

Eplerenone Prevents Salt-Induced Vascular Remodeling and Cardiac Fibrosis in Stroke-Prone Spontaneously Hypertensive Rats

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Abstract—We examined the effect of different levels of salt intake on the role of aldosterone on cardiac and vascular changes in salt-loaded stroke-prone spontaneously hypertensive rats (SHRSP). Eleven-week-old SHRSP were fed high-salt (4.2% NaCl), normal-salt (0.28%), or low-salt (0.03%) diets with or without eplerenone (100 mg/kg per day, in food) for 5 weeks. A group of high-salt SHRSP was also treated with hydralazine (25 mg/kg per day). Blood pressure increased more in high-salt rats than in other groups ($P<0.001$). Eplerenone prevented further blood pressure rise in salt-loaded rats, with little effect on control and low-salt SHRSP. Increased media-to-lumen ratio of mesenteric resistance arteries induced by salt ($P<0.01$) was prevented by eplerenone ($P<0.01$). Maximal acetylcholine-induced vasodilation was impaired under salt loading ($P<0.01$), but improved under eplerenone ($P<0.01$). Eplerenone prevented ($P<0.01$) increased heart weight and left and right ventricular collagen deposition induced by high salt. Blood pressure lowering by hydralazine in high-salt SHRSP did not influence endothelial function or left ventricular collagen. Our study demonstrates salt-dependency of aldosterone effects on severity of hypertension, endothelial dysfunction, and cardiac and vascular remodeling in SHRSP. These effects were attenuated by eplerenone, particularly in the salt-loaded state, underlining the pathophysiological role of aldosterone in salt-sensitive hypertension. (*Hypertension*. 2004; 43:1252-1257.)

Key Words: rats, stroke-prone SHR ■ aldosterone ■ resistance ■ arteries ■ remodeling ■ heart ■ collagen

Aldosterone is recognized as a target for therapeutic intervention in cardiac failure¹ and hypertension.^{2,3} Beneficial effects of aldosterone antagonism on end-organ damage have also been studied in animal models. In aldosterone-infused rats, spironolactone or the more specific antagonist eplerenone, prevented left ventricular inflammation and fibrosis,^{4–6} renal inflammation and proteinuria,⁷ stiffening of the carotid artery,⁸ resistance artery remodeling, endothelial dysfunction, and activation of NADPH oxidase.^{9,10} In stroke-prone spontaneously hypertensive rats (SHRSP), a model of genetic hypertension, spironolactone reduced renal damage and proteinuria.^{11,12}

Most studies using mineralocorticoid receptor antagonism in aldosterone-infused rats^{4–8} or in SHRSP were performed after salt loading.^{11,12} We questioned whether salt induces end-organ damage in SHRSP through mechanisms that involve mineralocorticoids. Aldosterone suppression may be impaired in salt-loaded SHRSP both with regard to plasma aldosterone concentrations¹³ and tissue specific aldosterone synthase activity,^{14,15} suggesting that aldosterone could play a role in the aggravation by salt of end-organ damage in SHRSP.

In the present study, we tested the hypothesis that salt-induced vascular and cardiac remodeling in SHRSP depends,

at least in part, on aldosterone-mediated actions by examining the effects of mineralocorticoid antagonism in SHRSP receiving different levels of salt in their diet.

Methods

Animal Experiments

The study was approved by the Animal Care Committee of the Clinical Research Institute of Montreal and was conducted in accordance with the recommendations of the Canadian Council of Animal Care. Male SHRSP, aged 9 to 11 weeks, were divided into 6 groups, $n=7$ /group plus 4 additional rats per group that were only used for blood pressure (BP) monitoring by radiotelemetry. They received either low-salt (0.03% NaCl), normal-salt (0.28%), or high-salt (4.2%) diets with or without eplerenone (100 mg/kg per day, in food; Pharmacia/Pfizer) for 5 weeks. In a second set of experiments, rats received a high-salt diet with or without hydralazine (25 mg/kg per day, in food, Sigma; $n=5$ per group plus 3 additional for telemetry). A third set of rats compared SHRSP and normotensive WKY, both on a normal salt diet, to assess whether plasma aldosterone levels and markers of oxidative excess are altered in SHRSP ($n=6$ per group). 24-hour sodium excretion was measured in urine collected in metabolic cages at the end of the experiment. Radiotransmitters (Data Sciences) were implanted 12 days before the end of the experiment, and BP was recorded for the last 7 days every 5 minutes for 10 seconds. At the end of the experiment, the mesenteric vasculature was dissected, and one segment was used for

