Long-term mineralocorticoid receptor blockade reduces fibrosis and improves cardiac performance and coronary hemodynamics in elderly SHR

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Susic, Dinko, Jasmina Varagic, Jwari Ahn, Luis Matavelli, and Edward D. Frohlich. Long-term mineralocorticoid receptor blockade reduces fibrosis and improves cardiac performance and coronary hemodynamics in elderly SHR. Am J Physiol Heart Circ Physiol 292: H175–H179, 2007. First published August 11, 2006; doi:10.1152/ajpheart.00660.2006.—Aldosterone has been implicated as one of the mediators of cardiovascular injury in various diseases. This study examines whether mineralocorticoid antagonism ameliorates or prevents the adverse cardiac effects of hypertension and aging. Male 22-wk-old spontaneously hypertensive rats (SHR) were divided into two groups, 15 rats in each. One group received no treatment; the other was given eplerenone (100 mg·kg⁻¹·day⁻¹). At the age of 54 wk, indexes of cardiovascular mass, systemic and regional hemodynamics, including coronary, left ventricular function, and myocardial collagen content, were determined in all rats. Hemodynamic studies were done in conscious rats. Arterial pressure was lowered only slightly in eplerenone-treated rats, and cardiac output and total peripheral resistance did not differ from control rats. Left and right ventricular and aortic mass indexes were unaffected by eplerenone; however, concentration of hydroxyproline in the right and left ventricle was decreased significantly (P < 0.05) by eplerenone. This was accompanied by an improvement in left ventricular diastolic function and coronary hemodynamics. In conclusion, long-term therapy with the mineralocorticoid receptor antagonist eplerenone ameliorated adverse cardiac effects of both hypertension and aging in SHR. Thus reduction in myocardial fibrosis, paralleled by improvements in left ventricular function and coronary hemodynamics, was observed in eplerenone-treated SHR.

MATERIALS AND METHODS

Animals. Adult male SHR rats obtained from Charles River Breeding Laboratories (Wilmington, MA) were housed in a temperature- and humidity-controlled facility with a 12-h:12-h light-dark cycle. Standard rat chow (PMI Nutrition International, St. Louis, MO) and tap water were provided ad libitum. The investigation conforms with the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996), and our Institutional Animal Care and Use Committee had approved the study in advance.

Experimental protocol and techniques. Male 22-wk-old SHR were divided randomly into two groups with 15 rats in each. The control group received no treatment; rats in the second group were given eplerenone mixed in food (calculated on the basis of food intake, average daily eplerenone intake was 104 ± 4 mg/kg). Rats were treated for over 6 mo, and, at the age of 54 wk, studies on cardiac function, systemic and regional hemodynamics, and ventricular hydroxyproline concentration, as an estimate of collagen, were performed. To this end, each rat was anesthetized with pentobarbital sodium (40 mg/kg) and a catheter-tip transducer (Millar Instruments, Houston, TX) introduced into the left ventricle via the right carotid artery. The catheter was connected to a preamplifier of a multichannel polygraph (Grass Instrument, Quincy, MA), and the signal was then fed to a data acquisition system (Emka Technologies, Paris, France). Several indexes of cardiac function, including end-diastolic pressure, maximum rates of pressure rise and decline (±dP/dmax and −dP/ dmax) and diastolic time constant (τ), were obtained from ventricular pressure tracing. A femoral artery polyethylene catheter (PE-50) was used for arterial pressure measurement. After measurement of ventricular function was completed, the catheter-tip transducer was removed, and the rats were then instrumented for the determination of systemic and regional hemodynamics (using the reference standard radiomicrosphere method) as detailed elsewhere (13, 14, 24). In brief, the costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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Hypertensive heart disease involves alterations in cardiac structure and function, including hypertrophy of myocytes, ischemia, and interstitial fibrosis, leading eventually to impaired myocardial performance and coronary hemodynamics, and apoptosis (8–10). Aging further aggravates each of these adverse events (17). It has been well established that pathogenesis of heart disease associated with hypertension and aging involves all components of the heart including myocytes and nonmyocytic cells, such as fibroblasts and endothelial cells, extracellular matrix proteins, such as fibrillar collagen, and coronary vessels (25, 33). It is also generally accepted that changes in cardiac myocytes and coronary vasculature participate significantly in the adverse effects of hypertension and aging on cardiac function, but there is also much evidence that changes in extracellular matrix, particularly in fibrillar collagen, also have an important role (3, 28, 35). Thus numerous reports (4, 26, 29) link changes in extracellular fibrillar collagen with development of diastolic dysfunction and diastolic heart failure. Recent experimental and clinical studies (5, 6, 20, 30) have also demonstrated that aldosterone, independently of the renin-angiotensin system, may have a role in the development of cardiac fibrosis and, in that way, may mediate cardiovascular damage. Moreover, mineralocorticoid receptor (MR) antagonists have been shown to affect cardiac and vascular fibrosis in various experimental models (2, 16, 32).

