Cardiac Hypertrophy and Performance of Dahl Hypertensive Rats on Graded Salt Diets

MARC A. PFEFFER, JANICE PFEFFER, ISRAEL MIRSKY, AND JUNICHI IWAI

With the Technical Assistance of CYNTHIA STEINBERG AND MARTHA HEINE

SUMMARY The relationship between arterial pressure and left ventricular (LV) functional capacity and LV mass during the natural development of cardiac hypertrophy was assessed in Dahl-resistant (R) and -sensitive (S) hypertensive rats maintained on three dietary NaCl regimens (0.4%, 4.0%, and 8.0% for 9 weeks, then 4.0%) from 5 until 20 weeks of age. In R rats, arterial pressure and LV mass were unaffected by diet. In contrast, S rats demonstrated levels of arterial pressure and LV hypertrophy that were graded according to dietary NaCl. Hemodynamic studies on rats under ether anesthesia demonstrated that the graded pressure elevation in S rats was produced by corresponding increases in total peripheral resistance, as cardiac output did not vary. During acute volume loading, the S rats on all diets achieved the same maximum stroke volume as did R rats, despite the marked increase in the arterial pressure of S rats. An analysis of the ejection fraction/afterload relationship demonstrated preserved contractile state. The ability of the left ventricle to generate pressure was increased in S rats in direct proportion to the degree of LV hypertrophy. Thus, in young adult S rats, cardiac performance was well compensated since pump and contractile functions were maintained and pressure-generating capacity was increased in relation to the degree of LV hypertrophy.

(Hypertension 6: 475-481, 1984)

KEY WORDS • hemodynamics • cardiac function • genetic hypertension • wall stress

THE level of arterial pressure of the Dahl hypertensive rats is a complex interaction between genetic and environmental factors. Although Dahl salt-sensitive (S) and salt-resistant (R) rats have been observed to have arterial pressure responses to a variety of environmental factors that are quantitatively different, these groups were originally selected and named for their respectivepressor responses to alterations in dietary sodium. While the arterial pressure level of the R rats is unperturbed even by large increases in dietary sodium, the S rats demonstrate a graded elevation of arterial pressure that is dependent on the sodium intake. Therefore, this study was undertaken to evaluate the cardiac responses to varying degrees of hypertension in Dahl R and S rats maintained on different salt diets and to determine whether left ventricular (LV) mass and functional capacity are related to the level of systemic hypertension.

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Supported by Grants HL 28238 and HL 12711 from the National Heart, Lung, and Blood Institute. Dr. M. Pfeffer is the recipient of an Established Investigatorship of the American Heart Association. Dr. J. Pfeffer is the recipient of National Institutes of Health Research Career Development Award HL 01186.

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Received July 19, 1983; revision accepted October 23, 1983.

Methods

Female Dahl R and S rats were obtained from the Brookhaven National Laboratories colony and were placed on either a 0.4%, 4.0%, or 8.0% NaCl diet (g NaCl/100 of food) at 5 weeks of age. The potassium content of all diets was 1.1 g/100 g of food. Since several early deaths were noted in the S rats on the 8% diet, the protocol was modified so that all of the R and S rats initially placed on the 8% diet for 8 to 10 weeks were then switched to the 4% diet. During the 36 hours of transit from Brookhaven to Boston, all rats were fed the 0.4% NaCl diet and had access to water. Except for the stipulated diets, all rats were housed under identical conditions. The dietary regimens of 0.4%, 4.0%, and 8%-to-4% NaCl have been termed low, moderate, and high, respectively.

