Myocardial fibrosis, impaired coronary hemodynamics, and biventricular dysfunction in salt-loaded SHR

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Varagic, Jasmina, Edward D. Frohlich, Javier Diez, Dinko Susic, Jwari Ahn, Arantxa González, and Begona López. Myocardial fibrosis, impaired coronary hemodynamics, and biventricular dysfunction in salt-loaded SHR. Am J Physiol Heart Circ Physiol 290: H1503–H1509, 2006.—Arterial pressure in most experimental and clinical hypertensions is exacerbated by salt. The effects of salt excess on right and left ventricular (RV and LV, respectively) functions and their respective coronary vasodilatory responses have been less explored. We therefore examined the effects of 8 wk of NaCl excess (8% in food) on arterial pressure, RV and LV functions (maximal rate of increase and decrease of ventricular pressure; dP/dt max and dP/dt min), coronary hemodynamics (microspheres), and collagen content (hydroxyproline assay and collagen volume fraction) in young adult normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR), aged 16 wk by the end of the study. Prolonged salt excess in WKY and SHR elevated pressure only modestly, but it markedly increased LV mass, especially in SHR. Moreover, salt excess significantly impaired RV and LV diastolic function in SHR but only LV diastolic function in WKY rats. However, salt loading affected neither RV nor LV contractile function in both strains. Intersitial and perivascular collagen deposition was increased, whereas coronary vasodilatory responses to dipryidamole diminished in both ventricles in the salt-loaded SHR but not in WKY rats. Therefore, accumulation of ventricular collagen as well as altered myocardial perfusion importantly interrelate in initiation and/or progression of ventricular dysfunction (3, 4, 27, 29), the collagen content and coronary hemodynamics of both ventricles were also determined.

METHODS

Experimental protocol. Male WKY and SHR, purchased from Harlan (Indianapolis, IN), were maintained in a temperature and humidity-controlled room with a 12-h:12-h light-dark cycle. All rats were handled in accordance with National Institutes of Health guidelines, and our Institutional Animal Care and Use Committee approved the protocol of the study in advance. Before the study was initiated, all rats were screened echocardiographically to exclude congenital cardiac abnormalities (31). At 8 wk of age, the rats of each strain were divided into two groups (15 rats/group). One group was given an 8% NaCl diet (Harlan, Teklad) for 8 wk; the respective control group was administered a standard laboratory diet containing 0.7% NaCl. According to food intake, the salt ingestion did not differ between the salt-loaded groups (6.54 ± 0.19 vs. 6.79 ± 0.39 g·day−1·100 g body wt−1 in salt-loaded WKY and SHR, respectively). All rats were permitted free access to chow and tap water. Two separate groups of each strain (5–7 rats each) were kept under identical experimental conditions and were used exclusively for pathological examination. During the course of this study, three SHR receiving the high-salt diet died prematurely. None of the rats in the other groups died during the study, but the final numbers of rats reflected surgical failure.

LV Structure by transthoracic echocardiography. At the end of the salt-loading period, rats were anesthetized with pentobarbital sodium excess; left and right ventricular function; spontaneously hypertensive rats; Wistar-Kyoto rats.
(50 mg/kg ip), and transthoracic echocardiography (TTE) analysis was performed by using a commercially available echocardiographic system (Sonos 2000 with a 7.5-MHz transducer, Agilent Technologies) to evaluate LV geometry as described previously (1, 31). Systemic and ventricular hemodynamics and aortic stiffness. After echocardiographic examination was concluded, the right carotid artery was cannulated with a transducer-tipped catheter (Micro-Tip 3F, Millar Instruments) that was advanced into the ascending aorta for recording of arterial pressure. A second catheter was placed into the abdominal aorta through the femoral artery. Both arterial catheters were connected to a multichannel recorder (Grass Instrument) interfaced to an IBM computer with digital data acquisition system (EMKA Technologies). Aortic distensibility was determined from (echo)diameter/pressure changes during cardiac cycle and was normalized for diastolic diameter. For determination of pulse-wave velocity (PWV), pulse contours from the two catheters were registered simultaneously on the same channel, and PWV was calculated from the aortic length between the two catheters (measured postmortem) and the time difference between their diastolic notches. After aortic functional measurements were made, the catheters already placed in the ascending aorta and right jugular vein were advanced further into LV and RV, respectively. The maximal and end-diastolic pressures of both ventricles as well as the first derivatives of pressure over time (dP/dt_max and dP/dt_min) as indexes of global contractility and relaxation were recorded. After recording LV and RV hemodynamics, Millar catheters were replaced with polyethylene catheters (PE-50) that were advanced into the abdominal aorta (through the left femoral artery), LV, and jugular vein for determination of systemic and coronary hemodynamics (using the reference standard microsphere method) as we described previously (33). After the coronary hemodynamic study, the rats were then euthanized by an overdose of pentobarbital sodium, their heart and lungs were removed, and the ventricles were separated and weighed. As an estimate of ventricular collagen content, hydroxyproline concentration of the LV and RV samples were determined and expressed as milligrams per grams of dry weight, as previously described (1, 33).

