Aldosterone Receptor Blockade Prevents the Transition to Cardiac Pump Dysfunction Induced by β-Adrenoreceptor Activation

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Abstract—The transition from compensated to decompensated left ventricular hypertrophy (LVH) in hypertension involves excessive β-adrenoreceptor (β-AR) stimulation. To explore whether aldosterone receptor activation contributes toward β-AR–induced left ventricular (LV) decompensation in hypertensive LVH, the effect of spironolactone (SPIRO; 80 mg · kg−1 · day−1) on LV cavity dimensions, function, and chamber remodeling mechanisms was evaluated in spontaneously hypertensive rats (SHR) receiving a low dose of the β-AR agonist isoproterenol (ISO) at 0.02 to 0.04 mg · kg−1 · day−1 for 4.5 months. ISO administered to SHR resulted in an increased 24-hour urinary aldosterone excretion and LV cavity dimensions, a right shift in LV diastolic pressure-volume relations, a decreased LV relative wall thickness, and increased total, noncross-linked, type I and type III myocardial collagen concentrations without further enhancing increased myocardial norepinephrine (NE) release. ISO reduced pump function without modifying intrinsic myocardial systolic function or inducing further myocyte necrosis or apoptosis. ISO only increased LV cavity volumes after prolonged periods of administration. SPIRO abolished ISO-induced chamber dilation, wall thinning, and pump dysfunction and reduced total, noncross-linked, type I and type III myocardial collagen concentrations but failed to modify blood pressure, volume preloads, intrinsic myocardial systolic function, myocardial NE release, or the degree of necrosis or apoptosis. In conclusion, these results suggest that aldosterone receptor blockade, through load-independent effects, may be useful in preventing the transition from compensated LVH to dilatation and pump dysfunction mediated by chronic β-AR activation. (Hypertension. 2005;45:914-920.)

Key Words: remodeling ■ collagen ■ sympathetic nervous system ■ aldosterone

There is substantial evidence to indicate that in hypertension, the transition from compensated left ventricular hypertrophy (LVH) to heart failure involves chronic β-adrenoreceptor (β-AR) activation. Indeed, in LVH, increased circulating norepinephrine (NE) concentrations1,2 as well as myocardial NE release3 precede heart failure. Moreover, in hypertensive LVH, prolonged β-AR agonist administration4 or genetically enhanced sympathetic effects5 increase the susceptibility to decompensation without blood pressure (BP) changes. Despite data to support a notion that β-AR activation induces the transition to heart failure in hypertensive LVH, whether the renin-angiotensin-aldosterone system (RAAS) mediates this effect is unknown. This question arises because β-AR stimulation provokes the RAAS, β-AR blockers inhibit the RAAS in hypertension,6 and aldosterone receptor antagonists attenuate left ventricular (LV) dilatation.7–9 Because β-AR blockers have a limited impact on LVH10 and increase the chance of new onset diabetes mellitus,11 identification of downstream targets from β-ARs responsible for LV decompensation that modify LVH but not metabolic pathways is desirable. Therefore, in the present study, we explored the role of aldosterone receptors as mediators of β-AR–induced decompensation in hypertensive LVH and the mechanisms thereof. For this purpose, the spontaneously hypertensive rat (SHR) was selected as a model of compensated LVH susceptible to β-adrenergic–induced LV decompensation4 and the impact of an aldosterone receptor antagonist on β-AR–mediated LV dilatation and pump dysfunction in SHR examined.

Methods

The present study was approved by the animal ethics screening committee of the University of the Witwatersrand (Approval numbers 99:01:2b, 2002:37:5, and 2002:39:5).
Groups, Treatment Regimen, and BP