Spontaneously hypertensive rats (SHR) develop, with aging, significant myocardial fibrosis, accompanied by impairment in left ventricular function and coronary hemodynamics (23). Thus they provide a good experimental model for studying the relationship between fibrosis and cardiovascular function. This study was designed to examine whether extended therapy with a selective MR antagonist, eplerenone, affects the adverse cardiovascular changes associated with aging in SHR.

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a jugular vein, femoral artery, and the left ventricle (via right carotid artery) were cannulated with polyethylene catheters (PE-50) filled with heparinized saline and exteriorized at the nape of the neck through a subcutaneous tunnel. Rats were then placed into nonrestrictive polyethylene cages and were allowed to recover for several hours (13, 14, 23).

Baseline measurements of systemic and coronary hemodynamics were obtained in unrestrained rats after full recovery from anesthesia. Cardiac output was measured by the reference sample microsphere method as reported previously (13, 14, 23). Cardiac index was calculated from cardiac output and body weight (expressed in ml·min⁻¹·kg⁻¹). Total peripheral resistance index (in U/kg) was calculated by dividing mean arterial pressure by cardiac index. Blood flow to different organs, including heart, lungs, liver, kidneys, skeletal muscle, skin, and brain, was determined on the basis of percent distribution of the radionuclide (¹⁸⁵Sc) microspheres to each organ at the end of the study (13, 14, 23). The method has been validated previously (13).

After the baseline measurements were obtained, maximal coronary vasodilatation was produced by dipyridamole infusion (4 mg·kg⁻¹·min⁻¹ iv for 10 min) (13, 23) using a Harvard infusion/withdrawal pump (Harvard Apparatus, South Natick, MA), and hemodynamic measurements were repeated using radiomicrospheres with a second radionuclide (¹⁰⁳Ru). At the conclusion of the study, rats were euthanized with an overdose of pentobarbital sodium, and, immediately thereafter, the heart, aorta, lungs, liver, kidneys, brain, and samples of skin and skeletal muscle were removed and weighed. Tissue samples, as well as blood reference samples, were placed in plastic scintillation vials and counted for 5 min in a deep-well gamma scintillation spectrometer (Packard, Downer Grove, IL) with a multichannel analyzer. Spillover correction between channels was achieved by matrix inversion software (Compusphere, Packard). Organ blood flows were calculated by multiplying the fractional distribution of radioactivity to each organ by cardiac output and normalized for the wet weight of the respective organ (expressed as ml·min⁻¹·g⁻¹). Regional vascular resistances were calculated by dividing the mean arterial pressure by organ blood flow and then normalized for organ weight (expressed as U/g). Blood flow reserve for the right and left ventricles was calculated as the difference between the baseline and dipyridamole infusion flows. Minimal vascular resistance was defined as vascular resistance achieved by dipyridamole (13, 14, 23, 24).

Myocardial collagen content. As an estimate of collagen content, hydroxyproline concentration was determined in left and right ventricular samples as described previously (23). Briefly, myocardial samples (~50 mg) were taken from both ventricles. Specimens were dried overnight in a 60°C oven and lipids then extracted with a 2:1 mixture of chloroform and methanol. Samples were dried again at 60°C, weighed, and hydrolyzed with 6 N hydrochloric acid at 110°C overnight. Hydrolyzates were neutralized (to pH 7.0) with NaOH, and, after extraction with activated charcoal, were treated with chloramine-T and para-dimethyl-aminobenzaldehyde solution. Absorbance was read at 560 nm; hydroxyproline concentration was determined from standard curve (expressed as mg/g dry wt).

