to decrease smooth muscle cell DNA synthesis and vessel growth.

The results of this study suggest that cardiac myocyte loading governs myocyte growth and myocardial mass while humoral systems, including AL, regulate the growth of cardiac fibroblasts and consequently the accumulation of collagen within the interstitial and vascular compartments of the myocardium in arterial hypertension. We further believe that it is the involvement of cardiac fibroblasts, and not cardiac myocytes, that alters the normally adaptive hypertrophic remodeling of the myocardium and sets the stage for pathological hypertrophy and ultimately ventricular dysfunction in hypertension. However, further analysis of the humoral or neurohumoral cascade that leads to myocardial fibrosis will be necessary.

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