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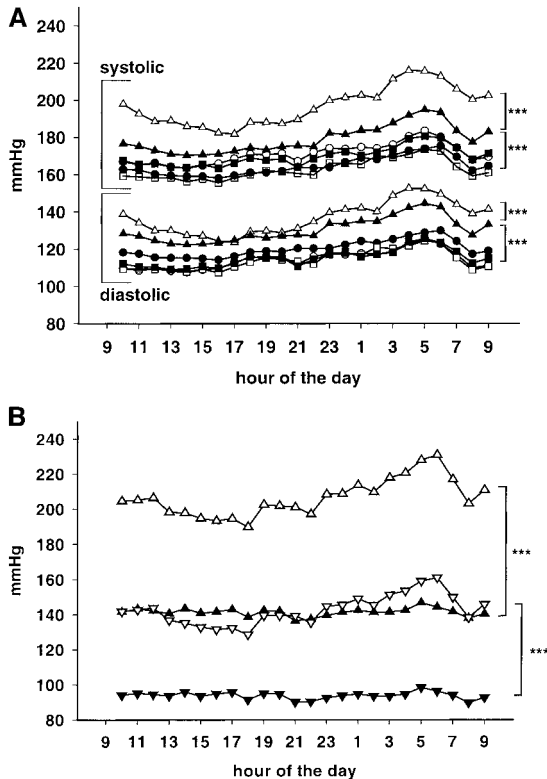


Figure 1. Twenty-four-hour blood pressure as assessed by telemetry over 7 days. Results are presented as means of the recordings of all animals of all 7 days for every hour of the day. A, circles indicate low salt; squares, normal salt; triangles, high salt; open symbols, without eplerenone; and filled symbols, with eplerenone. ANOVA for repeated measurements: *** $P < 0.001$, $n = 4$. B, triangle pointing to top indicates systolic blood pressure; triangle pointing down, diastolic blood pressure; open triangles, high salt without hydalazine; and filled triangles, high salt with hydalazine. $n = 3$.

preparation of small arteries. The rest of the vascular bed and a sample of the apex of the heart were used for measurement of NADPH oxidase activity. A mid cross-section of the heart was fixed in 4% paraformaldehyde.

Preparation and Study of Small Arteries

A third-order branch of the mesenteric artery was isolated and mounted on a pressurized myograph as described previously.¹⁶ After

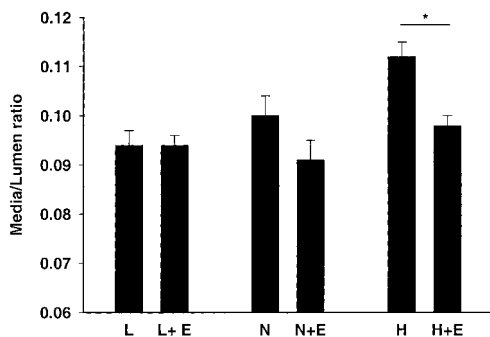


Figure 2. Media/lumen ratio from mesenteric resistance arteries. 2-way ANOVA: influence of eplerenone $P < 0.01$, influence of salt $P < 0.01$, interaction ns. * $P < 0.05$ post-hoc (Bonferroni). L indicates low salt; L+E, low salt plus eplerenone; N, normal salt; N+E, normal salt plus eplerenone; H, high salt; and H+E, high salt plus eplerenone.

precontraction with 10^{-5} mol/L norepinephrine, endothelium-dependent and independent relaxation was assessed with acetylcholine (10^{-9} – 10^{-4} mol/L) and sodium nitroprusside (10^{-9} – 10^{-3} mol/L), respectively. To study oxidative excess, vessels were pretreated with tempol (10^{-3} mol/L; 4-hydroxy-2,2,6,6-tetramethyl piperidinoxyl), superoxide dismutase (80 U/mL), and catalase (100 U/mL). Vascular morphology and mechanics were studied as previously described.¹⁶ At the end of the experiment, vessels were fixed for histological analysis.