SHR were studied over a time period (14 to 18.5 months of age) before that when LV decompensation occurs.\textsuperscript{12} SHR were assigned to groups that received no treatment; twice daily intraperitoneal injections of the β-AR agonist isoproterenol (ISO; Imuprel, Adcock Ingram; 0.02 mg · kg \(^{-1}\) · day \(^{-1}\); injection 1; \(\text{0.2 mL}\)) and daily spironolactone (SPIRO; 80 mg · kg \(^{-1}\) · day \(^{-1}\) suspended in a gelatin-meat extract cube), an aldosterone receptor antagonist; or ISO and SPIRO. Age-matched Wistar-Kyoto (WKY) control rats received no treatment because ISO has little effect on LV geometry and function in this rat strain.\textsuperscript{4} The model of LV dilatation studied has the advantage that in it is not associated with death related to heart failure when studied up to 5 months from the time that ISO is initiated\textsuperscript{4} and hence allows for a clear interpretation of cardiac remodeling end points. In the present study, noninvasive systolic BP was assessed on 3 separate occasions in each group\textsuperscript{12} and echocardiography performed at the end of the study as described below. The dose of SPIRO was selected from the outcome of a pilot study conducted in 14-month-old SHR in which SPIRO given orally at 80 mg · kg \(^{-1}\) · day \(^{-1}\) was compared with 1 or 2.5 mg · kg \(^{-1}\) · day \(^{-1}\) for 2 months decreased LV weight (in grams; SHR [\(n=7\)] = 1.13 ± 0.04; SHR + SPIRO [\(n=8\)] = 0.98 ± 0.02; WKY [\(n=8\)] = 1.00 ± 0.02; \(P<0.01\)) without reducing mean carotid arterial pressure (in mm Hg; SHR = 118 ± 2.6; SHR + SPIRO = 124 ± 4; WKY = 91 ± 2; \(P<0.01\)). Because aldosterone receptor blockade could modify LV cavity size through effects on myocardial NE release\textsuperscript{6} or through alterations in blood volume, we used a different series of SHR with β-AR–mediated LV dilatation to determine whether aldosterone receptor blockade produced short-term actions on myocardial NE release or LV cavity dimensions. Thus, once clear evidence of LV dilatation was noted on echocardiography in SHR receiving ISO (4.5 months), rats were assigned to be given daily SPIRO or no treatment 2 weeks before termination of the study. At 5 months of ISO injections, LV cavity dimensions and myocardial NE release were measured.

Urinary Aldosterone Excretion Rates

Urinary aldosterone excretion rates were determined after 2 months of daily ISO administration, when normal LV dimensions were documented on echocardiography. Urine was collected via metabolic chambers over 2 consecutive 24-hour periods in untreated SHR (\(n=8\)) and SHR receiving ISO (\(n=8\)) after rats had received a 72-hour adaptation period. Urine was acidic with glacial acetic acid (3 μL/30 mL urine), stored at −20°C for 2 weeks, and aldosterone concentration measured using a commercially available radioimmunoassay (Diagnostic Products). Because urinary aldosterone excretion rates are unaltered in SHR,\textsuperscript{13} comparisons between untreated SHR and WKY controls were not made.

Echocardiography

To assess LV dilatation and pump function in intact animals, 2D targeted M-mode echocardiography was performed using a 7.5-MHz transducer and a Hewlett Packard Sonos 2500 sector scanner.\textsuperscript{14–16} LV dimensions and posterior wall thickness at end diastole and end systole were measured and LV endocardial (fractional shortening [FS\(_{\text{em}}\)]) and midwall FS (FS\(_{\text{mwm}}\)) calculated to determine chamber and myocardial systolic function, respectively.\textsuperscript{14,16}

Isolated Perfused Hearts

Because LV dimensions and systolic function are influenced by loading conditions, LV remodeling and function were also determined ex vivo under controlled conditions. Hearts were removed, mounted on a perfusion apparatus, and retrogradely perfused at a constant flow (12 mL · min \(^{-1}\) · g wet heart weight).\textsuperscript{4,14,15} LV developed and diastolic pressures were measured via a balloon inserted into the left ventricle through the mitral valve. LV pressures were assessed at 0.01 mL increments in volume injected into the balloon via a micromanipulator. LV pressures over a range of volumes were initially measured in the absence of an isotropic stimulus, and then after exposure of the heart to 10\(^{-7}\) M ISO added to the perfusate to assess adrenergic–inotropic reserve. LV remodeling was determined by constructing LV diastolic pressure-volume (P-V) relations and comparing LV volumes obtained at a filling pressure of 0 mm Hg (LV \(V_0\)).\textsuperscript{4,14,15} Load-independent measures of chamber and intrinsic myocardial systolic function were assessed by constructing developing P-V and stress–strain relations and comparing the slopes of these relations (E and En, respectively).\textsuperscript{4,14} Developed stress and strain were calculated as described previously.\textsuperscript{4,14}

