Cardiovascular effects of inhibition of renin-angiotensin-aldosterone system components in hypertensive rats given salt excess

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Susic D, Varagic J, Frohlich ED. Cardiovascular effects of inhibition of renin-angiotensin-aldosterone system components in hypertensive rats given salt excess. Am J Physiol Heart Circ Physiol 298: H1177–H1181, 2010. First published January 29, 2010; doi:10.1152/ajpheart.00866.2009.—This study examined the role of the renin-angiotensin-aldosterone system (RAAS) in mediating cardiovascular and renal damage in spontaneously hypertensive rats (SHR) given salt excess. Since the circulating RAAS is inhibited in this model, it permits examination of the role of local tissue RAASs in mediating this injury. To this end, male 8-wk SHR were divided into 7 groups. The control group (C) received normal NaCl (0.6%) diet. All other groups were given 8% NaCl chow. In addition, group 2 was given placebo, group 3 the mineralocorticoid receptor blocker eplerenone (100 mg·kg⁻¹·day⁻¹), group 4 the angiotensin converting enzyme inhibitor quinapril (3 mg·kg⁻¹·day⁻¹), group 5 the angiotensin II type 1 receptor blocker candesartan (10 mg·kg⁻¹·day⁻¹), and groups 6 and 7 eplerenone and either quinapril or candesartan. The treatments lasted 8 wk. Compared with controls, mean arterial pressure (MAP), renal blood flow, coronary flow reserve, minimal coronary vascular resistance, diastolic time constant, and maximal rate of ventricular pressure fall were all adversely affected by salt loading. Left ventricular mass and fibrosis as well as proteinuria were also markedly increased by salt overload. Eplerenone induced only slight changes, whereas quinapril and candesartan normalized all indexes except MAP. Combination therapy also normalized all indexes, including MAP. These data suggest that 1) cardiovascular and renal damage induced by salt excess in the SHR were not pressure dependent; 2) mineralocorticoids were only marginally involved in this model; and 3) local tissue generation of angiotensin II may be, at least in part, responsible for the other adverse effects.

ventricular function; angiotensin-converting enzyme inhibitor; angiotensin receptor blockade; mineralocorticoid receptor blocker

IT IS WELL ACCEPTED that salt excess exerts adverse cardiovascular and renal effects both in essential hypertensive patients and in naturally developing hypertension of the spontaneously hypertensive rats (SHR) (5, 6, 14, 18, 19, 30, 34). Previous studies from our laboratory also demonstrated detrimental severe structural and functional derangements in heart, aorta, vessels, and kidney in the SHR (1, 9, 31). Some of these adverse effects of salt overload were pressure related, but many of these studies demonstrated that salt overload also exerted its adverse cardiovascular and renal effects through pressure-independent mechanisms (3, 7, 9, 14, 15, 17–19, 34). We have also reported that, in salt-loaded SHR, treatment with angiotensin receptor blockers, either candesartan or losartan, ameliorated adverse cardiovascular and renal effects without reducing arterial pressure and, therefore, we have suggested that in this model local tissue renin-angiotensin-aldosterone system (RAAS) may be responsible for cardiac and renal damage of salt overload (31, 32).

This study was designed to examine the effects of isolated or combined blockade of various components of the RAAS on the development of myocardial hypertrophy, left ventricular (LV) and renal dysfunction, and coronary circulatory impairment in this SHR model with hypertensive cardiovascular damage that was exacerbated by dietary salt excess. To this end, the effects of either an angiotensin converting enzyme inhibitor, an angiotensin II type 1 receptor blocker, or a mineralocorticoid receptor blocker, either alone or in combination, were examined. Activity of the RAAS was not examined in this study since in this experimental setting numerous factors may affect its activity (salt load, target organ damage, myocardial and renal ischemia, treatment with agents that may affect activity of RAAS), which would make interpretation of data very difficult and potentially misleading.

MATERIALS AND METHODS

Animals. Male, 7-wk-old, SHR were purchased from Harlan Laboratories (Indianapolis, IN) and were maintained in a temperature- and humidity-controlled room with a 12-h light-dark cycle. All rats were handled in accordance with National Institute of Health guidelines, and our Institutional Animal Care and Use Committee approved the study protocol in advance.

