Salt loading produces severe renal hemodynamic dysfunction independent of arterial pressure in spontaneously hypertensive rats

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Salt loading produces severe renal hemodynamic dysfunction independent of arterial pressure in spontaneously hypertensive rats. Am J Physiol Heart Circ Physiol 292: H814–H819, 2007. First published September 22, 2006; doi:10.1152/ajpheart.00671.2006.—We have previously shown that salt excess has adverse cardiac effects in spontaneously hypertensive rats (SHR), independent of its increased arterial pressure; however, the renal effects have not been reported. In the present study we evaluated the role of three levels of salt loading in SHR on renal function, systemic and renal hemodynamics, and glomerular dynamics. At 8 wk of age, rats were given 4% (n = 11), 6% (n = 9), or 8% (n = 11) salt-load diet for the ensuing 8 wk; control rats (n = 11) received standard chow (0.6% NaCl). Rats had weekly 24-h proteinuria and albuminuria quantified. At the end of salt loading, all rats had systemic and renal hemodynamics measured; glomerular dynamics were specially studied by renal micropuncture in the control, 4% and 6% salt-loaded rats. Proteinuria and albuminuria progressively increased by the second week of salt loading in the 6% and 8% salt-loaded rats. Mean arterial pressure increased minimally, and glomerular filtration rate decreased in all salt-loaded rats. The 6% and 8% salt-loaded rats demonstrated decreased renal plasma flow and increased renal vascular resistance and serum creatinine concentration. Furthermore, 4% and 6% salt-loaded rats had diminished single-nephron plasma flow and increased afferent and efferent arteriolar resistances; glomerular hydrostatic pressure also increased in the 6% salt-loaded rats. In conclusion, dietary salt loading as low as 4% dramatically deteriorated renal function, renal hemodynamics, and glomerular dynamics in SHR independent of a minimal further increase in arterial pressure. These findings support the concept of a strong independent causal relationship between salt excess and cardiovascular and renal injury.

MATERIALS AND METHODS

Forty-two male SHR were purchased from Harlan (Indianapolis, IN). A minimum of 1 wk was allowed to adjust to our animal-care facility. All rats were individually housed in a temperature- and humidity-controlled room with a 12-h:12-h light-dark cycle. Food and tap water were provided ad libitum. All rats were handled in accordance with National Institutes of Health guidelines, and our Institutional Animal Care and Use Committee approved the study in advance.

Experimental protocol. At 8 wk of age, rats weighing 180 to 200 g were divided randomly into four groups. The control rats (n = 11; group 1) were given standard chow (0.6% salt). The other groups were given chow with either 4% (n = 11; group 2), 6% (n = 9; group 3), or 8% salt loading (n = 11; group 4) over the ensuing 8 wk. All diets were obtained from Harlan-Teklad (Madison, WI). Rats had their body weight measured weekly. At the end of salt loading, all rats had systemic and renal hemodynamics determined. Additionally, those in groups 1, 2, and 3 were studied by renal micropuncture. Renal micropuncture was not performed in the 8% salt-loaded rats of group 4 due to their inability to endure this lengthy study.

Twenty-four-hour urinary measurements. Each week, each rat was placed in individual metabolic cages for three consecutive days and, on the third day, 24-h water intake and urinary output were measured, and 24-h urinary albumin (rat albumin EIA Kit, SPI-Bio), protein (Lowry method), sodium, and potassium (flame photometry) excretions were determined.

Renal hemodynamic and glomerular dynamic studies. At the end of the salt-loading period (16 wk of age), all rats were anesthetized with thiobutabarbital sodium (Inactin, 100 mg/kg ip; Sigma-Aldrich, St. Louis, MO) and placed on a heating pad to maintain rectal temperature at 37°C throughout the study. After tracheal intubation, a polyethylene catheter (PE-50) was inserted into the right femoral artery to permit blood sampling and measurement of mean arterial pressure and heart rate, which were recorded via a transducer (model...
SALT LOADING AND RENAL DYSFUNCTION

Table 1. Body and organ weights, 24-h water intake, urinary output, $U_{Na}$, and $U_K$