LV Dimensions at Controlled Filling Pressures

Because LV dilatation is thought to mediate pump dysfunction through reductions in wall thickness-to-radius ratios (h/r), h/r was determined over a range of filling pressures in vivo immediately before mounting hearts on a perfusion apparatus. To achieve this aim, LV end-diastolic pressures (LVEDPs) and short-axis external diameters were measured over a range of LVEDP values in anesthetized, ventilated, open-chest rats using techniques described and validated previously.\textsuperscript{3,12,17,18} LVEDP was determined via a fluid-filled catheter with amplitude-frequency responses uniform to 10 Hz inserted through the apex. LV short-axis diameter was measured using piezoelectric ultrasonic transducers placed across the short axis via a cradle designed in our laboratory.\textsuperscript{17,18} Filling volumes were modified by use of an iso-oncotic, isotonic solution as well as by inferior vena cava occlusion.\textsuperscript{4,11,17,18} Frequent extrasystolic beats below an LVEDP of 2 mm Hg prevented the collection of data over this pressure range. LVEDP \(r\) and LVEDP \(h\) were determined from previously described formulas\textsuperscript{17,18} and LVEDP–LVEDP \(r\) and LVEDP–P–LVEDP \(h\) relations constructed.

Myocardial NE

Before assessing function in isolated, perfused hearts, a sample of coronary effluent was collected for 1 minute in prechilled containers at a constant coronary flow (12 mL · min \(^{-1}\) · g wet heart weight). Because NE release decreased when measured at incremental LV filling volumes, all measurements were performed at 0.23 mL · Coronary effluent was stabilized with Na\(_2\)EDTA and HClO\(_4\) (0.01 mol · L \(^{-1}\) and 0.025%, respectively). NE was immediately extracted from 1 mL of coronary effluent using alumina (Sigma) adsorption with a Tris buffer at pH 8.6 eluted with 0.1 mol/L HClO\(_4\),\textsuperscript{19} stored at −70°C, and concentrations determined using reversed-phase, ion-exchange high performance liquid chromatography with electrochemical detection.\textsuperscript{19} Because all hearts were perfused at the same flow rates per gram of tissue, myocardial NE release was expressed as the concentration of NE in the effluent.

Myocardial Collagen

Samples of LV tissue were weighed and stored at −70°C before tissue analysis. Myocardial hydroxyproline concentration ([HPRO]) was determined after acid (HCl) hydrolysis.\textsuperscript{4,12,14,15,17} Myocardial collagen was also extracted and digested with cyanogen bromide (CNBr).\textsuperscript{4,12,15,17} A portion of the CNBr-digested collagen sample was subjected to acid hydrolysis and [HPRO] determination. The amounts of noncross-linked (soluble) and cross-linked (insoluble) collagen in the myocardium were ascertained based on the solubility of myocardial collagen to CNBr digestion.\textsuperscript{4,12,15,17} Using the remaining portion of the CNBr-digested sample, polyacrylamide gel electrophoresis was subsequently performed on vertical gels by stacking and separating gel concentrations of 3% and 12.5%, respectively, and the type I-to-type III collagen ratio was determined after gel scanning.\textsuperscript{12,15,17} Myocardial type I and III collagen concentrations were assessed from type I-to-type III ratios and the [HPRO] in myocardial tissue.\textsuperscript{12,15,17}

Myocyte Necrosis and Apoptosis

Before storing tissue for biochemical assessment, a longitudinal slice of the LV from the apex to the base through the LV free wall was obtained from all rats for histology. LV tissue was stored, prepared, and sectioned as described previously.\textsuperscript{3,12} After staining sections with van Gieson’s stain, a pathological grade was assigned in which 0 indicates no damage; 1 and 2, patchy fibrosis in 20% or >20% of the field, respectively; and 3 and 4, diffuse contiguous subendocardial fibrosis in <50% or >50% of the field, respectively; and 5 and 6,
full thickness fibrosis in <50\% or >50\% of the field, respectively.\textsuperscript{12,13} The degree of apoptosis was quantified on myocardial tissue sections obtained from the same tissue blocks used to assess the pathological score. For each tissue block, 5-\mu \text{m}-thick sections were stained and evaluated. Nuclear DNA fragments in the tissue sections were detected using a nonradioactive in situ apoptotic cell death detection kit (DeadEnd Colorimetric TUNEL system; Promega), where terminal deoxynucleotidyl transferase (TdT) was used to incorporate biotinylated nucleotide at the 3'-OH DNA ends. Horseradish peroxidase-labeled streptavidin binds to biotinylated nucleotides, which subsequently stain dark brown in response to hydrogen peroxide and diaminobenzidine.\textsuperscript{20} Positive (DNase-treated) and negative (no addition of TdT) control tissue sections were incorporated in each assay. The number of apoptotic cardiomyocyte nuclei and the total number of cardiomyocyte nuclei (hematoxylin and eosin stain) in each slide were counted on 10 evenly spaced fields from the apex to the base using a computer-based image acquisition and analysis system at \times 400 magnification (Axiovision 3; Carl Zeiss). Apoptotic cardiomyocyte nuclei were expressed as a percentage of the total number of cardiomyocyte nuclei.