Systolic arterial pressures were obtained at weekly intervals with an indirect tail cuff technique previously validated. When the rats were 18 to 22 weeks of age, hemodynamic studies were performed by a previously described technique. Each rat was anesthetized with ether, the right carotid artery and left jugular vein were cannulated, and the carotid catheter was advanced into the left ventricle and then withdrawn into the central aorta for the respective monitoring of the left ventricle and systemic arterial pressures. The trachea was cannulated and connected to a small animal respirator to sustain ventilation and ether anesthesia throughout the remainder of the study.
A midsternal thoracotomy was performed to allow placement of an electromagnetic flow probe around the ascending aorta. Mean flow through this vessel was designated as cardiac output (coronary flow was not included). Stroke volume was calculated by dividing cardiac output by heart rate; total peripheral resistance was calculated by dividing the difference between mean arterial and right arterial pressures by cardiac output. Weight-dependent variables such as cardiac output, stroke volume, and total peripheral resistance were normalized by dividing by body weight.

After the recording of stable baseline hemodynamic variables, maximal preload and afterload stresses were imposed on the left ventricle. 7 Maximal cardiac and stroke outputs were obtained during a 45-second infusion of Tyrode's solution (40 ml/min/kg) into a femoral vein. 6 The LV stroke work determined during the volume-loading procedures was calculated as the product of the difference between mean arterial and right atrial pressures, the stroke volume, and the conversion factor of 0.0136. With the return of blood flow and ventricular filling pressures to baseline values, the aortic flow probe was removed, and the carotid arterial catheter was advanced into the left ventricle for the continuous monitoring of systolic and end-diastolic pressures. Maximal afterload stress was then produced by a brief (1-second) aortic occlusion, which produced isovolumic ventricular contractions. 7 The peak developed pressure (LV systolic minus end-diastolic pressure) that was generated by each left ventricle was determined for every contraction during the aortic occlusion, and the average value served as an index of maximal pressure-generating capacity.

To characterize more fully the relationship between the flow and pressure-generating capacities of the left ventricle and its volume, the passive pressure-volume relationship of each left ventricle was determined following diastolic arrest of the heart with potassium chloride. A double lumen catheter was advanced across the aortic valve into the LV cavity and secured by a ligature around the atroventricular groove. Potential compressive effects of the right ventricle were eliminated by incising the right ventricular free wall. Duplicate pressure-volume curves were generated by emptying the ventricle by compression, then continuously infusing saline (0.68 ml/min) while simultaneously recording pressure over the range of negative 2 to 30 mm Hg. 8

LV pumping ability (peak stroke volume) was related to both chamber size (volume) and afterload. To derive an index of the ejection fraction for each rat, the peak stroke volume attained during the in vivo volume-loading procedure was divided by the LV volume at 20 mm Hg distending pressure obtained from the in vitro pressure-volume relationship. 8, 9 This ejection fraction index was related to LV afterload (systolic wall stress, \( \sigma_s \)) to more fully characterize the LV contractile state. Systolic wall stress, \( \sigma_s \), was defined as: \( \sigma_s = P_{AP}\left(\frac{2/3}{\pi}a^2(b^2_2 - a^2_2) + \left(\frac{1}{3}\right)a^2_2\right) \), where \( P_{AP} \) is the systolic arterial pressure; subscripts d and s denote end-diastole and end-systole, respectively; and a and b are the inner and outer radii, respectively, of a sphere. 10

Results

The body weights of the R and S rats on the various salt diets did not differ (Table 1). As anticipated from the selective breeding process, the arterial pressure of the R rats was unaffected by modifications in diet, whereas the arterial pressure of the S rats was increased in direct relation to the NaCl diet. Both indirect and direct techniques failed to detect any differences in systolic arterial pressure among the R rats on the three diets (Figure 1). In contrast, three distinct levels of systolic arterial pressure were detected in the S rats by both techniques (Figure 1). It should be emphasized that the S rats on the high salt diet actually were on 4% NaCl when these indirect pressures were taken and the hemodynamic studies performed.

