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Regression of myocardial fibrosis in hypertensive heart disease: diverse effects of various antihypertensive drugs

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Abstract

Objective: In left ventricular hypertrophy (LVH) due to systemic hypertension, myocardial fibrosis is an important determinant of pathologic hypertrophy. Therefore, it is most relevant to utilize an antihypertensive regimen that permits a regression in myocardial fibrosis along with blood pressure normalization and regression of LVH. **Methods:** To address this issue we examined 60 Sprague–Dawley rats. We treated 16-week-old rats having established LVH and myocardial fibrosis due to 8-week renovascular hypertension (RHT) with either 6 mg/kg/day zofenopril (ZOF), 30 mg/kg/day nifedipine (NIF) or 40 mg/kg/day labetalol (LAB) for 12 weeks. Systolic arterial pressure (SAP, mmHg), left ventricular/body weight ratio (LV/BW, mg/g), and left and right ventricular collagen volume fractions (LVCVF, RVCVF, %) were obtained and compared with age/sex matched untreated rats with RHT and sham-operated controls. **Results:** In RHT, SAP was significantly elevated compared with controls (188±11 vs. 125±5 mmHg; $P<0.001$) while in each treated group SAP was normalized. LV/BW was significantly increased in RHT (2.61±0.12 mg/g; $P<0.00001$) while in each treated group LVH was completely regressed ($P<0.002$ vs. untreated RHT) with LV/BW values comparable to controls (1.82±0.03 mg/g) irrespective of the utilized antihypertensive agent. In untreated RHT, myocardial fibrosis was present in the left (LVCVF: 12.3±1.9%; $P<0.0005$ vs. 4.5±0.2% of controls) and right ventricles (RVCVF: 20.6±2.5%; $P<0.00005$ vs. 8.8±0.4% of controls). In rats treated with ZOF or NIF, LVCVF was significantly reduced to 5.6±0.4 and 5.4±0.6%, respectively ($P<0.005$ vs. untreated RHT), and RVCVF was decreased as well (ZOF: 11.0±0.9%; NIF: 10.4±2.4%; $P<0.007$ vs. untreated RHT) where no significant difference to controls remained. In contrast, treatment with LAB did not affect myocardial fibrosis where LVCVF was 9.3±1.3% and RVCVF was 19.8±2.8%, i.e., remained significantly elevated compared with controls ($P<0.007$). **Conclusions:** In rats with renovascular hypertension and hypertensive heart disease that included LVH and fibrosis, equipotent doses of ZOF, NIF, and LAB normalized arterial pressure associated with regression of LVH while only ZOF and NIF were found to regress myocardial fibrosis.

Keywords: ACE inhibitors; Adrenergic (ant)agonists; Antihypertensive agents; Fibrosis; Hypertension; Hypertrophy

1. Introduction

In the adult rat with arterial hypertension, left ventricular hypertrophy (LVH) can be associated with a pathologic accumulation of fibrous tissue within the cardiac interstitium [1–4]. This reactive fibrosis, which occurs irrespective of myocyte necrosis [5], appears in the pressure overloaded, hypertrophied left ventricle and the normotensive, nonhypertrophied right ventricle [6]. The accumulation of fibrillar collagen adversely raises myocardial stiffness [1–4]. In previous studies using spontaneously hypertensive rats (SHR) with established LVH and myocardial fibrosis, we found that it was possible to regress myocardial fibrosis with chronic treatment by an angiotensin converting enzyme (ACE) inhibitor leading to normalization of diastolic stiffness [7,8] and prevention of systolic left ventricular (LV) dysfunction [9]. A similar fibrous tissue accumulation is found in both ventricles of the rat with renovascular hypertension due to unilateral ischemia (2-kidney/1-clip model), where it likewise accounts for abnormal myocardial stiffness [1–6]. Whether it is possible to regress fibrosis with an ACE inhibitor in this rat model of renovascular hypertension, and whether other

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antihypertensive therapies would prove equally cardioreparative are questions that remain to be addressed. Accordingly, this treatment trial was undertaken in the adult rat with established renovascular hypertension, LVH and myocardial fibrosis due to surgically-induced unilateral renal ischemia of eight weeks duration. These animals were then treated for 12 weeks with an oral antihypertensive regimen that included one of the following: (a) zofenopril, a sulfhydryl-containing ACE inhibitor; (b) nifedipine, a dihydropyridine Ca^{2+} channel blocker; or (c) labetalol, an antagonist of α- and β-adrenergic receptors. Age/sex matched untreated rats with renovascular hypertension and sham-operated treated and untreated rats served as controls.