**Data Analysis**

Regression analysis was used to determine the lines of best fit for the cardiac function and other relations. Differences between groups were assessed by a 1-factor ANOVA followed by a Tukey post hoc test. All values in the text are represented as mean\pm SEM.

**Results**

**BP, Heart Weight, and Urinary Aldosterone Excretion Rates**

Neither ISO nor SPIRO administration significantly modified the increased systolic BP noted in SHR (eg, at 18 months of age in mm Hg: SHR untreated=182\pm 8; SHR+ISO=178\pm 4; SHR+ISO+SPIRO=168\pm 8; SHR+SPIRO=164\pm 12; WKY=136\pm 6; \(P<0.001\) for WKY versus other groups). However, ISO enhanced and SPIRO decreased LV weight in SHR and SPIRO prevented ISO-induced augmentation of LVH (Table 1). ISO administration increased 24-hour urinary aldosterone excretion rates in SHR (in pg \cdot \text{g}^{-1} \cdot \text{body weight} \cdot \text{day}^{-1}): SHR=49\pm 2; SHR+ISO=66\pm 6; \(P<0.03\)).

**Chamber Dimensions in Intact Rats**

ISO administration increased LV end-diastolic diameter (LVEDD) and LV end-systolic diameters in SHR, an effect that was prevented by SPIRO (Figure 1; Table 1). Although ISO augmented LV weight (Table 1), as a consequence of increases in LVEDD, ISO failed to significantly modify LV wall thickness (Table 1). In contrast to the ability of SPIRO, when initiated at the beginning of the study, to prevent \(\beta\)-AR-induced LV dilatation, 2 weeks of SPIRO administration, once LVEDD had increased, failed to attenuate these changes (in cm: SHR \(n=18\)=0.70\pm 0.02; SHR+ISO \(n=10\)=0.82\pm 0.02; SHR+ISO+SPIRO \(n=9\)=0.79\pm 0.02; \(P<0.05\) versus untreated SHR).

**Chamber Remodeling**

As assessed in isolated, perfused heart preparations, ISO administered to SHR for 4.5 months resulted in a right shift in LV diastolic P-V relations and an increased LV \(V_0\) (Figure 2; Table 1). These effects were prevented by SPIRO (Figure 2; Table 1). Similarly, as assessed in vivo, ISO administered to SHR resulted in increases in LVEDr noted over a range of LVEDPs (\(P<0.01\)), an effect abolished by the coadministration of SPIRO (data not shown).

Consistent with concentric LVH, untreated SHR at 18.5 months of age had an increased LVEDh/r (relative wall thicknesses) compared with age-matched WKY controls (data not shown). As a consequence of LV dilatation, ISO administered to SHR resulted in relative wall thinning, with marked decreases in LVEDh/r noted over a range of LVEDPs (\(P<0.01\)), an effect abolished by the coadministration of SPIRO (data not shown).

**Systolic Function**

ISO administered to SHR decreased systolic chamber function as assessed in vivo (Figure 1; LV FS\textsubscript{end}) and ex vivo (Figure 3, systolic P-V relations; Table 1, slope of these relations [E] in the absence of an inotropic stimulus), an effect prevented by the coadministration of SPIRO (Figures 1 and 3; Table 1). In contrast, neither ISO nor SPIRO modified intrinsic myocardial systolic function as assessed either in vivo (Figure 1, LV FS\textsubscript{end}) or ex vivo (Figure 3, systolic stress–strain relations; Table 1, slope of these relations [E] in the absence of an inotropic stimulus). Similar data were obtained when systolic chamber and myocardial function were assessed in the presence of 10\textsuperscript{-7} M ISO (data not shown).