Experimental protocol. At 8 wk of age, these rats weighed up to 180 g and were divided randomly into seven groups, with 15 rats in each. The first (control) group received no treatment and was given standard diet, containing 0.6% NaCl, with tap water ad libitum. All other rats were given rat chow containing 8% NaCl. All diets were obtained from Harlan-Teklad (Madison, WI). The second group was given an inert vehicle (distilled water). The third group was given the mineralocorticoid receptor blocker eplerenone (100 mg·kg⁻¹·day⁻¹). The fourth group was given the angiotensin converting enzyme inhibitor quinapril (3 mg·kg⁻¹·day⁻¹). Rats in the fifth group were given angiotensin II type 1 receptor blocker candesartan (10 mg·kg⁻¹·day⁻¹). Finally, the sixth and seventh groups were given eplerenone and either quinapril or candesartan. All agents were administered by gastric gavage.

Rats received their respective treatments for 8 wk. Body weight was measured weekly in all rats. During the last week of the experiment all rats were housed in metabolic cages for 24-h urine collections. Arterial pressure, LV function, cardiovascular mass indexes, extent of cardiac fibrosis, and renal functional indexes were examined at the end of the 8-wk study.

Six rats in the second and four in the third group died during the course of salt overloading with signs of heart failure (labored breathing, increased lung and cardiac masses at autopsy). These rats were replaced to maintain the size of the respective groups. We have previously reported increased mortality in salt-loaded young adult SHRs (1, 9). Technical failure was encountered in a few rats during determination of hemodynamics and heart function so that the final number of rats per group ranged from 10 to 14.

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Twenty four-hour urinary measurements. During the last week of treatment, all rats were placed in individual metabolic cages for 3 consecutive days. Urine was collected during the second and third days, urinary output was measured, and 24-h urinary protein (Lowry method) excretions were determined.

Systemic and regional hemodynamics and LV function. At the end of the study, all rats were anesthetized with pentobarbital (40 mg/kg ip) and the right carotid artery was cannulated with a transducer-tip catheter (Micro-Tip 3F, Millar Instruments) that was advanced into the left ventricle (LV). A second polyethylene catheter (PE-50) was placed into femoral artery. Both catheters were connected to a multichannel recorder (Grass Instrument) interfaced to an IBM computer with digital data acquisition system (EMKA Technologies) (32). Arterial pressure was measured via femoral artery catheter, and indexes of LV function, including LV end-diastolic pressure, the diastolic time constant (τ), and maximal rates of pressure rise and fall (dP/dt\text{max} and dP/dt\text{min}, were determined from LV pressure tracing (32). Both indexes of LV diastolic function, maximal rate of pressure decline and diastolic time constant, actually quantify LV isovolumic relaxation and are, therefore, better estimates of active ventricular relaxation than ventricular stiffness. When the natural log of LV diastolic pressure is plotted vs. time, τ equals the inverse slope of this linear relation. Stated in more simplified terms, τ is the time that it takes for LV pressure to fall by approximately two-thirds of its initial value.

After these measurements were obtained, the Millar catheter was withdrawn and a jugular vein and left ventricle were cannulated with polyethylene catheters (PE-50) for determination of systemic, coronary, and renal hemodynamics as described previously (26, 27). In brief, cardiac output was measured by the reference sample microsphere method as reported previously (26, 27). Cardiac index was calculated from cardiac output and body weight (expressed in ml/min 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 radiolabeled (⁴⁶Sc) microspheres to each organ at the end of the study (24, 26, 27). After the baseline measurements were obtained, maximal coronary vasodilation was produced by dipyridamole infusion (4 mg·kg⁻¹·min⁻¹ iv for 10 min) (26, 27) by using a Harvard infusion/withdrawal pump (Harvard Apparatus, South Natick, MA), and hemodynamic measurements were repeated with use of radiomicrospheres with a second radionuclide (⁹⁰Ru).