2. Methods

2.1. Animal models

In total, 60 8-week-old male Sprague–Dawley rats (Harlan Sprague–Dawley, Inc., Indianapolis, IN) weighing 160–180 g at the onset of the experiment were studied. In 40 anesthetized animals, renovascular hypertension was established by abdominal aorta banding with constriction of the right renal artery, as previously reported [5,6,10], while 20 rats were sham-operated. This model of renovascular hypertension is associated with progressive atrophy of the right kidney [11] and an elevation in circulating angiotensin (Ang) II and aldosterone [6] according to the 2 kidney/1 clip model. After 8 weeks, rats with renovascular hypertension were randomly assigned to the following treatment groups (Table 1): (a) 6 mg/kg/day oral zofenopril (ZOF; $n=8$); (b) 30 mg/kg/day oral nifedipine (NIF; $n=8$); (c) 40 mg/kg/day oral labetalol (LAB; $n=8$); and d) no treatment (CTR; $n=16$). At the same time, sham-operated animals were randomly assigned to identical treatment groups ($n=5$; each group). Before treatment started, one randomly picked animal from each group was killed after arterial pressure had been measured (vide infra) to assure established renovascular hypertension, LVH, and myocardial fibrosis in aorta banded animals as expected from previous studies using the same experimental conditions and observation period [5,6,10]. The treatment period was 12 weeks where rats received their assigned treatment via tap water. Drug dosages were adjusted according to weekly measurements of body weight and daily measurements of water uptake. While zofenopril and labetalol dissolved directly in water, 10 mg of nifedipine were dissolved in 15 g of 95% ethyl alcohol and 15 g of polyethylene glycol subsequently mixed with water. In addition, drinking water containers with nifedipine had to be covered with aluminum foil because nifedipine is extremely sensitive to light. All rats were housed under standardized conditions (two to three rats/cage) receiving standard rat chow and water ad libitum. All rats were killed at their 28th week of age. At the time of sacrifice, the presence of renal atrophy was confirmed in rats with abdominal aorta banding and constriction of the right renal artery.

The investigation conformed with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.2. Experimental protocol

At sacrifice, body weight was measured and animals were anesthetized with methohexital (45 mg/kg i.p.), intubated and mechanically ventilated. Arterial pressure was measured by carotid artery cannulation using a Statham pressure transducer (Gould-Statham, Oxnard, CA, USA) in the lightly anesthetized state. Following supplemental anesthesia the chest was opened by median sternotomy, and the ascending aorta was cannulated for perfusion fixation of the heart using 2.5% phosphate-buffered glutaraldehyde (pH 7.4) at 100 mmHg perfusion pressure for 15 min. Thereafter, heart and lungs were removed. The left (including the interventricular septum) and right ventricles were weighed and coronal sections of both ventricles, taken at the equator of the heart, were immediately fixed in 10% formalin and kept until histological study. LVH was determined by LV weight normalized to body weight (LV/BW).

Table 1
Animal models

RHT	Four groups of Sprague–Dawley rats with abdominal aorta banding with constriction of the right renal artery for 20 weeks
ZOF	After 8 weeks of RHT, treatment with 6 mg/kg/day zofenopril for 12 weeks ($n=7$)
NIF	After 8 weeks of RHT, treatment with 30 mg/kg/day nifedipine for 12 weeks ($n=7$)
LAB	After 8 weeks of RHT, treatment with 40 mg/kg/day labetalol for 12 weeks ($n=7$)
CTR	Control group with no treatment ($n=15$).
Sham	Four groups of sham-operated Sprague–Dawley rats followed for 20 weeks
ZOF	After 8 weeks, treatment with 6 mg/kg/day zofenopril for 12 weeks ($n=4$)
NIF	After 8 weeks, treatment with 30 mg/kg/day nifedipine for 12 weeks ($n=4$)
LAB	After 8 weeks, treatment with 40 mg/kg/day labetalol for 12 weeks ($n=4$)
CTR	Control group with no treatment ($n=4$)