LV weight reflected the respective level of arterial pressure of the R and S rats, since these weights did not differ among the R rats on the various diets, but were increased in relation to diet (and therefore arterial blood pressure) in the S rats (Table 1). The weight of the right ventricular free wall demonstrated the same pattern as that of the left ventricle in that it remained

### Table 1. Body and Ventricular Weights of Salt-Resistant (R) and Salt-Sensitive (S) Rats

<table>
<thead>
<tr>
<th>Weight</th>
<th>Salt intake: R rat</th>
<th>Salt intake: S rat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Moderate</td>
</tr>
<tr>
<td>No. of rats</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>262 ± 4</td>
<td>263 ± 7</td>
</tr>
<tr>
<td>Left ventricle (mg)</td>
<td>580 ± 18</td>
<td>590 ± 20</td>
</tr>
<tr>
<td>Right ventricle (mg)</td>
<td>140 ± 6</td>
<td>154 ± 7</td>
</tr>
</tbody>
</table>

*p < 0.01 vs respective low group.
†p < 0.01 vs respective moderate group.
unchanged in R rats and increased in relation to NaCl diet in S rats (Table 1). Although right ventricular pressures were not obtained in each hemodynamic study, it is of interest that these pressures were increased in S rats (pooled S vs pooled R, p < 0.001), most strikingly in those S rats on the high NaCl diet (Table 2).

Despite the progressive increase in LV systolic pressure in the S rats, LV end-diastolic pressure was not elevated (Table 2). The mean arterial pressure of S rats on the low NaCl diet was slightly higher than that of any R group. The increase in mean arterial pressure within the S rats in response to dietary NaCl was especially marked in rats on the high NaCl regimen; they exhibited levels of mean arterial pressure over 190 mm Hg (Table 2). The LV systolic and end-diastolic and mean arterial pressures of the three groups of R rats did not differ.

Baseline hemodynamic values indicated that the amount of dietary NaCl did not alter arterial pressure or cardiac output (index) and, therefore, total peripheral resistance (index) of the R rats (Figure 2). In contrast, the graded elevations in arterial pressure of the S rats were produced by corresponding elevations in the total peripheral resistance (index), since cardiac output (index) was not altered by diet (Figure 2). Despite the

**Table 2. Prethoracotomy Pressure and Heart Rate**

<table>
<thead>
<tr>
<th>Pressure and heart rate</th>
<th>Salt intake: R rat</th>
<th>Salt intake: S rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>Low</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td>121 ± 5</td>
<td>118 ± 3</td>
</tr>
<tr>
<td>LVP systolic</td>
<td>139 ± 4</td>
<td>136 ± 3</td>
</tr>
<tr>
<td>end-diastolic (mm Hg)</td>
<td>3 ± 0.4</td>
<td>3 ± 0.6</td>
</tr>
<tr>
<td>RVP systolic (mm Hg)</td>
<td>39 ± 3 (6)</td>
<td>37 ± 3 (5)</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>478 ± 10</td>
<td>437 ± 12</td>
</tr>
</tbody>
</table>

*p < 0.01 vs respective low group.
†p < 0.01 vs respective moderate group. n = sample size for right ventricular pressure. The sample size for other variables is the same as in Table 1. MAP = mean arterial pressure; LVP = left ventricular pressure; RVP = right ventricular pressure.
FIGURE 2. Baseline cardiac index (CI, upper panel) and total peripheral resistance index (TPRI, lower panel) of Dahl R (salt-resistant) and S (salt-sensitive) rats on the three dietary NaCl regimens. Within each group, alterations in NaCl did not produce changes in cardiac index. S rats demonstrated a graded effect of NaCl on total peripheral resistance (p < 0.001). * = different than respective low NaCl group, p < 0.01; † = different than respective low NaCl group, p < 0.05.

increased arterial pressure of the S rats, the heart rate of the group on high NaCl was faster than that of the S rats on low and moderate NaCl diets (Table 2). This observation was supported by analysis of the heart rates obtained in the unanesthetized state (during the determination of indirect tail cuff pressures) at which time the S rats on high NaCl had the fastest heart rate (R: low, 449 ± 9; moderate, 446 ± 11; high, 436 ± 6; S: low, 453 ± 6; moderate, 461 ± 6; and high, 484 ± 11 bpm, p < 0.05).