Statistical analysis. Values are expressed as means ± SE. Student’s t-test was used to evaluate the significance of differences between the groups (1). A probability value of <0.05 was considered significant.

RESULTS

Body weight and cardiovascular mass indexes. No difference in body weight was observed between the groups (control, 411 ± 4 vs. eplerenone, 401 ± 4 g). Similarly, there were no differences between left ventricular (3.16 ± 0.07 vs. 3.05 ± 0.05 mg/g, P = 0.2188), right ventricular (0.48 ± 0.02 vs. 0.47 ± 0.02 mg/g), and aortic (1.43 ± 0.04 vs. 1.39 ± 0.04 mg/g) weight indexes, control vs. treated, respectively. However, hydroxyproline concentration, as an estimate of collagen content, was significantly (P < 0.01) lower in the eplerenone-treated rats than in their control counterparts with respect to both the left (5.4 ± 0.2 vs. 6.2 ± 0.2 mg/g) and right (6.4 ± 0.2 vs. 8.1 ± 0.1 mg/g) ventricles. Histological examination also demonstrated that eplerenone reduced myocardial collagen (Fig. 1).

Systemic and organ hemodynamics. Systolic (191 ± 3 vs. 207 ± 5 mmHg) and mean (165 ± 3 vs. 179 ± 6 mmHg) arterial pressures were significantly (P < 0.05) lower in the eplerenone-treated rats, but there was no significant difference in diastolic pressure (143 ± 5 vs. 156 ± 5 mmHg). Furthermore, there was no difference in heart rate (388 ± 7 vs. 398 ± 11 beats/min), cardiac index (257 ± 8 vs. 285 ± 20 ml·min⁻¹·kg⁻¹), or total peripheral resistance (0.65 ± 0.03 vs. 0.68 ± 0.09 U) between the eplerenone and control groups. Blood flow and vascular resistance in liver, kidneys, skin, skeletal muscle, and brain were similar in the two groups (data not shown). Similarly, basal blood flow to the right (3.86 ± 0.17 vs. 4.12 ± 0.27 ml·min⁻¹·g⁻¹) and left (4.25 ± 0.21 vs. 4.89 ± 0.32 ml·min⁻¹·g⁻¹) ventricles was no different between the two groups. The same holds true for basal vascular resistance (43.7 ± 2.5 vs. 46.1 ± 4.8 and 39.5 ± 1.7 vs. 38.7 ± 3.8 U) in the right and left ventricles, respectively. However, when compared with that in the control group, minimal vascular coronary resistance (determined after dipyridamole infusion) was significantly (P < 0.05) lower in both ventricles of the eplerenone-treated rats (Fig. 2). Simultaneously, coronary flow reserve was significantly (P < 0.05) greater in both ventricles of the eplerenone-treated animals (Fig. 2).
Left ventricular function. No difference in end-diastolic pressure or the \( +\frac{dP}{dt_{\text{max}}} \) during systole was observed between the two groups (Fig. 3). However, two indexes of diastolic function, \( \theta \) and \( -\frac{dP}{dt_{\text{max}}} \), were significantly \((P < 0.05)\) improved in eplerenone-treated rats (Fig. 3). Left ventricular hydroxyproline concentration correlated significantly \((P < 0.01)\) with \( \theta \) \((r = 0.601)\) and \( -\frac{dP}{dt_{\text{max}}} \) \((r = 0.580)\).

DISCUSSION

The present data, showing a decrease in myocardial hydroxyproline concentration in eplerenone-treated rats, demonstrate that long-term MR antagonism reduced cardiac fibrosis in aging SHR, a finding in agreement with previous reports (2, 16, 32). Interestingly, this is accomplished without any effect on overall ventricular mass. Furthermore, our data also imply that the effect of eplerenone is not mediated by virtue of hemodynamic unloading but, rather, was due to the direct inhibition of MRs. Three separate sets of data actually support this concept. First, reduction in arterial pressure in eplerenone-treated animals was quite modest, a finding in agreement with a previous report from Frohlich’s laboratory (33) and those from other laboratories (2, 32). Furthermore, total peripheral resistance was not decreased in rats given eplerenone, which together with the finding of a very modest decrease in arterial pressure suggests that left ventricular afterload was not reduced or that it was only slightly affected. In addition, left ventricular mass was not decreased in the eplerenone-treated rats, thereby providing further evidence that the hemodynamic load was not reduced significantly in these animals. Finally, eplerenone also reduced collagen in the right ventricle, a chamber that was not exposed to systemic hemodynamic overload. Thus our findings clearly indicate that MR antagonism reduces cardiac fibrosis in elderly hypertensive rats and that this effect is, at least in part, independent of its hemodynamic effects. Similar findings (16)