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>4% NaCl</th>
<th>6% NaCl</th>
<th>8% NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>11</td>
<td>11</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>337±5</td>
<td>341±5</td>
<td>329±10</td>
<td>290±14*</td>
</tr>
<tr>
<td>Left ventricle, mg/g</td>
<td>2.75±0.04</td>
<td>3.01±0.07</td>
<td>3.60±0.09*</td>
<td>3.84±0.19*</td>
</tr>
<tr>
<td>Right ventricle, mg/g</td>
<td>0.59±0.02</td>
<td>0.57±0.01</td>
<td>0.56±0.02</td>
<td>0.65±0.04</td>
</tr>
<tr>
<td>Left kidney, mg/g</td>
<td>3.41±0.10</td>
<td>3.72±0.04</td>
<td>3.84±0.11*</td>
<td>4.02±0.19*</td>
</tr>
<tr>
<td>Aorta, mg/g</td>
<td>3.05±0.09</td>
<td>3.50±0.12*</td>
<td>3.76±0.14*</td>
<td>4.51±0.23*</td>
</tr>
<tr>
<td>Water intake, ml/dl</td>
<td>32.9±1.9</td>
<td>48.1±1.8*</td>
<td>79.7±3.1*</td>
<td>86.3±6.7*</td>
</tr>
<tr>
<td>Urinary output, ml/dl</td>
<td>10.0±0.6</td>
<td>24.8±1.5*</td>
<td>49.9±3.3*</td>
<td>58.9±5.1*</td>
</tr>
<tr>
<td>$U_{Na}$, meq/dl</td>
<td>2.2±0.1</td>
<td>12.3±0.7*</td>
<td>19.3±1.1*</td>
<td>22.8±1.1*</td>
</tr>
<tr>
<td>$U_K$, meq/dl</td>
<td>4.3±0.2</td>
<td>3.1±0.2*</td>
<td>3.2±0.2</td>
<td>3.2±0.2*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of rats. $U_{Na}$ and $U_K$, sodium and potassium excretions, respectively. *P < 0.05 vs. control.

RESULTS

Body and organ weights and water balance. Body weight of the 8% salt-loaded rats decreased, presumably due to severe renal disease (Table 1). Left ventricular, aortic, and left kidney masses were increased in the 6% and 8% salt-loaded rats; aortic mass was also increased in the 4% salt-loaded rats. As expected, 24-h water intake, urinary output, and urinary sodium progressively increased in the rats from groups 1 to 2 and to 3 and to 4; and urinary potassium decreased in each salt-loaded group (Table 1).

Systemic and renal hemodynamics. Mean arterial pressure increased significantly, but minimally, in each salt-loaded group by the end of study (Fig. 1). Heart rate was not different between groups (control, 340 ± 5; 4%, 353 ± 6; 6%, 342 ± 6; and 8%, 356 ± 11 beats/min; not significant), and hematoctrit decreased significantly only in the 8% salt-loaded group [control, 50.1 ± 0.4%; 4%, 50.2 ± 0.9%; 6%, 47.5 ± 1.1%; and 8%, 46.2 ± 1.4% (P < 0.05 vs. control)]. Although filtration fraction did not differ, GFR decreased significantly in all salt-loaded rats (Fig. 2). Furthermore, the 6% and 8% salt-loaded rats had a decreased RPF and RBF [control, 6.42 ± 1.1%; and urinary sodium progressively increased in the rats from groups 1 to 2 and to 3 and to 4; and urinary potassium decreased in each salt-loaded group (Table 1)].

Glomerular dynamics. The 4% and 6% salt-loaded rats demonstrated a decreased single-nephron plasma flow and an
increased single-nephron filtration fraction, arteriolar efferent pressure, as well as afferent and efferent arteriolar resistances, although SNGFR, tubular pressure, and \( K_f \) did not diminish (Table 2). Additionally, stop-flow and glomerular hydrostatic pressures increased in the 6% salt-loaded rats (Table 2).

**Proteinuria and albuminuria.** By the second week of salt-loading 24-h proteinuria [2nd wk: control, 21.9 ± 1.0; 4%, 28.9 ± 1.5 (\( P < 0.05 \) vs. control); 6%, 30.0 ± 1.1 (\( P < 0.05 \) vs. control); and 8%, 33.2 ± 0.8 (\( P < 0.05 \) vs. control)] and albuminuria [2nd wk: control, 0.48 ± 0.04; 4%, 0.49 ± 0.05; 6%, 0.80 ± 0.07 (\( P < 0.05 \) vs. control); and 8%, 1.24 ± 0.18 mg/dl (\( P < 0.05 \) vs. control)] were increased in the 6% and 8% salt-loaded rats. For the 4% salt-loaded SHR, although proteinuria was significantly increased by week 2, it did not achieve statistical significance in the following weeks of salt loading. The differences among groups during the overall 8 wk follow-up period were even more evident as to proteinuria [8th