To assess the peak-pumping ability of the LV, the rats were subjected to acute volume loading. The maximum stroke volume (both absolute and indexed for body weight) attained by all of the R groups did not differ, and, despite the elevated arterial pressures of the S rats, these indexes of ventricular pumping ability also did not differ (Figure 3). Thus, even in the presence of severe systemic hypertension in the S rats on the high NaCl diet, normal values of baseline and peak flows were sustained. Calculations of external LV stroke work were therefore unaffected by diet in R rats and were progressively increased in S rats (Figure 3).

The ejection fraction index (derived from the passive pressure-volume relationship and the peak stroke volume of each rat) did not differ among all of the groups of R and S rats, except for the S rats on high NaCl in which this index was reduced (Figure 4). Similarly, all of the R and S groups had comparable values of systolic wall stress, except for the S rats on high NaCl in which wall stress was increased (Figure 4). The increased ratio of LV mass to cavitary volume of the S rats on low and moderate NaCl diets offset the elevated levels of pressure since systolic wall stress was within the range of that of the R rats. Only the S rats on the high NaCl diet demonstrated an elevation in systolic wall stress. Thus, the concomitant reduction in ejection fraction index appears to be appropriate for the increase in afterload since these values (and those of the other R and S groups) fell within the range (Figure 4, dotted line) of the ejection fraction index-afterload relationships.
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Figure 4. The ejection fraction index-afterload (peak systolic wall stress) relationship of the R (salt-resistant) and S (salt-sensitive) rats on the three NaCl diets (subscripts L, M, H refer to low, moderate, and high NaCl diets, respectively). The dashed line represents the ejection fraction index-afterload relationship of a separate group of 32 female normotensive rats (see reference 22) and is provided to demonstrate that the reduced ejection fraction index of the S high-NaCl group is appropriate for the level of wall stress.

Figure 5. The relationship of left ventricular developed pressure (Dev Pressure) to left ventricular weight to body weight ratio (LV/BW) of R (salt-resistant, X) and S (salt-sensitive, •) rats.

Discussion

The hemodynamic characteristics of only young Dahl R and S rats have been described previously. Ganguli and co-workers demonstrated a higher total peripheral resistance in 6-week-old Dahl S rats on an 8% NaCl diet for 1 week compared to R rats on the high salt diet or S rats on a low salt diet. The observation that the vascular resistance of perfused hindquarters of S rats on high NaCl was greater than that of S rats on low NaCl or that of R rats on either diet further supports the concept of a genetic difference in the response of vascular resistance to NaCl intake in the Dahl rats. In the present study, Dahl R and S rats were fed several dietary NaCl regimens. The graded hypertension that was produced in the S rats was associated with a normal cardiac output and an increased total peripheral resistance that was related to the magnitude of the pressure elevation. The levels of both arterial pressure and total peripheral resistance were unaltered in R rats on all diets and were increased in S rats in relation to the dietary NaCl regimen. It should be emphasized that, despite the fact that the S rats on high and moderate NaCl diets were both receiving 4% NaCl at the time of the hemodynamic study, the previous short-term (9-week) exposure to 8% NaCl in these S rats produced a lasting and measurable effect on arterial pressure levels.

It should also be noted that the S rats maintained on the low NaCl diet had increases in both arterial pressure and LV mass with respect to R rats (12% and 14%, respectively). These observations are congruent with the past experience of others with these colonies and are discussed in a recent excellent review.