Histology

Tissue sections of mesenteric arteries and hearts were stained with Sirius red F3BA (0.5% in saturated aqueous picric acid, Aldrich Chemical Company) for assessment of collagen, as previously described.⁴

Measurement of NADPH Oxidase Activity

Activity of NADPH oxidase was measured in a lucigenin luminescence assay as previously described.¹⁷

Measurement of Plasma Thiobarbituric Acid-Reacting Substances and Plasma Aldosterone Concentration

Plasma thiobarbituric acid-reacting substances (TBARS) were measured colorimetrically.¹⁸ Plasma aldosterone concentration was measured using a radioimmunoassay kit (ICN Diagnostics) according to the manufacturer's instructions.

Data Analysis

For telemetric BP, the means of all readings every hour were calculated. For 7 days the means of the same hours of the day were pooled. Data are presented as mean \pm SEM. Groups were compared using Student *t* test, 1-way ANOVA, 2-way ANOVA, or ANOVA for repeated measurements as appropriate. Post-hoc testing was performed using Bonferroni (2-way ANOVA) or Newman-Keuls (1-way and repeated measures) test. $P < 0.05$ was significant.

Results

Blood Pressure, Heart Weight, and Plasma Aldosterone Concentration

After 4 weeks of salt-loading, BP increased significantly in SHRSP ($P < 0.001$; Figure 1). Treatment with eplerenone blunted ($P < 0.001$) the rise in systolic and diastolic BP in salt-treated SHRSP, with greater effect on systolic BP. There were no important differences between low- and normal-salt diets with and without eplerenone. The typical day-night BP rhythm was present in all groups, with highest values at approximately 5:00 AM, at which time the animals are most active. Hydralazine reduced systolic and diastolic blood pressure in salt-loaded SHRSP (Figure 1).

Heart:body weight ratio was significantly increased in salt-loaded SHRSP (Table 1). Eplerenone prevented ($P < 0.05$) its increase and was most effective in animals on a high-salt diet. Hydralazine significantly reduced relative heart weight to a similar extent as eplerenone in salt-loaded SHRSP (Table 2).

Twenty-four-hour sodium excretion increased according to salt intake (data not shown), with no differences between eplerenone-treated and untreated animals. Plasma aldosterone concentration decreased ($P < 0.0001$) with increased salt intake (Table 1). Eplerenone treatment resulted in significantly ($P < 0.0001$) increased plasma aldosterone. SHRSP have significantly higher plasma aldosterone levels compared with

TABLE 1. Physiological Parameters, Characteristics of Mesenteric Resistance Arteries, Cardiac Collagen Content, and Markers of Oxidative Stress

Parameter	Low Salt	Low Salt+ Eplerenone	Normal Salt	Normal Salt+ Eplerenone	High Salt	High Salt+ Eplerenone	Salt <i>P</i>	Eplerenone <i>P</i>	Interaction <i>P</i>
BW, g	303±4	308±5	318±5	301±3	299±6	300±5	NS	NS	0.049
HW/BW, mg/g	4.4±0.1	4.3±0.1	4.5±0.2	4.4±0.1	4.9±0.1	4.6±0.04	<0.001	<0.05	NS
PAC, pg/mL	445±64	976±90*	371±72	706±78†	223±44	376±36‡	<0.0001	<0.0001	<0.05
Media, μm	20.0±0.4	19.8±0.4	20.7±0.6	20.0±0.7	22.4±0.7	20.1±0.2‡	<0.05	<0.05	NS
Lumen, μm	213±8	211±5	208±6.0	221±7.1	200±6	206±4	NS	NS	NS
CSA, $10^3 \times \mu\text{m}^2$	14.7±0.7	14.4±0.5	14.8±0.6	15.1±0.8	15.7±0.9	14.3±0.3	NS	NS	NS
Collagen media, % of area	9.0±0.7	7.3±0.5	9.1±0.6	7.7±0.6	10.1±0.9	8.2±0.4	NS	<0.01	NS
Interstitial collagen, % of area									
Lv subepicardial	1.82±0.07	1.78±0.07	2.25±0.11	1.75±0.09‡	2.50±0.09	1.78±0.05*	<0.01	<0.001	<0.01
Lv midmyocardial	2.27±0.18	2.40±0.14	2.92±0.20	2.50±0.15	3.41±0.06	2.89±0.14	<0.001	NS	NS
Lv subendocardial	2.84±0.06	2.58±0.16	3.27±0.20	3.14±0.14	4.20±0.13	3.17±0.12*	<0.0001	<0.01	<0.05
Right ventricle	2.97±0.10	2.58±0.16	3.27±0.20	3.14±0.06	4.59±0.09	3.17±0.12*	<0.001	<0.001	<0.01
TBARS, nmol/mL	2.23±0.14	2.47±0.15	2.47±0.15	2.68±0.2	2.90±0.16	2.40±0.15	NS	NS	NS
NADPH Ox. Act.									
Mes. Art.	19.1±5.9	17.6±3.2	18.1±5.8	15.9±5.8	18.9±9.1	20.4±8.9	NS	NS	NS
Heart	7.9±3.5	2.7±4.2	6.3±1.9	5.6±3.0	6.8±2.1	6.1±2.3	NS	NS	NS