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**TABLE 1. Effect of Chronic Administration of ISO and SPIRO on LV Weight, Dimensions, and Systolic Function in SHR**

<table>
<thead>
<tr>
<th></th>
<th>WKY (n=9)</th>
<th>SHR (n=9)</th>
<th>SHR+ISO (n=9)</th>
<th>SHR+SPIRO (n=6)</th>
<th>SHR+ISO+SPIRO (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV weight (g)</td>
<td>1.08\pm 0.04</td>
<td>1.32\pm 0.06\dagger</td>
<td>1.56\pm 0.09\ddagger</td>
<td>1.18\pm 0.05</td>
<td>1.32\pm 0.04\dagger</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>438 \pm 12</td>
<td>359 \pm 6\dagger</td>
<td>353 \pm 10\dagger</td>
<td>352 \pm 12\dagger</td>
<td>355 \pm 7\dagger</td>
</tr>
<tr>
<td>LV/body weight \times 10\textsuperscript{4}</td>
<td>2.45 \pm 0.14</td>
<td>3.66 \pm 0.14\dagger</td>
<td>4.41 \pm 0.34\ddagger</td>
<td>3.35 \pm 0.10\dagger</td>
<td>3.65 \pm 0.11\dagger</td>
</tr>
<tr>
<td>LV end systolic diameter (cm)</td>
<td>0.24 \pm 0.03</td>
<td>0.26 \pm 0.03</td>
<td>0.39 \pm 0.02\ddagger</td>
<td>0.24 \pm 0.05</td>
<td>0.25 \pm 0.03</td>
</tr>
<tr>
<td>LV posterior wall thickness (cm)</td>
<td>0.19 \pm 0.01</td>
<td>0.28 \pm 0.01\dagger</td>
<td>0.21 \pm 0.02\ddagger</td>
<td>0.24 \pm 0.03*</td>
<td>0.29 \pm 0.03\dagger</td>
</tr>
<tr>
<td>LV V\textsubscript{0} (mL)</td>
<td>0.19 \pm 0.01</td>
<td>0.20 \pm 0.01</td>
<td>0.28 \pm 0.02\ddagger</td>
<td>0.19 \pm 0.01</td>
<td>0.19 \pm 0.01</td>
</tr>
<tr>
<td>LV E (mm Hg \cdot mL\textsuperscript{-1})</td>
<td>1650 \pm 170</td>
<td>1670 \pm 109</td>
<td>910 \pm 56\dagger</td>
<td>1352 \pm 88</td>
<td>1285 \pm 93</td>
</tr>
<tr>
<td>LV En (g \cdot cm\textsuperscript{-2})</td>
<td>896 \pm 34</td>
<td>961 \pm 14</td>
<td>836 \pm 27</td>
<td>955 \pm 28</td>
<td>950 \pm 32</td>
</tr>
</tbody>
</table>

\# indicates at end diastole; LV V\textsubscript{0}, volume intercepts of LV diastolic pressure–volume relations (Figure 2); E, end-systolic chamber elastance; En, end-systolic myocardial elastance.

\*\(P<0.05\); \dagger\(P<0.01\) vs WKY group; \ddagger\(P<0.01\) vs other SHR groups.
Myocardial Collagen

Compared with WKY, myocardial [HPRO] was greater in untreated SHR (Figure 4). However, because of a reduction in collagen solubility (increased cross-linking) in SHR, only insoluble (cross-linked) collagen concentrations were increased (Figure 4). ISO administered to SHR potentiated the increment in myocardial [HPRO] and increased the solubility of collagen (decreased cross-linking) to WKY values (Figure 4). ISO increased soluble but not insoluble collagen concentrations (Figure 4). SPIRO coadministration prevented ISO-mediated increases on myocardial [HPRO], collagen solubility, and soluble collagen concentrations (Figure 4). The ratios of myocardial type I-to-type III collagen ratios of all groups of SHR were similar compared with WKY, although type I and type III concentrations were increased (Table 2). ISO augmented the increments in type I and type III collagen concentrations in SHR, an effect prevented by SPIRO (Table 2).

Regression analysis in data obtained for all SHR demonstrated significant relationships between myocardial soluble (noncross-linked) collagen concentrations and LV $V_0$ ($r=0.53; P<0.01$); soluble collagen and LVED r (eg, at 2 mm Hg: $r=0.41; P<0.05$); total collagen and LV $V_0$ ($r=0.45; P<0.02$); and total collagen and LVED r (eg, at 2 mm Hg: $r=0.38; P<0.05$).