2.3. Collagen morphometry

The coronal sections were dehydrated and embedded in paraffin. Six sequential, 5-μm thick sections, containing a complete cross sectional cut of either ventricle, were obtained from each ventricle: half of the sections were stained with hematoxylin and eosin, and the other half with the collagen specific stain Sirius Red F3BA (Pfaltz & Bauer, Stamford, CT, USA) as reported elsewhere [2–10].

Collagen volume fractions of the left and right ventricles (LVCVF and RVCVF, respectively) of the Sirius Red stained tissue were determined by computerized videodensitometry (Quantimet 520, Cambridge Instruments, Inc., Deerfield, IL, USA) as previously reported [7–10], and based on the following assumptions: (1) distribution of interstitial collagen is homogenous within each ventricle and (2) the equatorial, coronal sections of the left and right ventricles are representative of the entire ventricle in each animal. Connective tissue and muscle areas were identified according to their respective gray levels, where collagen fibers appear black, myocytes gray, and interstitial space white. The digitized profiles were transferred to a computer that calculated collagen volume fraction as the sum of all connective tissue areas in the entire section divided by the sum of all connective tissue and muscle areas in %. 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 [10,12]. The investigator responsible for the morphometrical analysis was blinded as to each experimental group.

2.4. Statistical analysis

All grouped data are expressed as mean±S.E.M. Before the treatment period started, rats with 8 weeks of renovascular hypertension were compared with sham-operated controls using Student's *t*-test for unpaired data. At the end of the treatment period, all grouped data were compared by Kruskal–Wallis test (Statistica 6.0, StatSoft, Inc., Tulsa, OK, USA). For significant test results, Mann–Whitney *U*-test was applied as post-hoc test. Due to multiple comparisons ($n=7$: RHT-CTR vs. Sham-CTR; RHT-ZOF vs. RHT-CTR; RHT-NIF vs. RHT-CTR; RHT-LAB vs. RHT-CTR; RHT-ZOF vs. Sham-ZOF; RHT-NIF vs. Sham-NIF; RHT-LAB vs. Sham-LAB) levels of significance were required at $P<0.05/7$, i.e. $P<0.007$.

3. Results

3.1. Baseline evaluation

After 8 weeks of abdominal aortic banding with constriction of the right renal artery, atrophy of the right kidney, renovascular hypertension, LVH and myocardial fibrosis has been confirmed in four animals randomly picked from each treatment group (Table 2), i.e. arterial hypertension was present, and LVH and myocardial fibrosis were established before the treatment period of 12 weeks started. During the entire observation period of 20 weeks, five rats died in the RHT-CTR group, one in the NIF-RHT group, and two in the LAB-RHT group following the surgical procedure or due to premature, presumably stroke-related death. In addition, growth development of untreated rats with renovascular hypertension (RHT-CTR) was retarded compared with all other groups (Table 3).

Table 2
Baseline conditions[a]

	SAP (mmHg)	LV/BW (mg/g)	LVCVF (%)	RVCVF (%)
AAB	174±9*	2.29±0.06†	9.1±0.9†	12.8±0.7†
CON	135±2	1.77±0.08	3.6±0.3	6.8±0.7

[a] Values are means±S.E.M. AAB=8 weeks of abdominal aortic banding with constriction of the right renal artery ($n=4$); CON=sham-operated controls after 8 weeks ($n=4$). SAP, systolic arterial pressure; LV/BW, left ventricular weight normalized to body weight; LVCVF, RVCVF, left and right ventricular collagen volume fractions, respectively. * $P<0.05$, † $P<0.001$ vs. CON.