Values are mean±SEM. BW indicates body weight; HW/BW, relative heart weight; PAC, plasma aldosterone concentration; CSA, cross-sectional area; Lv, left ventricle; Mes. Art., mesenteric arteries; NADPH Ox. Act., NADPH oxidase activity. TBARS are expressed as malonyl dialdehyde equivalents. NADPH oxidase activity is expressed as counts/min per mg dry tissue weight.

Bonferroni post-hoc testing: * $P<0.001$, † $P<0.01$, ‡ $P<0.05$ vs same salt loading without eplerenone.

normotensive WKY rats (484 ± 51 versus 272 ± 66 pg/mL; $P<0.05$), indicating upregulation of the aldosterone system in SHRSP.

Morphology, Mechanical Properties, and Endothelial Function of Mesenteric Arteries

Media thickness of mesenteric resistance arteries increased significantly ($P<0.05$) with salt-loading in SHRSP. Eplerenone prevented the increase of media thickness ($P<0.05$), with the most pronounced effects in animals on high-salt diet (Table 1). Lumen diameter tended to be smaller

in salt-loaded animals and higher under eplerenone treatment. Media-to-lumen ratio was increased ($P<0.01$) with greater salt intake. This was prevented ($P<0.01$) by eplerenone (Figure 2). Treatment with eplerenone was most effective in animals on high-salt diet. These results paralleled changes in media thickness and relative heart weight. Hydralazine significantly reduced media-to-lumen ratio (Table 2).

Strain was significantly reduced with increased salt ($P<0.001$ between the different salt treatments without eplerenone, Figure 3A). Eplerenone treatment prevented decrease of strain significantly ($P<0.001$) in salt-loaded

TABLE 2. Results SHRSP+Salt+/-Hydralazine on Heart and Mesenteric Arteries

Parameter	High Salt	High Salt+ Hydralazine	<i>P</i>
BW, g	296±15	278±6	NS
HW/BW, mg/g	5.1±0.1	4.7±0.1	<0.01
Media, μm	22.3±0.9	21.5±0.8	NS
Lumen, μm	202±11	227±8	NS
M/L ratio	0.111±0.006	0.095±0.003	<0.05
CSA, $10^3 \times \mu\text{m}^2$	15.8±1.3	16.9±1.2	NS
Max. vasodilation	0.92±0.02	0.92±0.02	NS
Interstitial cardiac collagen content, collagen area/total area			
Subepicardial	1.92±0.04	1.94±0.04	NS
Midmyocardial	2.80±0.05	2.67±0.13	NS
Subendocardial	3.17±0.10	3.14±0.03	NS
Right ventricle	3.43±0.07	3.14±0.07	<0.01

Values are mean±SEM. BW indicates body weight; HW/BW, relative heart weight; CSA, cross-sectional area.