The functional response of the left ventricle to experimental hypertension remains controversial. Some investigators believe that ventricular dysfunction occurs early and is inherent in the hypertrophy process, but others believe that a phase of compensated ventricular performance and hypertrophy can be demonstrated. The Dahl S rat provides a genetic model of systemic hypertension in which graded levels of arterial pressure can be produced. In the present study,
we have demonstrated that the LV performance of 20-week-old female Dahl S rats is well compensated. In these animals with mild, moderate, and severe systemic hypertension (mean arterial pressures of 135 ± 4, 148 ± 4, and 193 ± 6, mm Hg, respectively), baseline cardiac output was maintained at normal levels without an elevation in LV filling pressure or volume. The magnitude of this compensatory response can be illustrated by the following contrasting observations: the left ventricle of S rats on high NaCl ejected a normal stroke volume against an aortic systolic pressure of 222 ± 8 mm Hg whereas the left ventricle of R rats did not even shorten at comparable levels of pressure (peak systolic pressure during isovolumic contractions = 214 ± 4 mm Hg). Moreover, S rats with the most severe hypertension maintained the same reserve of pumping ability as R rats, since the peak cardiac output and stroke volume attained during acute volume loading in S rats were similar to those in R rats.

An analysis of the ejection fraction-index-aftload relationship confirmed the contention that contractile function was preserved in S rats. As in young spontaneously hypertensive rats (SHR) with compensated ventricular hypertrophy, the ejection fraction-aftload relation of the Dahl S rats fell within the range of that observed in female normotensive rats. This relationship is sensitive to alterations in the contractile state of the myocardium since SHR of advanced age demonstrated a reduction in ejection fraction index in the presence of normal systolic wall stress.

The increase in LV weight in the S rats was related to the level of arterial pressure: the LV weights of the S rats were 14%, 32%, and 54% greater than those of the R rats on their respective low, moderate, and high NaCl diets. In the R rats, alterations in NaCl diet did not affect either arterial pressure or LV weight. It is noteworthy that the weight of the right ventricular free wall of S rats was also increased, compared to that of R rats. Although measurements of right ventricular pressures were not obtained in all animals, S rats appeared to exhibit pulmonary as well as systemic hypertension. In the 20-week-old S rats studied, this elevation in right ventricular pressure could not be attributed to LV dysfunction. This unique observation of increased right ventricular pressure and weight in S rats suggests that the response to NaCl may be more generalized than previously considered and may involve the pulmonary as well as systemic vasculature.

In summary, Dahl S rats developed graded systemic hypertension and ventricular hypertrophy in response to NaCl intake. At the age of the rats examined in the present study, the cardiac response to systemic hypertension was adaptive in that the capacity of the ventricle to perform external work had been expanded beyond the range of the normotensive ventricle without encroaching on reserve capacity. In a sense, this adaptive cardiac response played a permissive role in the development of hypertension since the nonhypertrophied normotensive ventricle was not capable of sustaining forward output against the pressures observed in the S rats on high NaCl. The 20-week-old Dahl S rat and spontaneously hypertensive rat demonstrated that the presence of ventricular hypertrophy per se is not indicative of ventricular dysfunction, but is a compensatory response to a pathological cardiac-loading condition. The experimental manipulation of dietary sodium in the Dahl S rat was used to produce three levels of systemic hypertension and LV hypertrophy in genetically similar animals. This experimental design eliminated the controversy of appropriateness of control groups often encountered in studies of the SHR. The magnitude of the hypertrophic response and the augmentation of ventricular performance in the Dahl S rat are related to the extent of the systemic hypertension. However, as with any compensatory mechanism, there are inherent limitations to the adaptation. In the SHR, dysfunction of the left ventricle becomes apparent with advanced age and more marked ventricular hypertrophy. 6, 9, 10, 18, 21, 22 The ventricular performance of the aging Dahl rats has not been examined, and it remains to be determined whether the compensated hypertrophy observed in these 20-week-old animals can be sustained against longer durations of the pathologic load imposed by the severe hypertension.

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Cardiac hypertrophy and performance of Dahl hypertensive rats on graded salt diets.
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Hypertension. 1984;6:475-481
doi: 10.1161/01.HYP.6.4.475

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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