3.2. Arterial hypertension and left ventricular hypertrophy

At the end of the study, i.e. after 20 weeks, systolic arterial pressure (SAP) was significantly elevated ($P<0.001$) above sham-operated controls (125±5 mmHg) in untreated rats with abdominal aortic banding and constriction of the right renal artery (188±11 mmHg) (Fig. 1). In all treatment groups of rats with abdominal aortic banding and constriction of the right renal artery, blood pressure was normalized where no significant difference in SAP was found compared with sham-operated controls (RHT-ZOF: 136±6 mmHg; Sham-ZOF: 131±10 mmHg; RHT-NIF: 125±6 mmHg; Sham-NIF: 145±5 mmHg; RHT-LAB: 113±11 mmHg; and Sham-LAB: 120±8 mmHg).

Table 3
Body and ventricular weights[a]

	BW (g)	LVW (mg)	RVW (mg)	RV/BW (mg/g)
RHT-CTR	416±8*	1080±42	232±13	0.53±0.02
Sham-CTR	687±21	1250±43	320±9	0.47±0.02
RHT-ZOF	581±12	1118±78	263±18	0.42±0.02
Sham-ZOF	611±16	1075±49	262±10	0.43±0.03
RHT-NIF	550±10	755±69	213±36	0.39±0.04
Sham-NIF	618±16	1178±70	283±10	0.46±0.02
RHT-LAB	608±16	1086±109	278±15	0.46±0.02
Sham-LAB	731±37	1319±74	316±15	0.43±0.01

[a] Values are means±S.E.M. BW, body weight; LVW, left ventricular weight; RVW, right ventricular weight; RV/BW, right ventricular weight normalized to body weight; for abbreviations of experimental groups see Table 1. * $P<0.0005$ vs. Sham-CTR.

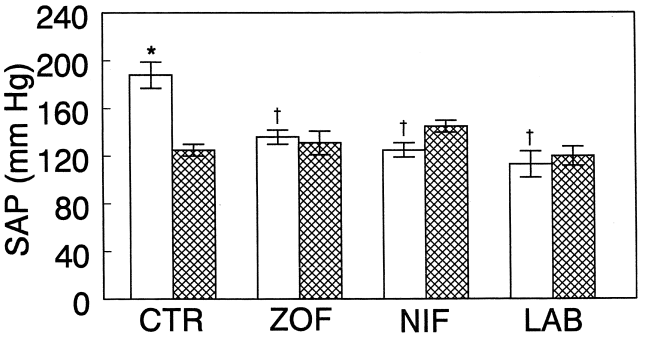

Fig. 1. Systolic arterial pressure (SAP) in 28-week-old Sprague–Dawley rats for the different experimental groups: CTR=no treatment; ZOF= after 8 weeks of renovascular hypertension, treatment with 6 mg/kg/day zofenopril for 12 weeks; NIF=after 8 weeks of renovascular hypertension, treatment with 30 mg/kg/day nifedipine for 12 weeks; and LAB= after 8 weeks of renovascular hypertension, treatment with 40 mg/kg/day labetalol for 12 weeks. Open bars: rats with abdominal aortic banding and constriction of the right renal artery for 20 weeks (RHT); cross-hatched bars: sham-operated rats (Sham). SAP was significantly elevated in untreated rats with abdominal aortic banding and constriction of the right renal artery, and was normalized in all treatment groups. * $P<0.001$ vs. Sham-CTR; † $P<0.005$ vs. RHT-CTR.

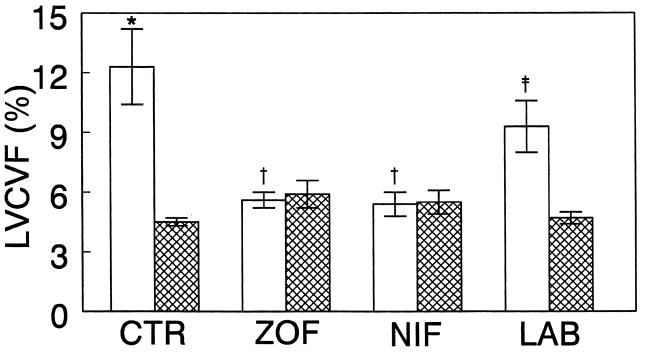

Fig. 3. Left ventricular collagen volume fraction (LVCVF) in 28-week-old Sprague–Dawley rats for the different experimental groups: for abbreviations and bar assignment see legend of Fig. 1. LVCVF was significantly elevated in untreated rats with abdominal aortic banding and constriction of the right renal artery. It was normalized in rats treated with either zofenopril or nifedipine. In contrast, no regression of myocardial fibrosis of the left ventricle was found in rats treated with labetalol. * $P<0.0005$ vs. Sham-CTR; † $P<0.005$ vs. RHT-CTR; ‡ $P<0.007$ vs. Sham-LAB.