### Table 1. Body mass, LVMI and RVMI and hydroxyproline concentrations in WKY and SHR receiving control or high-salt diet

<table>
<thead>
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<th>WKY</th>
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<th>SHR</th>
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<td>Control</td>
<td>Salt</td>
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<td>Salt</td>
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<tr>
<td>n</td>
<td>13</td>
<td>12</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>Body mass, g</td>
<td>309±4</td>
<td>321±6</td>
<td>346±7†</td>
<td>253±18†</td>
</tr>
<tr>
<td>LV mass, mg</td>
<td>748±12</td>
<td>994±13*</td>
<td>990±27†</td>
<td>1,159±56†</td>
</tr>
<tr>
<td>RV mass, mg</td>
<td>159±8</td>
<td>204±11*</td>
<td>173±4†</td>
<td>191±12†</td>
</tr>
<tr>
<td>LVMI, mg/g</td>
<td>2.42±0.02</td>
<td>3.11±0.05*</td>
<td>2.85±0.05†</td>
<td>4.70±0.22*†</td>
</tr>
<tr>
<td>RVMI, mg/g</td>
<td>0.50±0.02</td>
<td>0.63±0.03*</td>
<td>0.52±0.01</td>
<td>0.71±0.04*†</td>
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<tr>
<td>LV hydroxyproline, mg/g</td>
<td>3.36±0.11</td>
<td>3.5±0.19</td>
<td>4.32±0.22†</td>
<td>5.37±0.21*†</td>
</tr>
<tr>
<td>RV hydroxyproline, mg/g</td>
<td>5.71±0.28</td>
<td>5.38±0.39</td>
<td>5.41±0.22</td>
<td>6.87±0.52*†</td>
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Values are means ± SE; n, number of rats. LVMI and RVMI, left ventricular (LV) and right ventricular (RV) mass indexes, respectively; WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats. *P < 0.05, salt vs. control; †P < 0.05, SHR vs. WKY.

Fig. 1. Effects of salt loading on systemic hemodynamics in Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR). *P < 0.05, salt vs. control; †P < 0.05, SHR vs. WKY.
Pathological studies. After receiving their respective diets for 8 wk, the rats were euthanized with an overdose of pentobarbital sodium, and the hearts were removed and fixed in 10% formalin solution, dehydrated by routine methods, and embedded in paraffin for subsequent histological evaluation (35). The myocardial volume fraction occupied by collagen volume fraction (CVF) was determined by quantitative morphometry (35) using an automated image analysis system (AnalySYS, Soft Imaging System) in sections stained with collagen-specific picrosirius red.

Statistical analysis. All values are expressed as means ± SE. Data were analyzed by the use of ANOVA, followed by Newman test for multiple comparison. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

Salt loading promoted significantly decreased body weight in the SHR, but it had no such effect in the WKY rats (Table 1). When compared with the rats on the control diet, both WKY and SHR, maintained on the high salt intake, had higher mean arterial pressure (MAP) and total peripheral resistance index; cardiac output did not differ between groups (Fig. 1).

In both strains, LV and RV mass indexes increased with salt loading (Table 1). Echocardiographic LV examination revealed that the prolonged salt excess increased both the septal and posterior wall thickness in SHR; but in the WKY, only septal thickness increased (Table 2). This high dietary salt...
intake did not affect the LV diameter of either strain. Calculated relative wall thickness was markedly elevated only in the salt-loaded SHR (Table 2).

There was no difference between the groups with respect to LV end-diastolic pressure and contractility (dP/dmax/LVPmax), whereas LV dP/dmin/LVPmax was lower in the salt-loaded groups, suggesting impaired LV relaxation in both strains (Fig. 2). Salt excess in SHR significantly decreased RV dP/dmin/RVPmax, suggesting that it altered RV diastolic function as well. There were no differences in the RV hemodynamic indexes between the two WKY groups (Fig. 2).

Salt loading increased arterial pulse pressure in both strains, and this response was associated with diminished aortic distensibility (Table 3). These changes were more pronounced in the SHR (Table 3). These changes were more pronounced in the SHR (Table 3). There were no differences in the RV hemodynamic indexes between the two WKY groups (Fig. 2).

