Differential Effect of Dietary Salt on Renal Growth in Dahl Salt-Sensitive and Salt-Resistant Rats

Cheryl Paschal McCormick, Albert L. Rauch, and Vardaman M. Buckalew Jr.

A high salt diet has been shown to increase renal mass of intact rats, although the mechanism by which this occurs has not been investigated. We used Dahl rats that are sensitive (DS) or resistant (DR) to the hypertensinogenic effect of salt to examine changes in renal size and composition caused by a high salt diet. Renal index, deoxyribonucleic acid (DNA), protein, water content, protein/DNA ratio, and cell number and size were measured in age-matched DR and DS on a high salt diet for 7, 14, or 28 days. The results were compared with those obtained from respective rats on a low salt diet. High salt diet elevated renal index and protein in DR and DS rats at each time point. After 7 days of a high salt diet, DNA increased in DS only. Protein/DNA ratio was progressively decreased by a high salt diet in DS and remained unchanged in DR rats. Cell number was increased 35% in DS versus only 13% in DR rats at 4 weeks. Cell size decreased 24% in DS and only 11% in DR rats. These results indicate that renal growth due to hyperplasia accompanies ingestion of a high salt diet in both DR and DS rats, but the rate of growth and the mechanism through which it occurs differ between strains. This difference may be important in delineating salt sensitivity and future development of hypertension.

Numerous studies of renal enlargement have been conducted after reduction of renal mass. The mechanism by which compensatory renal growth occurs has been ascribed mainly to hypertrophy, although a component of hyperplasia has been found in at least one study. Compensatory renal growth occurs independent of changes in blood pressure and many factors are known to modify its extent, including age, hormones, and diet.

We found only one published report of the effect of salt intake on renal growth in the absence of renal ablation. That study demonstrated increased renal mass after ingestion of a high salt diet by normal rats, but the mechanism of growth was not investigated. In order to gain further insight into this question, we have studied the effect of salt consumption on renal size and composition in Dahl rats sensitive (DS) or resistant (DR) to the hypertensinogenic effect of salt. The data permit analysis of the degree to which renal growth on a high salt diet is due to hyperplasia or hypertrophy and the detection of differences between the two strains.

Materials and Methods

Male DR and DS rats were obtained as 26- to 32-day-old weanlings from Brookhaven National Laboratories (Upton, New York), housed individually, and maintained on low salt chow containing 0.4% NaCl until introduction of high salt chow containing 8.0% NaCl. All animals in this study consumed standard rodent laboratory chow (5001, Purina Laboratories, Richmond, Indiana) with a protein content of 23% and a potassium content of 1.1%. The NaCl content of this chow was adjusted to yield either a low or high salt diet. Three experimental groups and one control group were designated with 10 rats from each strain in each group for a total of 80 rats.

High salt diet was introduced in a staggered manner so that all groups were studied at 8–9 weeks of age. The control group remained on low salt chow for the entire 28-day period of the experiment, and the experimental groups consumed high salt chow for 7, 14, or 28 days.
Blood Pressure

Systolic blood pressure was measured weekly by a photoelectric pulse device (Gilson Medical Electronics Inc., Middleton, Wisconsin) placed on the tail of unanesthetized, restrained rats warmed to 38°C for 10 minutes. Values for blood pressure were defined from the average of four measurements with variability not exceeding ±5% for each rat. Systolic blood pressure and body weights obtained immediately before decapitation were used for statistical comparison.

Tissue Harvest and Sample Preparation

Rats were decapitated and kidneys were excised immediately, blotted, and weighed. Renal index was calculated as the combined left and right kidney weight in grams divided by body weight in grams. The right kidney was frozen at −4°C for future deoxribonucleic acid (DNA) and protein determination, and the left kidney was dried for 48 hours at 100°C and reweighed. Renal water content was determined as the difference between wet and dry kidney weight divided by wet kidney weight.

Renal tissue for protein and DNA content was sliced perpendicular to the renal poles while still frozen. Tissue (70-415 mg) was homogenized in a total volume of 3 ml of distilled, deionized water in a glass-glass homogenizer (Kontes, Vineland, New Jersey) until a uniform suspension was obtained.