Associated with renovascular hypertension, LVH was present in untreated rats with abdominal aortic banding and constriction of the right renal artery, measured by a significantly ($P<0.00001$) elevated LV/BW ratio (2.61±0.12 mg/g) above sham-operated controls (1.82±0.03 mg/g) (Fig. 2). In all groups of rats with abdominal aortic banding and constriction of the right renal artery and antihypertensive treatment, LV/BW ratio was normalized where no significant difference remained in comparison to sham-operated controls (RHT-ZOF: 1.80±0.06 mg/g; Sham-ZOF: 1.76±0.06 mg/g; RHT-NIF: 1.74±0.05 mg/g; Sham-NIF: 1.91±0.10 mg/g; RHT-LAB: 1.78±0.14 mg/g; and Sham-LAB: 1.81±0.05 mg/g). Due to significant differences of body weight among experimental groups absolute values of LV weight could not be compared. RV weight normalized to body weight did not show significant differences among groups (Table 3).

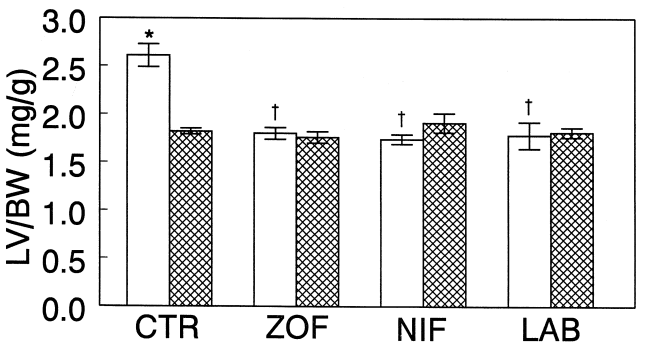

Fig. 2. Left ventricular weight normalized to body weight (LV/BW) in 28-week-old Sprague–Dawley rats for the different experimental groups: for abbreviations and bar assignment see legend of Fig. 1. LV/BW was significantly elevated in untreated rats with abdominal aortic banding and constriction of the right renal artery, and was normalized in all treatment groups. * $P<0.00001$ vs. Sham-CTR; † $P<0.002$ vs. RHT-CTR.

3.3. Myocardial fibrosis

In untreated rats with renovascular hypertension, myocardial fibrosis was present in the pressure overloaded, hypertrophied left and normotensive right ventricle (Figs. 3 and 4). LVCVF (12.3±1.9%) and RVCVF (20.6±2.5%) were each significantly ($P<0.0005$) elevated compared with untreated sham-operated controls (LVCVF:

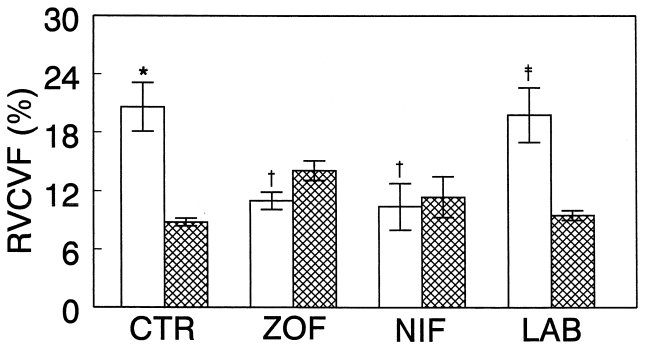

Fig. 4. Right ventricular collagen volume fraction (RVCVF) in 28-week-old Sprague–Dawley rats for the different experimental groups: for abbreviations and bar assignment see legend of Fig. 1. RVCVF was significantly elevated in untreated rats with abdominal aortic banding and constriction of the right renal artery. It was normalized in rats treated with either zofenopril or nifedipine. In contrast, no regression of myocardial fibrosis of the right ventricle was found in rats treated with labetalol. * $P<0.00005$ vs. Sham-CTR; † $P<0.007$ vs. RHT-CTR; ‡ $P<0.007$ vs. Sham-LAB.