There were no differences between baseline coronary blood flows and vascular resistances in the salt-loaded groups compared with their respective controls (Table 4). However, minimal coronary vascular resistance, after dipyridamole infusion, was significantly higher in both ventricles of the salt-loaded SHR, and coronary flow reserve was correspondingly reduced in these rats (Table 4). The coronary vasodilatory responses to dipyridamole infusion in both ventricles were similar in the two WKY groups.

DISCUSSION

The results of this study confirmed our previous findings that salt loading in the SHR significantly impaired LV function associated with increased arterial pressure and LV mass (1). However, three novel findings emerged from this study. First, to the best of our knowledge, this is the first study to demonstrate impaired RV function in the SHR receiving increased salt intake. Second, in the salt-loaded SHR, impaired relaxation of both ventricles was associated not only with increased ventricular mass and greater fibrosis but also with profound impairment in coronary vasodilatory response to dipyridamole, thereby resulting in reduced coronary flow reserve. Finally, in the normotensive WKY rats, salt loading elicited only a modest increase in MAP and LV mass without increasing collagen content further; it also significantly impaired LV diastolic function.

The changes observed in the SHR were not obtained in the normotensive WKY rats. Until relatively recently, attention has not been directed to assess RV structure and function in systemic hypertension (2, 5, 24). Consequently, reports concerning RV involvement in response to salt loading are rare and even contradictory (19, 37). Moreover, its performance in response to dietary salt loading is unknown. In the present study, we clearly identified impaired RV relaxation in the SHR given high salt intake; this was not observed in WKY rats. We previously reported increased accumulation of collagen in the LV wall of salt-loaded SHR, and coronary flow reserve was correspondingly reduced in these rats (Table 4). The coronary vasodilatory responses to dipyridamole infusion in both ventricles were similar in the two WKY groups.

Finally, dietary salt excess significantly increased both RV and LV hydroxyproline concentration that was paralleled by histological findings of interstitial and perivascular fibrosis in the SHR but not in the WKY rats (Tables 1 and 5 and Fig. 3).

### Table 3. The effects of high dietary salt intake on PP, AMI AD and PWV in WKY and SHR

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<th>WKY</th>
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<tr>
<td>Control</td>
<td>Salt</td>
<td>Control</td>
</tr>
<tr>
<td>n</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>PP, mmHg</td>
<td>43 ± 2</td>
<td>61 ± 2*</td>
</tr>
<tr>
<td>AMI, mm/µg</td>
<td>0.85 ± 0.02</td>
<td>1.01 ± 0.02*</td>
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<tr>
<td>AD, mmHg⁻¹</td>
<td>7.64 ± 0.84</td>
<td>5.80 ± 0.59*</td>
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<tr>
<td>PWV, cm/s</td>
<td>357 ± 14</td>
<td>392 ± 13</td>
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Values are means ± SE; n, number of rats; PP, pulse pressure; AMI, aortic mass index; AD, aortic distensibility; PWV, pulse-wave velocity; *P < 0.05, salt vs. control; †P < 0.05, SHR vs. WKY.

### Table 4. LV and RV coronary hemodynamic indexes in control and salt-loaded WKY and SHR

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<th>WKY</th>
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<tr>
<td>n</td>
<td>13</td>
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<tr>
<td>CBF, ml/min/100g⁻¹</td>
<td>5.30 ± 0.69</td>
<td>5.53 ± 0.49</td>
</tr>
<tr>
<td>CVR, U/g</td>
<td>30 ± 4</td>
<td>31 ± 3</td>
</tr>
<tr>
<td>CVRmin, U/g</td>
<td>10 ± 1</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>CFR, ml/min/100g⁻¹</td>
<td>7.12 ± 0.84</td>
<td>5.56 ± 0.85</td>
</tr>
<tr>
<td>CBF, ml/min/100g⁻¹</td>
<td>4.25 ± 1.01</td>
<td>3.91 ± 0.52</td>
</tr>
<tr>
<td>CVR, U/g</td>
<td>44 ± 8</td>
<td>47 ± 4</td>
</tr>
<tr>
<td>CVRmin, U/g</td>
<td>7 ± 1</td>
<td>9 ± 1</td>
</tr>
<tr>
<td>CFR, ml/min/100g⁻¹</td>
<td>9.46 ± 1.42</td>
<td>9.34 ± 0.61</td>
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</table>

Values are means ± SE; n, number of rats; CBF, coronary blood flow; CVR, coronary vascular resistance; CVRmin, minimal coronary vascular resistance; CFR, coronary flow reserve; *P < 0.05, salt vs. control; †P < 0.05, SHR vs. WKY.
sin-aldosterone system (either systemically or locally produced) in salt-induced-cardiomyocyte hypertrophy, exaggerated extracellular matrix protein accumulation, as well as ventricular functional disturbances should be strongly considered (17, 32, 34, 38). However, the role of other local (10, 37) or circulating factors (13, 19, 20, 23, 28) cannot be excluded. Even direct hypertrophic effects of sodium per se on cardiomyocytes and vascular smooth muscle cells have been described (15).