**Table 2. Glomerular dynamic changes after salt loading**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>4% NaCl</th>
<th>6% NaCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>( n )</td>
<td>11</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>SNGFR, nl/min</td>
<td>27.3±2.6</td>
<td>24.2±1.1</td>
<td>23.6±2.2</td>
</tr>
<tr>
<td>SNPF, nl/min</td>
<td>102±10</td>
<td>75±6*</td>
<td>70±6*</td>
</tr>
<tr>
<td>SNFF, %</td>
<td>27.3±1.8</td>
<td>34.6±2.3*</td>
<td>34.2±2.5*</td>
</tr>
<tr>
<td>( P_T ), mmHg</td>
<td>12.0±0.4</td>
<td>11.8±0.5</td>
<td>11.9±0.7</td>
</tr>
<tr>
<td>( P_E ), mmHg</td>
<td>15.9±0.2</td>
<td>17.8±0.5*</td>
<td>18.3±0.5*</td>
</tr>
<tr>
<td>SFP, mmHg</td>
<td>31.1±0.7</td>
<td>32.6±0.8</td>
<td>35.1±0.9*</td>
</tr>
<tr>
<td>( P_O ), mmHg</td>
<td>49.2±0.9</td>
<td>50.7±0.8</td>
<td>53.6±1.0*</td>
</tr>
<tr>
<td>( R_A, U )</td>
<td>5.6±0.5</td>
<td>7.8±0.6*</td>
<td>8.8±0.9*</td>
</tr>
<tr>
<td>( R_E, U )</td>
<td>1.6±0.2</td>
<td>2.2±0.2*</td>
<td>2.6±0.3*</td>
</tr>
<tr>
<td>( K_u ), nl·s^{-1}·mmHg^{-1}·g^{-1} )</td>
<td>0.037±0.005</td>
<td>0.040±0.005</td>
<td>0.029±0.004</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n \), number of rats. SNGFR, single-nephron glomerular filtration rate; SNPF, single-nephron plasma flow; SNFF, single-nephron filtration fraction; \( P_T \), tubular pressure; \( P_E \), efferent pressure; SFP, stop-flow pressure; \( P_O \), glomerular hydrostatic pressure; \( R_A \), afferent arteriolar resistance; \( R_E \), efferent arteriolar resistance; \( K_u \), ultrafiltration coefficient. *\( P < 0.05 \) vs. control.
wk: control, 27.1 ± 0.8; 4%, 33.2 ± 2.1; 6%, 108.3 ± 28.6 (P < 0.05 vs. control); and 8%, 113.9 ± 38.2 mg/dl (P < 0.05 vs. control)] as well to albuminuria [8th wk: control, 0.84 ± 0.09; 4%, 1.47 ± 0.23; 6%, 33.0 ± 11.0 (P < 0.05 vs. control); and 8%, 48.8 ± 23.2 mg/dl (P < 0.05 vs. control)]. It was of particular interest that when all the 24-h proteinuria and albuminuria data were analyzed over the entire course of the study, there was a significant difference in the slopes of proteinuria and albuminuria between each group of salt-loaded rats (Fig. 3). Thus salt loading in each group promoted significant renal damage, including the 4% salt-loaded rats.

Serum creatinine and histopathology. Serum creatinine concentration at the end of study was significantly increased in both 6% and 8% salt-loaded rats [control, 0.48 ± 0.02; 4%, 0.55 ± 0.04; 6%, 0.71 ± 0.08 (P < 0.05 vs. control); and 8%, 0.62 ± 0.04 mg/dl (P < 0.05 vs. control)]. Of particular interest, histological examination showed that all three salt-loaded groups developed nephrosclerosis, which was progressively more severe in the 6% and 8% salt-loaded rats (Fig. 4).

DISCUSSION

This study was designed to investigate the effects of salt loading on renal structure and hemodynamics, glomerular dynamics, and parenchimal function. The data clearly demonstrated that increments of dietary salt loading of separate groups only slightly, but significantly, increased arterial pressure as the renal functions became impaired. Thus salt excess promoted profound early renal dysfunction with high degrees of proteinuria and albuminuria as early as within 2 wk of salt loading and increased serum creatinine concentration at the end of the study.

Our data provide strong support for the concept that the detrimental effects of salt loading cannot be attributed only to rising arterial pressure (14, 15). We have been intensively investigating this subject for many years and have demonstrated that salt-induced exacerbation of hypertensive disease is far in excess of the increasing arterial pressure and total peripheral resistance (4, 5). We have also reported that, independent of the rise in arterial pressure, salt loading produced severe structural and functional cardiac changes in both normotensive and hypertensive rats (1, 13, 34, 35). Moreover, increased aortic mass, pulse pressure, and pulse-wave velocity and diminished aortic distensibility also occurred in the salt-loaded SHR (34). Others, in experimental and clinical studies, have also demonstrated an independent role of salt loading in determining structural and functional cardiovascular changes (20, 26, 38). These changes may also be reflected by an exacerbation of the clinical manifestations of heart disease in hypertension, including ventricular perforance, cardiac failure, and even endothelial dysfunction (12). Associated with the nonpressure-related effects of salt loading on cardiovascular structure and function, we have previously reported massive...
proteinuria after 8 wk of 8% salt loading that was prevented by angiotensin II type 1 (AT1) receptor antagonism, independent of arterial pressure changes (35).