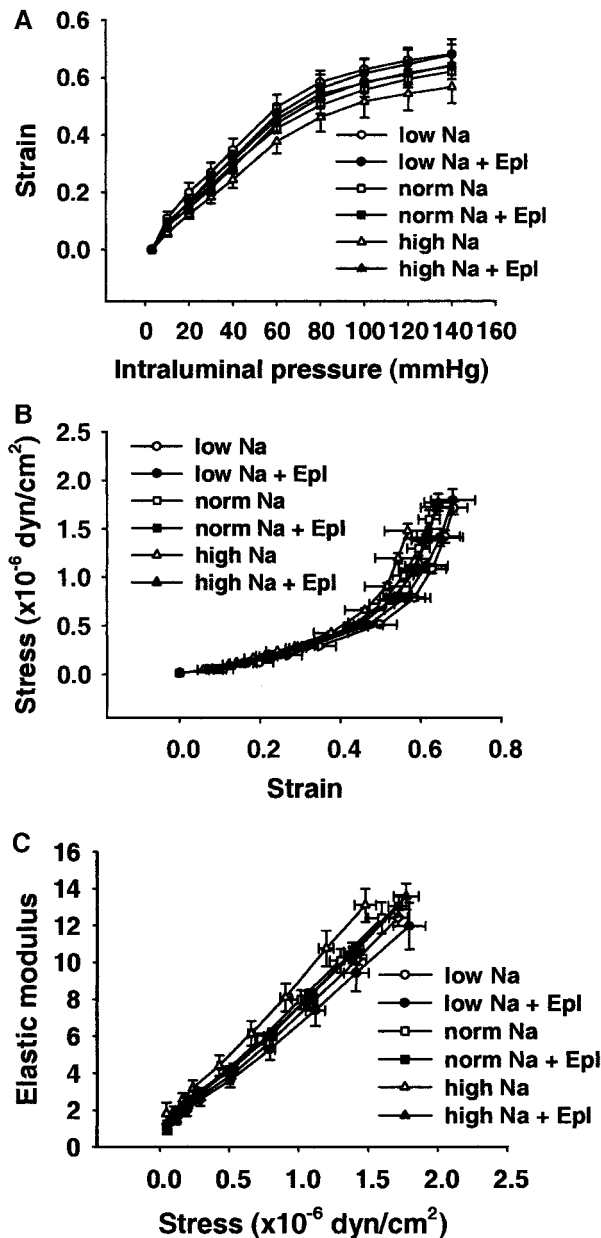


Figure 3. Mechanical properties of mesenteric resistance arteries. A, Strain-pressure relationship. ANOVA for repeated measurements: high salt vs normal salt $P < 0.001$, high salt vs high salt plus eplerenone $P < 0.001$, high salt vs low salt $P < 0.001$, and normal salt vs low salt $P < 0.001$. B, Stress-strain diagram. C, Incremental elastic modulus plotted against stress.

SHRSP, but not in rats receiving normal or low-salt diet. There were minor changes in the stress-strain relationship with slight displacement to the left with greater salt intake (Figure 3B) and increase in the slope of the elastic modulus-stress relationship (Figure 3C), both prevented by eplerenone, but which, however, did not reach statistical significance. Thus the significant changes in vessel mechanics may have been mostly because of vessel geometry.

There were no significant differences between groups in endothelium-independent vasorelaxation curves after sodium nitroprusside stimulation (data not shown). Endothelium-dependent vasorelaxation was similar in all groups (full