After the regional hemodynamic study, rats were killed with an overdose of pentobarbital and their heart, aorta, and kidneys were removed and weighed.

Left ventricular collagen concentration. As an estimate of ventricular collagen content, hydroxyproline concentration in the LV samples was determined and expressed as mg/g of dry weight (24).

Statistical analysis. All values are expressed as means ± SE. The differences between different groups were analyzed by ANOVA followed by a Bonferroni post hoc test for multigroup comparison (2).

All tests were two sided, and a value of \( P < 0.05 \) was considered to be of statistical significance.

RESULTS

Body weight and cardiovascular mass indexes. SHR given salt overload had a significantly lower body weight compared with controls, and therapy with any of these agents restored body weight to level seen in controls (Table 1). A great increase in LV mass was observed in salt-overloaded rats; eplerenone somewhat reduced LV mass, but other agents were more effective in reducing mass than eplerenone alone, although none of the applied therapies returned LV mass to that of the controls (group 1) (Table 1). Similarly, eplerenone only partially improved aortic and kidney weight indexes, but the other therapies were more effective. LV hydroxyproline, as an index of cardiac fibrosis, was significantly increased in the salt-overloaded SHR. This was unaffected by eplerenone, but it returned to the level seen in the controls in the groups given the other therapies (Table 1).

Systemic hemodynamics. Salt overload increased arterial pressure, and only the combination therapy (eplerenone plus quinapril or candesartan) reduced pressure to the level seen in controls (Table 2). Heart rate and cardiac index were not different among groups. Total peripheral resistance was increased (\( P < 0.05 \)) in salt-loaded SHRs, and it was restored to control values by combination therapy (Table 2).

Renal hemodynamics. Renal blood flow and vascular resistance were significantly (\( P < 0.05 \)) impaired by the salt overload (Fig. 1). Eplerenone only somewhat improved both variables, but the other therapies normalized kidney hemodynamics (Fig. 1).

Coronary hemodynamics. Coronary hemodynamics at basal conditions was not statistically affected either by salt overload or by various RAAS blockers. Thus coronary blood flow was 5.3 ± 0.5 ml-min⁻¹·g⁻¹ in the control group receiving regular-salt diet, 4.8 ± 0.8 in salt-loaded rats given placebo, 5.1 ± 0.5 in eplerenone-treated rats, 5.0 ± 0.5 in the quinapril group, 4.6 ± 0.3 in rats given candesartan, and 4.9 ± 0.4 and 5.0 ± 0.5 in the groups treated with eplerenone and either quinapril or candesartan, respectively. Similarly, coronary vascular resistance was not different among groups (i.e., 27.6 ± 2.3 ml-min⁻¹·g⁻¹ in the control group receiving regular-salt diet, 34.8 ± 2.3 in salt-loaded rats given placebo, 33.1 ± 2.6 in eplerenone-treated rats, 33.2 ± 2.4 in the quinapril group, 35.6 ± 2.5 in rats given candesartan, and 33.7 ± 2.1 and 30.5 ± 2.2 in the groups given combination therapy). However, minimal vascular resistance (determined after maximal coronary vasodilation with dipyridamole) was

<table>
<thead>
<tr>
<th>Table 1. Body weight and organ mass indexes</th>
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<tr>
<td>0.6% NaCl in Diet</td>
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<tr>
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</tr>
<tr>
<td>Body weight, g</td>
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<tr>
<td>LV weight index, mg/g</td>
</tr>
<tr>
<td>LV hydroxyproline, mg/g</td>
</tr>
<tr>
<td>RV weight index, mg/g</td>
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<tr>
<td>Aortic weight index, mg/mm</td>
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<td>Kidney weight index, mg/g</td>
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Values are means ± SE. E, eplerenone; Q, quinapril; C, candesartan; LV, left ventricular; RV, right ventricular. *\( P < 0.05 \) compared with controls; †\( P < 0.05 \) compared with salt-loaded, otherwise untreated rats (placebo).
greatly increased ($P < 0.05$) and coronary flow reserve (defined as the difference between flow at maximal vasodilation and basal flow) was greatly reduced ($P < 0.05$) in salt-overloaded and otherwise untreated rats (Fig. 2). Again, eplerenone alone was less effective in improving coronary hemodynamics than other therapies (Fig. 2).