In the present study, four groups of SHR were chronically fed with four different salt loads. All three salt-loaded groups (4%, 6%, and 8%) slightly, but significantly, increased arterial pressure while promoting dramatic deterioration of renal hemodynamics, glomerular dynamics, and parenchymal function. Although arterial pressure elevation increased progressively from one group to the next high-salt-loading group, those receiving the greatest salt load fared worse. In fact, these 8% salt-loaded rats decreased body weight and were unable to support the prolonged renal micropuncture procedure. Importantly, the 4% and 6% salt-loaded rats had severe glomerular dysfunction (even in the group that received the lesser amount of salt loading), demonstrating increased afferent and efferent arteriolar resistances associated with increased glomerular hydrostatic pressure, each important factors favoring the development and progression of nephrosclerosis in hypertensive patients. We emphasize that despite the great challenge of comparing the diet of rodents with that of human beings, the sodium content in the salt-loaded rats (especially the 4% load) may be comparable with the excessive salt intake in certain human populations (10, 28).

Early renal dysfunction was determined by the 24-h urinary protein and albumin excretions measured weekly during salt loading. These data clearly show an early and concomitant rise in both proteinuria and albuminuria after salt loading (i.e., by the second week), despite more profound increases over the entire course of study. Interestingly, several reports have also shown an increased proteinuria in untreated SHR with aging (11, 12). Taken together, these data indicate that proteinuria is a marker as sensitive as albuminuria in SHR and reflect the severity of vascular disease in the kidney. A high-salt diet has also been shown to be an independent factor promoting proteinuria or albuminuria in rats submitted to kidney transplantation (29), a high dose of angiotensin II infusion (16), and intrauterine stress (27) and in SHR that have a reduced number of nephrons (18).

In clinical studies, a high-salt diet has also been associated with renal injury. A large cohort of normotensive subjects and never-treated patients with essential hypertension has shown a progressive increase of albuminuria associated with high urinary sodium excretion (9). Moreover, in a population-based study, an independent association between high urinary sodium excretion and increased albuminuria has been documented and was even more evident in overweight subjects (36). In black patients with hypertension, salt reduction diminished blood pressure as well as proteinuria, but only the proteinuria was correlated significantly with urinary sodium excretion (31). Furthermore, a high-salt diet was not as effective in suppressing the intrarenal renin-angiotensin-aldosterone system (RAAS) in normotensive black subjects as in white subjects (25). The term “sodium glomerulopathy” was recently proposed to explain the independent effects of high-salt diet in determining renal damage in African Americans (2).

The mechanisms by which a high-salt diet promotes renal injury must be elucidated. The RAAS has been implicated as an important determinant of renal injury with salt loading, and participation of a local renal RAAS has been postulated (23, 33). Salt loading is well known to supress renin release from the renal juxtaglomerular apparatus and, hence, a reduction in systemically generated angiotensin II. However, experimental studies have demonstrated local participation of the RAAS in cardiac (37), vascular (23), and kidneys (22, 30, 32), suggesting that, despite systemic supression of the RAAS with salt loading, locally generated cardiac and renal RAAS may be stimulated by salt loading (33). In fact, improvement of proteinuria and renal hemodynamics after AT1 receptor antagonism (35) or aldosterone inhibition (6) have been reported in salt-loaded rats, unrelated to arterial pressure changes. Another mechanism whereby salt loading may operate in the kidney is by extracellular fluid expansion. Salt excess may promote fluid retention, thereby expanding plasma volume and blood pressure. Since the pressure rise is far less than the deterioration of renal and glomerular functions, this possibility now seems less likely. The transforming growth factor-β1 (TGF-β1) has been also implicated as an important factor for mediating the development of cardiac and renal fibrosis associated with salt loading, and overexpression of TGF-β1 has been reported in studies with salt-loaded rats (28). Others factors have also been associated with renal injury after salt loading, including oxidative stress (19), nitric oxide (3), and genetic mechanisms (17).

In conclusion, our data demonstrate a remarkable causal relationship between salt loading and renal hemodynamic, glomerular dynamic, and parenchymal functional injury independent of a minimal further increase in arterial pressure that reflects dramatic and severe pathophysiological changes. Salt loading produced a powerful and independent glomerular dysfunction and, hence, severe nephrosclerosis. These findings have important clinical applications, since even the lowest amount of salt intake (i.e., 4% NaCl), the most equivalent to increased salt consumption by patients, was a determinant of renal injury.

ACKNOWLEDGMENTS

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REFERENCES