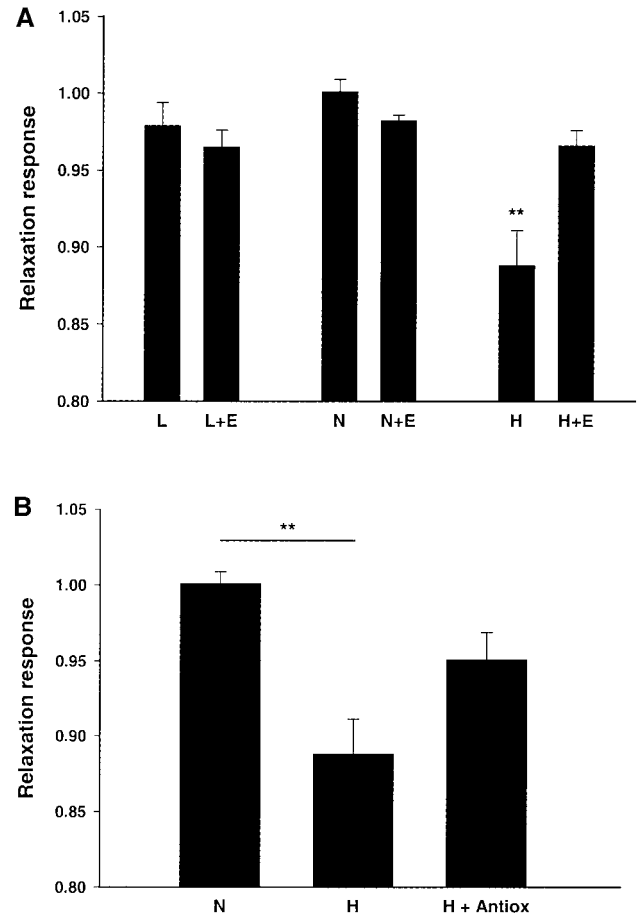


Figure 4. Endothelial function of mesenteric resistance arteries. Results are presented as means \pm SEM of the ratio between the lumen after maximal vasodilation with acetylcholine (10^{-4} mol/L) after precontraction with norepinephrine (10^{-5} mol/L), compared with that of the relaxed vessels. A, L indicates low salt; L+E, low salt plus eplerenone; N, normal salt; N+E, normal salt plus eplerenone; H, high salt; and H+E, high salt plus eplerenone. 1-way ANOVA: $**P < 0.01$ vs all other groups. B, Maximal vasodilatory response to acetylcholine (10^{-4} mol/L) \pm preincubation of the vessels with antioxidants (tempol 10^{-3} mol/L plus superoxide dismutase 80 U/mL plus catalase 100 U/mL). N indicates normal salt; H, high salt; and H+Antiox, high salt plus preincubation with antioxidants. 1-way ANOVA: $**P < 0.01$.

curves not shown). However, maximal vasodilation to acetylcholine (10^{-4} mol/L) was significantly less ($P < 0.01$) in rats on high-salt diet without eplerenone compared with other groups (Figure 4A). Pretreatment of vessels with antioxidants partially restored maximal vasodilatory responses (Figure 4B). Hydralazine did not improve endothelial dysfunction in salt-loaded SHRSP (Table 2).

Collagen Content of Mesenteric Arteries and the Left Ventricle

Collagen content in the media of mesenteric resistance arteries tended to increase with salt loading (Table 1). Eplerenone treatment significantly reduced ($P < 0.01$) media collagen content.

In the left ventricle, subepicardial ($P < 0.01$), midmyocardial ($P < 0.001$), and subendocardial ($P < 0.0001$), as well as

right ventricular ($P<0.0001$) interstitial collagen, increased significantly with salt loading (Table 1). These effects were prevented by eplerenone ($P<0.001$). Changes in collagen content paralleled changes in relative heart weight. Hydralazine had a small but significant inhibitory effect on collagen content in the right ventricle (Table 2).

TBARS and NADPH Oxidase Activity

Plasma TBARS, an indirect marker of global oxidative excess, were not different in the groups with or without eplerenone (Table 1). When comparing SHRSP and WKY on normal salt diet, the mean plasma TBAR levels tended to be higher in SHRSP (3.29 ± 0.64 versus 2.20 ± 0.32 nmol/mL malonyl dialdehyde equivalents, respectively), but statistical significance was not achieved. NADPH oxidase activity in mesenteric arteries and heart was unaltered (Table 1).

Discussion

In the present study, we show for the first time that in SHRSP receiving different levels of salt intake, mineralocorticoid antagonism with eplerenone prevented vascular remodeling and cardiac fibrosis, particularly in the rats exposed to the high-salt diet. Studies from our laboratory showed a beneficial effect of spironolactone on vascular hypertrophic remodeling and vascular function of mesenteric resistance arteries in aldosterone-infused rats.^{9,10} Eplerenone reduced carotid artery stiffness in aldosterone-infused salt-loaded rats,⁸ associated with differences in fibronectin, not in collagen or elastin density. To our knowledge, this is the first time that the effect of the more selective mineralocorticoid antagonist eplerenone has been investigated in resistance arteries. In high-salt conditions, the increased stiffness of SHRSP vessels was blunted, at least in part because of changes in vessel geometry, as shown by the lack of statistical significance in stress-strain and elastic modulus-stress relationships. However, these curves result from complex mathematical transformations with significant loss of statistical power. Because these findings were paralleled by the results of vascular collagen content, we cannot rule out some effect of salt on vascular collagen content and geometry-independent stiffness.