4.5±0.2%; RVCVF: 8.8±0.4%). In rats with abdominal aortic banding and constriction of the right renal artery treated with either zofenopril or nifedipine, myocardial fibrosis was completely regressed in both ventricles ($P<$ 0.007 vs. RHT-CTR). In RHT-ZOF and RHT-NIF groups, LVCVF was 5.6±0.4 and 5.4±0.6%, respectively. In these groups, LVCVF was not significantly different from their sham-operated controls (Sham-ZOF: 5.9±0.7%; Sham-NIF: 5.5±0.6%). The same was true for RVCVF. In RHT-ZOF and RHT-NIF groups, RVCVF was 11.0±0.9 and 10.4±2.4%, respectively (Sham-ZOF: 14.1±1.0%; Sham-NIF: 11.4±2.1%). In contrast, in rats with abdominal aortic banding and constriction of the right renal artery treated with labetalol, myocardial fibrosis was present in both ventricles irrespective of blood pressure normalization and absence of LVH, and where no significant difference was found compared with untreated rats with renovascular hypertension. LVCVF and RVCVF were 9.3±1.3 and 19.8±2.8%, respectively ($P<0.007$ vs. Sham-LAB where LVCVF and RVCVF were 4.7±0.3 and 9.5±0.5%, respectively).

4. Discussion

According to the Framingham heart study LVH is associated with all major adverse cardiovascular events, especially the appearance of symptomatic heart failure where arterial hypertension is still one of the most important etiologic factors [13]. Morphologic studies indicated that the increment in myocardial mass in LVH is primarily related to an increase in myocyte size [14]. However, a structural remodeling of the myocardial collagen matrix may also be involved depending on the nature of the hypertrophic stimulus. For example, in man [15–18], as well as in various experimental and genetic models of arterial hypertension [1–10,12], an interstitial and perivascular myocardial fibrosis has been observed. On the other hand, myocardial fibrosis was not seen in the hypertrophy associated with athletic training [19] or that accompanies anemia [20], chronic volume overload [21,22], or hyperthyroidism [23]. These findings suggest that the growth of cellular constituents of the various myocardial tissue compartments, namely cardiac myocytes and fibroblasts, which are responsible for myocardial collagen metabolism, may each have different regulatory mechanisms. Differences in the growth of fibroblasts and their synthesis of collagen, relative to myocyte hypertrophy, may lead to intercompartmental disequilibrium and thereby pathologic hypertrophy [24].

In arterial hypertension, the type of myocardial fibrosis is termed reactive fibrosis (versus reparative fibrosis or scarring), because it is not secondary due to myocyte necrosis [5]. At 8 weeks of renovascular hypertension in the rat and in comparison to age/sex matched controls, diffuse perivascular and interstitial fibrosis leads to a threefold increase in collagen volume fraction of the hypertrophied left ventricle and a twofold increase in the normotensive right ventricle [6]. In the present study, untreated rats with abdominal aortic banding and constriction of the right renal artery for 8 weeks, i.e. the same 2-kidney/1-clip model of renovascular hypertension as previously used [5,6,10], showed a 2.5-fold increase in LVCVF and a twofold rise in RVCVF compared with normotensive sham-operated controls. In previous experimental studies, it has been demonstrated that the development of myocardial fibrosis and not muscle mass per se is the major determinant of myocardial diastolic stiffness in hypertensive heart disease [8] that leads to LV diastolic and ultimately to LV systolic dysfunction and the appearance of heart failure [9,25–27]. Conversely, no sign of diastolic dysfunction of the left ventricle has been reported in forms of LVH when myocardial fibrosis is absent [19–23]. Therefore, regression of myocardial fibrosis or reversal of cardiac organ damage appears to be a primary treatment goal in arterial hypertension.