Pathological Score and Apoptosis

A trend for an increase in pathological score occurred in untreated SHR compared with WKY controls, an effect that
In the present study, SPIRO prevented dial NE release (in nmol SHR at 14 months of age had a marked increase in myocardial NE release, a modification consistent with those reported on in human LVH, neither ISO nor SPIRO influenced this change. and pump dysfunction in SHR. The beneficial effect occurred in association with an attenuated increase in total, noncross-linked, type I and type III myocardial collagen concentrations and myocardial collagen solubility. Neither an impact on BP, volume preloads (as determined from dimension measurements made in vivo after short-term SPIRO administration), intrinsic myocardial systolic function (determined in vivo and ex vivo), myocardial type I-to-type III collagen ratios, necrosis, apoptosis, nor NE release could explain the advantageous actions of SPIRO on LV dilatation and pump function.

The present study provides the first direct evidence to suggest that a fundamental mechanism responsible for the ability of chronic β-AR activation to promote the transition from compensated LVH to cardiac dilatation and pump dysfunction is through aldosterone receptor stimulation. The results of the present study are consistent with the favorable impact of aldosterone receptor antagonists on cardiac chamber dimensions in alternative forms of human and animal models of cardiac disease. Because ISO and SPIRO modified the intercept but not the slope of diastolic pressure–dimension relations and androgenic steroids determine the slope but not the intercept, the impact of SPIRO in the present study is unlikely to be attributed to androgen receptor effects. Because glucocorticoids act as antagonists of aldosterone receptors in cardiac tissue and thus protect against the ability of aldosterone to promote fibrosis and LVH, the favorable effect of SPIRO is unlikely to be attributed to blockade of glucocorticoid actions on mineralocorticoid receptors.

The mechanisms involved in the beneficial actions of SPIRO could include effects on volume preloads through renal changes or on afterload via BP. Importantly, in the present study, SPIRO given to rats with a dilated LV 2 weeks before assessing LV remodeling failed to modify LV diameters. Moreover, SPIRO did not alter BP. These data would suggest that load-induced effects play little role in the model assessed.

Aldosterone receptor activation promotes apoptosis and necrosis, as well as nongenomic effects on the myocardium. The consequences of these changes could be either reductions in myocardial contractility with secondary remodeling or primary remodeling as a result of myocyte death. However, in the present study, although systolic chamber function was altered by ISO and SPIRO, intrinsic myocardial systolic function was unchanged. Moreover, SPIRO modified neither apoptotic nor necrotic scores. These data would suggest that in the present study, β-AR activation mediates pump dysfunction, and SPIRO prevents these effects through primary remodeling processes unrelated to myocyte death rather than secondary to contractile disturbances, apoptosis, or necrosis. Nevertheless, whether apoptosis was potentiated by ISO earlier on in the study, an effect that may have been attenuated by SPIRO, cannot be ruled out.

SPIRO decreases excessive myocardial NE release in heart failure, an effect that may prevent adverse remodeling. However, in the present study, although SHR with LVH at 14 months of age had marked increases in myocardial NE release, a modification consistent with those reported on in human LVH, neither ISO nor SPIRO influenced this change.

Myocardial NE

SHR at 14 months of age had a marked increase in myocardial NE release (in nmol · mL⁻¹; SHR at 14 months of age [n = 12] = 0.27 ± 0.04 nmol · mL⁻¹; SHR at 7 months of age [n = 16] = 0.09 ± 0.02 nmol · mL⁻¹; WKY [n = 6] = 0.08 ± 0.02 nmol · mL⁻¹; P < 0.002). Neither ISO (SHR + ISO at 14 months of age [n = 10] = 0.23 ± 0.06 nmol · mL⁻¹) nor SPIRO given for 2 weeks before the termination of the study (SHR + ISO + SPIRO [n = 8] = 0.32 ± 0.07 nmol · mL⁻¹) modified myocardial NE release.