Endothelial dysfunction in salt-loaded SHRSP was partly restored by antioxidant pretreatment, suggesting that reactive oxygen species may contribute to the impaired endothelial function. Previous studies demonstrated oxidative excess in SHRSP.¹⁹ Plasma TBARS, an indirect marker of global oxidative excess tended to be higher in salt-loaded SHRSP. Vascular and cardiac NADPH oxidase activity, a major source of reactive oxygen species in cardiovascular tissues, was however not significantly altered in salt-loaded SHRSP. Oxidative excess thus probably plays only a minor role in salt-induced changes observed in our study. Sources other than NADPH oxidase may contribute to reactive oxygen species in this experimental paradigm.

The protective effect of eplerenone on cardiac fibrosis is in agreement with other studies. In humans, eplerenone reduced death in heart failure¹ and left ventricular hypertrophy in hypertension.²⁰ In dogs with left ventricular dysfunction, eplerenone attenuated left ventricular remodeling.²¹ In con-

trast, a recent study showed severe heart failure and cardiac fibrosis in a knock-down model of the mineralocorticoid receptor, suggesting that mineralocorticoid receptors may be cardioprotective.²² However, in that study, cardiomyocyte-specific mineralocorticoid receptors were downregulated, without consideration of the noncardiac aldosterone system. Accordingly, it is difficult to reconcile those findings with data obtained in the present study.

There are few studies simultaneously addressing the effect of different levels of salt intake on aldosterone-induced end-organ damage in hypertension. We demonstrate significant effects of salt on target organ damage, prevented partly by eplerenone. Spironolactone prevented high salt-induced cardiac hypertrophy and fibrosis in normotensive Wistar rats²³ and media hypertrophy in salt-fed normotensive Sprague-Dawley rats.²⁴ Although in the present study blood pressure lowering with hydralazine improved media to lumen ratio and cardiac hypertrophy, it did not influence endothelial function or left ventricular fibrosis. Thus, it is likely that eplerenone had additional blood pressure-independent beneficial actions on target organs. This is supported by the reduction by eplerenone of cardiac collagen content in the right ventricle similar to that in the left ventricle. Studies on human²⁵ and rat²⁶ cardiac myofibroblasts support a direct and blood pressure-independent effect of aldosterone on cardiac fibrosis. A small reduction in right ventricular collagen content by hydralazine may be related to the profound blood pressure-lowering effect of this drug.

One mechanism of salt-induced hypertension is impaired pressure natriuresis, in which the renin-angiotensin-aldosterone system has been implicated.²⁷ Blockade of these effects could contribute to beneficial actions of mineralocorticoid antagonism. However, 24-hour sodium excretion was unchanged by eplerenone, although there may have been initial differences in natriuresis under treatment. Other studies have shown impaired suppression or a rise of plasma aldosterone in SHRSP under high-salt, stroke permissive diets. In the present study, plasma aldosterone levels decreased with salt loading. Enhanced local cardiac aldosterone production has been reported in SHRSP compared with WKY rats¹⁵ and under salt treatment in SHRSP¹⁴ and WKY rats.²⁸ Thus, aldosterone associated end-organ damage may be attributed to direct effects of aldosterone on cardiovascular tissues. Under low-salt conditions aldosterone levels are much higher with less end-organ damage and less effect of aldosterone antagonism. This underscores the degree to which high salt sensitizes tissues to aldosterone-induced cardiovascular injury.

Perspectives

Findings from the present study indicate a pivotal role of the level of salt intake on the effect of aldosterone on cardiac and vascular changes in hypertension. Our data provide important insights into the pathophysiological significance of salt in the role of mineralocorticoids such as aldosterone, and the therapeutic potential for mineralocorticoid receptor blockade for protection of the vasculature and the heart in salt-sensitive hypertension.

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