This study sought to distinguish the relative importance of different antihypertensive drugs in regressing myocardial fibrosis of rats having experimental arterial hypertension. Since the coronary circulation supports both the right and left heart, circulating factors responsible for myocardial fibrosis would reach each ventricle based on this in-parallel arrangement while the importance of elevated arterial and LV systolic pressures could be distinguished by the in-series arrangement of the ventricles. Therefore, we analyzed the fibrous tissue response in both the left and right ventricles in response to various antihypertensive regimens. Our results indicate the heterogeneity of changes in myocardial structure depending on the utilized antihypertensive therapy. Myocyte growth and myocardial hypertrophy was most closely related to elevations in myocyte loading created by a rise in LV afterload. LVH was present when arterial pressure was chronically elevated and was absent when blood pressure was normalized as found in all treatment groups irrespective of the utilized antihypertensive agent. Cooper et al. [28] have previously reported that myocyte loading is the major determinant of hypertrophy in the intact animal. In the in vitro heart, Schreiber et al. [29] 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 [30].

With renovascular hypertension diffuse myocardial fibrosis was observed in the pressure overloaded, hypertrophied left ventricle. This remodeling was also found in the nonoverloaded, nonhypertrophied right ventricle. We selected entire coronal sections at the equator of the left and right ventricles for LVCVF and RVCVF measurements, respectively, 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 [15]

and that there exists a good correlation between hydroxyproline concentration and collagen volume fraction of the left ventricle [12]. At birth, the collagen concentration of the ventricles are 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 the left ventricle [31]. Therefore, given its smaller sized myocytes, the accumulation of collagen within the right ventricle in renovascular hypertension was greater than the left ventricle.

In rats with abdominal aortic banding and constriction of the right renal artery treated with the ACE inhibitor zofenopril, no myocardial fibrosis was present neither in the left nor in the right ventricle. In previous studies on SHR, the ACE inhibitor lisinopril was able to regress myocardial fibrosis in early and advanced hypertensive heart disease associated with improvement of LV diastolic dysfunction and prevention of LV systolic dysfunction [7–9]. Following this pioneering work broad evidence has been provided by many studies that ACE inhibitors, such as captopril, enalapril and trandolapril, regress myocardial fibrosis in SHR associated with reduction of ventricular arrhythmias and improvement of myocardial function [32–34]. Finally, those experimental findings could be confirmed in patients with hypertensive heart disease due to primary hypertension where the ACE inhibitor lisinopril proved to regress myocardial fibrosis along with improvement of LV diastolic dysfunction [35,36]. The mechanism by which chronic ACE inhibition might decrease myocardial collagen concentration has been partly addressed in previous studies. Any removal of collagen from the cardiac interstitium can be achieved either by inhibition of collagen synthesis or by enhanced collagen degradation. At the cellular level, we have shown that AngII significantly stimulates collagen synthesis in cultured adult rat cardiac fibroblasts in a dose-dependent manner [37] that has been confirmed in several laboratories [38–40]. In in vivo experiments on rats with abdominal aorta banding and chronic ACE inhibition, Linz et al. [41] found decreased myocardial mRNA expression for types I and III collagens, the major fibrillar collagens in the myocardium. Furthermore, we have found that AngII significantly inhibits matrix metalloproteinase (MMP)-1 activity in cultured adult rat cardiac fibroblasts [37], where MMP-1 is the key enzyme of interstitial collagen degradation. In vivo in SHR, myocardial MMP-1 activity was increased with oral lisinopril treatment [9]. Likewise, in patients with primary hypertension, treatment with lisinopril resulted in increased serum concentrations of MMP-1 [42].

In rats with abdominal aortic banding and constriction of the right renal artery treated with the Ca^{2+}-channel-blocker nifedipine, myocardial fibrosis could be also regressed. This is in accordance with results of others who used nifedipine, amlodipine or nisoldipine in SHR or stroke-prone SHR regression trials [43–46]. The mechanism by which Ca^{2+}-channel-blocking agents might decrease myocardial collagen concentration are largely unexplored. However, it is intriguing that AngII appears to use Ca^{2+} as second messenger in cardiac fibroblasts leading to enhanced collagen synthesis [47] that might be compromised by blockade of Ca^{2+} inflow. In vivo studies with the AngII type 1 (AT_1) receptor antagonists losartan and candesartan have clearly shown that myocardial fibrosis can be attenuated by AT_1 receptor antagonism in SHR [48,49]. In rats receiving AngII, treatment with the Ca^{2+}-channel-blocking agent mibefradil was able to prevent AngII-mediated myocardial fibrosis [50]. Preliminary results of our ongoing cell culture studies show that the Ca^{2+}-channel-blocker verapamil attenuates the AngII-mediated increase in intracellular Ca^{2+} of adult cardiac fibroblasts and thereby abolishes an AngII-mediated increase in collagen synthesis. These findings may explain the beneficial effects of Ca^{2+}-channel-blockers on myocardial fibrosis in the present study although RAAS is activated in rats with abdominal aortic banding and constriction of the right renal artery.