Fig. 2. Minimal coronary vascular resistance (MVR) and coronary flow reserve (CFR) in the right (RV) and left (LV) ventricle of the control (C) and eplerenone-treated (E) spontaneously hypertensive rats (SHR). Values are means ± SE; \( n = 15 \) rats/group. *\( P < 0.05 \).

Fig. 3. Left ventricular end-diastolic pressure (LVEDP), maximal rate of pressure rise \((+\frac{dP}{dt_{\text{max}}})\) and fall \((-\frac{dP}{dt_{\text{max}}})\), and Tau index in control and eplerenone-treated SHR. Values are means ± SE; \( n = 15 \) rats/group. *\( P < 0.05 \).
were reported in elderly normotensive rats in which spironolactone reduced aortic and cardiac fibrosis without affecting arterial pressure. However, we cannot exclude the possibility that neurohumoral factors or changes in the activity of local, cardiac, renin-angiotensin-aldosterone system may be involved. The exact mechanism of this antifibrotic action of MR antagonism is still not clear and, as suggested by others, may involve alterations in intracellular signaling, changes in activation of transcription and growth factors, decreased endothelin production, increased endothelial nitric oxide generation, and anti-inflammatory action and reduction of oxidative stress (7, 19, 21, 22, 30).

As already mentioned, eplerenone did not affect left ventricular mass in the present study. However, in hypertensive models involving salt excess, such as salt-overloaded Wistar rats or Dahl salt-sensitive rats, blockade of MRs with spironolactone or eplerenone prevented left ventricular hypertrophy and remodeling (15, 18). These divergent results indicate that MRs may not be involved in the pathogenesis of ventricular hypertrophy in SHR as opposed to salt-overload models. This notion is further supported by the results of a recent in vitro study demonstrating that the effect of aldosterone on cardiomyocyte hypertrophy depends on extracellular sodium concentration, the effect being significantly greater in the presence of elevated extracellular sodium (31).

This study also demonstrates that MR antagonism improves coronary hemodynamics in aging SHR, as suggested by a reduction in minimal coronary vascular resistance and an increase in coronary flow reserve in both ventricles of eplerenone-treated rats. Frohlich’s laboratory (25) has previously reported that eplerenone also improves coronary hemodynamics in young adult SHR. Impairment in coronary hemodynamics is very pronounced in elderly SHR (23), and Frohlich’s laboratory (24) has previously shown that it may be partially corrected by antihypertensive drugs. Again, it appears that the observed improvement in coronary hemodynamics was not a consequence of changes in hemodynamic load. It may be due to improved endothelial function, as suggested by findings in two-kidney, one-clip hypertensive rat model (12). The present results are also in agreement with the findings that coronary dysfunction is present in transgenic mice overexpressing aldosterone synthase (11).

Of particular importance is the finding that parallel with a decrease in myocardial fibrosis, an improvement in left ventricular diastolic function occurred in eplerenone-treated rats. Thus two indexes of diastolic function, −dP/dtmax and θ, improved in rats given eplerenone, whereas systolic function remained unaltered. Furthermore, a good correlation between left ventricular collagen, as estimated by hydroxyproline concentration, and indexes of diastolic function was observed. Diastolic dysfunction and diastolic heart failure are common findings in both hypertensive and elderly populations (27, 34) and contribute significantly to overall morbidity and mortality. Results of experimental and clinical studies (4, 26, 29, 34) suggest that increased myocardial accumulation of fibrillar collagen may participate in the pathogenesis of diastolic dysfunction, most likely by affecting ventricular relaxation and stiffness. The present results further support this concept.

In conclusion, extended therapy with eplerenone improved coronary hemodynamics and myocardial function and reduced myocardial collagen, suggesting that MR activation may be involved in mediating age- and hypertension-related cardiac fibrosis.

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GRANTS

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