Discussion

In the present study, SPIRO prevented β-AR–mediated LV dilatation (increased LVEDD as well as right shifts in LV diastolic pressure–dimension relations with wall thinning) and pump dysfunction in SHR. The beneficial effect occurred in association with an attenuated increase in total, noncross-linked, type I and type III myocardial collagen concentrations and myocardial collagen solubility. Neither an impact on BP, volume preloads (as determined from dimension measurements made in vivo after short-term SPIRO administration), intrinsic myocardial systolic function (determined in vivo and ex vivo), myocardial type I-to-type III collagen ratios, necrosis, apoptosis, nor NE release could explain the advantageous actions of SPIRO on LV dilatation and pump function.

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In the present study the beneficial effects of SPIRO on β-AR-mediated LV dilatation were partly attributed to alterations in myocardial collagen concentrations, a notion supported by previous findings in heart failure. An interstitial change that could explain the beneficial actions of SPIRO is through alterations in noncross-linked (soluble) myocardial collagen concentrations, because SPIRO prevented β-AR-mediated increases in soluble collagen concentrations. Noncross-linked myocardial collagen is susceptible to degradation by matrix metalloproteinases and an accumulation of noncross-linked collagen could therefore lead to side-to-side slippage of myocytes and hence chamber dilatation. The association between noncross-linked myocardial collagen concentrations and chamber dimensions in our study supports this notion, but the correlation coefficients suggest that alternative factors still need to be identified.

A potential limitation of the present study is that the technique used to assess apoptosis not only labels apoptotic, but also oncotic and cells undergoing DNA repair. This finding is entirely consistent with data obtained by other groups in SHR and WKY and could reflect intrinsic differences between rat strains.

Perspectives

The present results suggest that the aldosterone receptor antagonist SPIRO may prevent the transition to LV dilatation and pump dysfunction in hypertensive LVH associated with β-adrenergic overactivation. The beneficial effects on the heart induced by SPIRO appear to be determined by interstitial changes but are independent of BP and volume preloads and hence represent a load-independent target in hypertension. This finding suggests a useful therapeutic approach in hypertensives with LVH, in whom β-AR blockers have limited beneficial effects on LV mass and indeed may modified the index of apoptosis used. The high percentage of biotinylated-labeled cells in SHR is not inconsistent with data obtained by other groups in SHR.

In the present study, systolic function, as determined using echocardiography in SHR and WKY, was higher than that reported previously by us in alternative rat strains. This finding is entirely consistent with data obtained by other groups in SHR and WKY and could reflect intrinsic differences between rat strains.

### Table 2. Effect of Chronic Administration of ISO and SPIRO on Myocardial Necrosis, Apoptosis, and Collagen Characteristics in SHR

<table>
<thead>
<tr>
<th></th>
<th>WKY</th>
<th>SHR</th>
<th>SHR+ISO</th>
<th>SHR+SPIRO</th>
<th>SHR+ISO+SPIRO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathological score</td>
<td>1.00±0.19</td>
<td>1.90±0.32</td>
<td>3.10±0.06*</td>
<td>2.33±0.33</td>
<td>2.67±0.44*</td>
</tr>
<tr>
<td>% Apoptotic/normal nuclei#</td>
<td>1.99±0.37</td>
<td>5.12±0.84*</td>
<td>5.10±0.89*</td>
<td>5.37±1.23</td>
<td>4.34±0.68*</td>
</tr>
<tr>
<td>Type I-to-type III collagen ratio</td>
<td>2.89±0.23</td>
<td>3.06±0.13</td>
<td>3.02±0.19</td>
<td>2.92±0.20</td>
<td>2.61±0.21</td>
</tr>
<tr>
<td>Collagen type I (μg · mg⁻¹ dry LV)</td>
<td>23±3</td>
<td>46±3*</td>
<td>65±10†‡</td>
<td>32±8</td>
<td>40±5*</td>
</tr>
<tr>
<td>Collagen type III (μg · mg⁻¹ dry LV)</td>
<td>8±1</td>
<td>15±†</td>
<td>21±2†‡</td>
<td>11±3</td>
<td>16±2*</td>
</tr>
</tbody>
</table>

Numbers in parentheses represent sample numbers.
# indicates apoptotic nuclei expressed as a % of the No. of normal nuclei.
*P<0.05; †P<0.01 vs WKY group; ‡P<0.01 vs other SHR groups.
See Table 1 for sample sizes.
induce deleterious metabolic actions. In this regard, aldosterone receptor antagonists regress LVH and mediate no documented adverse metabolic effects.

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References

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Induced by β-Adrenoreceptor Activation

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