Other antihypertensive drugs, e.g. minoxidil [51], α-methyldopa [52], hydrochlorothiazide [53] or hydralazine [49,54], have failed to show any significant effect on regression of myocardial fibrosis or even increased myocardial collagen concentration in experimental models of chronic LV pressure overload [51] and where minoxidil accelerated the development of heart failure [55]. The lack of antifibrotic effects appears to be also true for antiadrenergic agents. In this study, we utilized labetalol in order to fully antagonize the adrenergic system by blockade of both β- and α-adrenergic receptors. Using this antihypertensive drug, we were not able to regress myocardial fibrosis neither in the left nor in the right ventricle although blood pressure was controlled as well as with the other antihypertensive agents and although LVH could be regressed. This is in accordance with findings in SHR and rats with renovascular hypertension, where treatment with the β-adrenergic receptor blocker atenolol resulted in an adverse remodeling of the left ventricle with increased hydroxyproline concentration in dilated hearts [56]. Indeed, in cultured human fetal lung fibroblasts, the β-adrenergic receptor agonists epinephrine and isoproterenol suppressed collagen synthesis normalized to total protein production in a dose-dependent manner that could be abolished by the β-receptor antagonist propranolol [57]. In contrast, norepinephrine has been found to be a mitogenic stimulus for cultured fibroblasts [58]. However, its release into the cardiac interstitium is related to ventricular systolic pressure [59,60] and may be, therefore, not involved in the development of myocardial fibrosis irrespective of blood and ventricular pressures. In normotensive male Sprague–Dawley rats, atenolol even promoted the development of myocardial fibrosis [61]. In contrast, Ooshima and co-workers [62,63] have found that reserpine, an agent which depletes tissue norepinephrine, and/or hypophysectomy reduced collagen synthesis in both the

myocardium and systemic vasculature of normotensive rats or rats with deoxycorticosterone/saline hypertension. Likewise, in stroke-prone SHR, the antisympathotonic agent moxonidine was able to regress myocardial fibrosis [44].

Disproportionate growth between myocyte and nonmyocyte cells sets the stage for abnormal myocardial function. Recognition of trophic factors that regulate the growth of these different cell lines, and which appear to be quite different, may permit the development of pharmacologic strategies that would regress LVH and adverse structural changes of the cardiac interstitium, i.e. myocardial fibrosis. Based on the diverse effects of antihypertensive agents on the various tissue compartments in the heart, three classes of antihypertensive agents may be considered: (a) drugs with no effects on LVH and fibrosis (direct vasodilators); (b) drugs with clear effects on LVH, i.e. myocyte regression (diuretics, α- and β-adrenergic receptor antagonists); and (c) agents with proven effects on regression of LVH and fibrosis (ACE inhibitors, AT_1 receptor antagonists, Ca^{2+}-channel-blockers, and centrally acting antiadrenergic agents). In addition, aldosterone receptor antagonists may prove valuable to regress myocardial fibrosis where at least prevention trials provided evidence for their antifibrotic effects [10,64,65]. Removal of reactive myocardial fibrosis represents a means by which myocardial failure due to collagen accumulation would be reversible. Agents that would restore myocardial structure to normal and thereby alleviate abnormalities in myocardial stiffness would have cardioreparative properties. The findings of the present study indicate that not all antihypertensive agents are associated with an involution in collagen mass. The opportunity is at hand to determine which antihypertensive agents have antifibrotic effects and are cardiovascular tissue specific. In so doing such agents may have the potential to restore myocardial structure and function to normal and eliminate pathologic hypertrophy as a major determinant of myocardial failure.

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