Furthermore, an abnormal coronary vasodilatory response to dipyridamole was observed in both ventricles, and this might also have a role in deteriorating the ventricular functions. Increased deposition of interstitial CVF was associated with impaired ventricular relaxation and, hence, may have affected the coronary circulation because coronary blood flow occurs primarily during diastole. Moreover, perivascular fibrosis may have also impaired the vasodilatory ability. The importance of periarteriolar collagen for diminished coronary flow reserve was elucidated by Isoyama et al. (16). Normalization of arterial pressure after debanding of the rat aorta induced regression of medial hypertrophy, but normalization of coronary flow reserve was achieved only after the additional reversal of colla-
gen accumulation in the adventitia (16). Schwartzkopff et al. (29) reported that treatment with perindopril promoted an improvement in coronary flow reserve in hypertensive patients, and this hemodynamic response was associated with significant regression in periarteriolar fibrosis and slight, but not significant, reduction in medial thickness. Our data are in agreement with those of Yu et al. (37), who also noted increased perivascular and interstitial fibrosis in the LV of salt-loaded SHR; however, in their study, LV function and coronary hemodynamics were not examined. However, they did not observe any differences in RV mass index or fibrosis associated with salt loading in the SHR. We have no conclusive explanation for these divergent results, because the experimental conditions and animal ages were nearly identical. Additional studies are clearly necessary to resolve the question of RV involvement in the overall cardiac response to salt excess in SHR and other substrains.

Our results demonstrating that high salt intake only modestly increased arterial pressure and LV mass index in normotensive WKY rats are in agreement with previously published studies from other laboratories (19, 25, 37). Furthermore, we also demonstrated that salt loading promoted LV diastolic dysfunction. Of particular significance was the pronounced LV functional impairment in WKY rats receiving the high dietary salt intake compared with the SHR on the control diet (P < 0.05), even though arterial pressure, aortic distensibility, coronary hemodynamics, and LV mass of these two groups were similar. Thus, our data suggest an additional role of salt loading on ventricular performance. Because in this study salt loading neither produced ventricular fibrosis nor altered coronary hemodynamics in the WKY rats, other mechanisms must be considered. Interestingly, altered myocardial relaxation in compensated LVH was found even in the absence of significant changes in several molecular markers [e.g., sarcoplasmic reticulum Ca$^{2+}$-ATPase SERCA2, myosin heavy chain isoforms, and fibrillar collagen level changes] (26). In addition, our findings emphasize that a dietary salt load also increased RV mass but not cardiac index, and these findings support the previous notion about nonhemodynamic effects of salt loading (11, 12, 36). Furthermore, we have shown that salt loading did not provoke LV or RV fibrosis in normotensive WKY rats; and these results are in contrast with those studies of Yu et al. (37) and Lal et al. (19). Yu et al. demonstrated LV, but not RV, fibrosis in salt-loaded WKY rats, and Lal et al. reported salt-related fibrosis in both ventricles of Wistar rats. Nevertheless, the LV functional data of the present study still strongly support the contention of the important role that salt loading had in exacerbating effects of increased afterload on ventricular function.

The high-salt diet increased MAP as well as pulse pressure in both normotensive and hypertensive strains. Significant arterial stiffening was demonstrated by decreased aortic distensibility and increased PWV in the salt-loaded SHR. These changes contributed further to the observed LV diastolic dysfunction by increasing pulsatile load to the ventricle. Our results, therefore, are in agreement with reports from several laboratories (14, 18, 21, 30), underscoring the important relationship between dietary sodium intake and altered structure and function of large arteries.

In summary, our findings demonstrated that salt loading not only further increased arterial pressure and LV mass in SHR but also affected both RV and LV diastolic functions associated with impaired coronary hemodynamics and enhanced fibrosis. However, in the WKY rats, salt excess only modestly increased MAP and LV mass and did not promote ventricular collagen deposition or coronary hemodynamic alterations, although it did induce significant LV diastolic dysfunction.

Thus we believe primarily that the findings from the present study underline the necessity to explore the clinical as well as experimental scope of the adverse effects of salt on the RV as well. Second, the unique effects of salt loading on both ventricles in SHR, but not WKY rats, strongly suggest that nonhemodynamic mechanisms in hypertensive disease participate pathophysiologicaly with salt-loading hypertension. Therefore, these findings point to an inescapable conclusion that salt loading in hypertension should not be concerned only in terms of sensitivity of arterial pressure, but, perhaps even more importantly, there are a myriad of responses of the heart and other organs.

**GRANTS**

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**REFERENCES**