**LV function.** There were no differences in LV end-diastolic pressure between the groups (Fig. 3). LV diastolic function was impaired significantly in the salt-loaded SHR, as indicated by their high mortality, increased arterial pressure, LV mass increase, myocardial fibrosis, impaired organ hemodynamics including renal and coronary, LV diastolic dysfunction, and gross proteinuria (1, 9, 17, 31). This is also consistent with findings in salt-loaded SHR, stroke-prone SHR, genetically hypertensive rats, and Dahl’s salt-sensitive rats (3, 13–15, 18, 34).

Arterial pressure rose modestly in salt-loaded rats, a finding consistent with previous reports (1, 9, 14, 17, 18, 31, 34). However, none of the three antihypertensive agents given alone lowered arterial pressure, which is in agreement with our previous results (32) but at variance with other reports (30, 33). It appears that “salt sensitivity” of arterial pressure depends on the age at which salt excess was given as well as on the time when interventions that modify the activity of the RAAS were initiated. Thus Mercier et al. (18) reported that if salt excess was given to SHR at an early age (4 wk) the increase in arterial pressure was not prevented by therapy with an angiotensin receptor blocker, valsartan. On the other hand, if salt load was initiated after 10 wk of age, the valsartan attenuated the salt-induced rise in arterial pressure (18). Indeed, modification of the renin-angiotensin system early in life is also known to affect salt sensitivity (8, 21). Thus lifelong administration of an angiotensin-converting enzyme inhibitor, captopril, increased salt sensitivity in SHR and Wistar-Kyoto rats, whereas administration of an angiotensin receptor blocking agent (losartan) during suckling reduced salt sensitivity in stroke-prone SHR (8, 21).

The mineralocorticoid receptor antagonist eplerenone was only modestly effective in reducing the adverse cardiovascular effects of salt overload in SHR in the present study. This is

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**Table 2. Systemic hemodynamics.**

<table>
<thead>
<tr>
<th></th>
<th>0.6% NaCl in Diet</th>
<th>Placebo, n = 12</th>
<th>Eplerenone, n = 11</th>
<th>Quinapril, n = 12</th>
<th>Candesartan, n = 10</th>
<th>E + Q, n = 12</th>
<th>E + C, n = 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic Pressure, mmHg</td>
<td>180 ± 4</td>
<td>209 ± 9</td>
<td>215 ± 7*</td>
<td>211 ± 7*</td>
<td>215 ± 7*</td>
<td>194 ± 5</td>
<td>187 ± 6</td>
</tr>
<tr>
<td>Diastolic Pressure, mmHg</td>
<td>131 ± 5</td>
<td>147 ± 6*</td>
<td>148 ± 6*</td>
<td>145 ± 4*</td>
<td>150 ± 6*</td>
<td>142 ± 6</td>
<td>130 ± 5†</td>
</tr>
<tr>
<td>Mean Pressure, mmHg</td>
<td>149 ± 4</td>
<td>173 ± 5*</td>
<td>168 ± 5*</td>
<td>171 ± 5*</td>
<td>169 ± 5*</td>
<td>163 ± 4</td>
<td>151 ± 6†</td>
</tr>
<tr>
<td>Heart Rate (beats/min)</td>
<td>402 ± 7</td>
<td>430 ± 7*</td>
<td>444 ± 8*</td>
<td>439 ± 8*</td>
<td>410 ± 5</td>
<td>409 ± 4†</td>
<td>411 ± 5†</td>
</tr>
<tr>
<td>Cardiac Index (ml/min/kg)</td>
<td>322 ± 11</td>
<td>304 ± 9</td>
<td>320 ± 13</td>
<td>314 ± 13</td>
<td>312 ± 14</td>
<td>309 ± 12</td>
<td>321 ± 12</td>
</tr>
<tr>
<td>Total Peripheral Resistance (u)</td>
<td>0.52 ± 0.03</td>
<td>0.64 ± 0.03*</td>
<td>0.69 ± 0.02*</td>
<td>0.59 ± 0.04</td>
<td>0.61 ± 0.04</td>
<td>0.56 ± 0.04</td>
<td>0.54 ± 0.03†</td>
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Values are means ± SE. *$P < 0.05$ compared with controls; †$P < 0.05$ compared with salt-loaded, otherwise untreated rats (placebo).
somewhat at odds with the results of our previous studies, which showed that eplerenone was very effective in ameliorating cardiovascular injury in adult SHR, as well as hypertension- and age-related cardiovascular injury in elderly SHR (25, 26). Furthermore, eplerenone was found to be effective in a number of other studies, particularly in salt/mineralocorticoid models (4, 16, 20, 22). In this regard, two factors are pertinent. First, in the previous studies eplerenone did reduce cardiovascular and renal injury in rat models of hypertension; however, it never completely prevented adverse effects (4, 15, 20, 22). These findings are similar to our results that eplerenone somewhat improved cardiovascular and renal consequences of salt overload. Second, our model is a model of very severe hypertension, characterized by body mass wasting and great target organ damage, in which the renin-angiotensin system plays a significant role. This is probably a reason for greater effectiveness of quinapril and candesartan over eplerenone.

Both the angiotensin converting enzyme inhibitor and the angiotensin II type 1 receptor antagonist prevented the adverse effects of salt loading in SHR, suggesting that angiotensin II mediates salt-related cardiovascular and renal injury. Plasma renin activity has been shown to be decreased in SHR given salt excess (10, 34). Yet, after 4 wk of salt loading, plasma angiotensin II concentration and urinary angiotensinogen excretion were found to be increased in SHR (10, 28). Thus it appears that systemic and/or local tissue RAAS in mediates adverse cardiovascular effects of salt. Furthermore, both quinapril and candesartan prevented cardiovascular and renal injuries in salt-loaded rats without affecting arterial pressure, also suggesting that the demonstrated adverse effects of salt overload are, at least partially, pressure independent. Combination therapy of eplerenone and candesartan was the only one intervention that was effective in preventing both salt-induced cardiovascular and renal structural and functional injury and arterial pressure increase.

A number of studies have suggested that local tissue RAAS may be involved in the pathophysiology of cardiovascular injury related to salt excess (11, 12, 23). Furthermore, our most recent results on the time course of changes in RAAS activity during salt loading support the notion that RAAS may mediate the adverse cardiovascular and renal effects of salt loading (28). Eight-week-old SHRs were given either regular rat chow or high-salt diet for 2 or 4 wk and they were studied when either 10 or 12 wk old. After 2 wk of salt loading a decrease in plasma renin activity and renal angiotensin II concentration was observed. However, no difference in plasma and heart angiotensin II concentration was observed between groups. Furthermore, urinary angiotensinogen excretion was increased in salt-loaded rats. Thus, at the beginning of salt loading, activity of the RAAS (originating from the juxtaglomerular apparatus) seems suppressed, although in some target organs, such as heart, angiotensin II concentration did not diminish. After 4 wk of salt loading plasma angiotensin II concentration increased three- to fourfold and urinary angiotensinogen excretion increased tenfold, and these changes were partially ameliorated by an angiotensin II receptor blocker, losartan. These findings of augmented plasma angiotensin II and urinary angiotensinogen levels in SHR on high salt suggest that an augmented RAAS contributes to the adverse cardiovascular and renal effects of salt loading.

In conclusion, excessive dietary salt intake has been shown epidemiologically to be associated with increased arterial pres-
sure in developed countries; however, structural and functional pathophysiological consequences of salt overload have not been fully appreciated. This study demonstrated that salt excess adversely affects cardiovascular and renal structure and function independent of pressure elevation. Our data further demonstrated that blockade of the RAAS prevented that harmful structural and functional effects of excessive salt intake. Finally, our presented findings suggest that systemic and/or local tissue RAAS participates importantly in the adverse effects of salt excess on heart and kidney.

GRANTS

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DISCLOSURES

No conflicts of interest are declared by the author(s).

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