Protein Assay

Total renal protein was determined by Coomassie brilliant blue dye method. Coomassie dye (Biorad, Richmond, California) was diluted 1:4 with distilled, deionized water. Duplicate 5 μl aliquots of kidney suspension were added to 5 μl of the diluted dye reagent and absorbance was measured against a reagent blank at 595 nm. A standard curve was generated with bovine serum albumin.

DNA Assay

Total renal DNA was measured by modified Burton method after modified Schneider extraction. Perchloric acid (1.5 ml of 0.5N) was added to duplicate 400 μl aliquots of homogenized kidney suspension and calf thymus DNA standards (Sigma, St. Louis, Missouri). All tubes were incubated at 70°C for 30 minutes and cooled to room temperature. Diphenylamine reagent (2.5 ml) was then added to all tubes and incubated overnight in the dark. The next day, absorbance of all tubes was read at 600 nm against a reagent blank, and total DNA concentration was calculated.

Calculations and Statistical Analysis

Protein/DNA ratio was expressed as micrograms of total renal protein divided by micrograms of total renal DNA. Total number of nuclei per kidney and weight per nucleus were calculated by the methods of Enesco and LeBlond, assuming a constant DNA content of 6.2 pg per diploid nucleus.

All results are presented as mean±SEM. Each high salt group was compared with its respective low salt group of the same strain by analysis of variance and Dunnett’s multiple comparison test. Interstrain comparisons were made for rats on the same diet at the same time point by unpaired t test. Probability values <0.05 were considered significant.

Results

The body weights, measured before decapitation, of all groups are shown in Table 1. Similar weight gain during the course of the experiment indicates that all animals consumed similar quantities of the chow. Body weights of DR rats varied only in the 28-day high salt group, which displayed slightly lower body weight compared with DR rats of the same age on a low salt diet (p<0.05). DS rats on a high salt diet for 2 weeks gained more weight than DS of the same age on a low salt diet (p<0.05). However, consumption of the high salt diet for 4 weeks resulted in lower body weight in DS rats compared with those on the low salt diet (p<0.05). Table 1 also shows changes in group systolic blood pressure due to high salt diet. When compared with the respective strain at the same age on

<table>
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<tr>
<th>Table 1. Body Weight, Systolic Blood Pressure, and Kidney Water Content in Dahl Salt-Resistant and Salt-Sensitive Rats</th>
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<td><strong>Group</strong></td>
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<td><strong>Dahl salt-resistant rats</strong></td>
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All values are mean±SEM, n=10 for all groups.

*p<0.05; †p<0.01 compared with low salt groups of the same strain.
the low salt diet, systolic blood pressure of DR and DS rats increased after consumption of the high salt diet for 7 days and continued to rise in DS at a higher rate than DR rats. Blood pressure in DR rats after 4 weeks ranged from 118±2 mm Hg for the group on low salt chow to 144±4 mm Hg (p<0.01) for the group on high salt chow. The DS rats on the high salt diet for 4 weeks became hypertensive with systolic blood pressure increasing to 194±6 mm Hg compared with 131±2 mm Hg (p<0.01) for the DS rats on low salt diet. In addition, Table 1 displays renal tissue water content. Percent water content did not change in any high salt group when compared with respective low salt controls, with the exception of the DS rats on the high salt diet for 28 days.

The effect of a high salt diet on renal index is illustrated in Figure 1. The renal index was significantly elevated by NaCl in DR and DS rats at each time point examined when compared with respective rats on the low salt diet. The index, after 4 weeks, increased 12% in DR rats from a mean value of 9.1±0.08 for the group on low salt chow to 10.4±0.13 (p<0.01), for the group on high salt chow. In DS rats, renal index, after 4 weeks, increased 21% from a mean of 9.4±0.16 for the low salt group to 11.9±0.19 (p<0.01) for the high salt group. After 4 weeks of the high salt diet, a mean renal index of 11.9 in DS rats was significantly greater than 10.4 in DR rats (p<0.01).

Total renal protein is presented in Figure 2 for control and experimental groups from each strain. After only 1 week of high salt chow, the mean total protein was significantly increased in both strains compared with low salt controls. Two weeks of the high salt diet increased total renal protein in DR rats from 135±4.6 to 155±4.8 mg (p<0.01) and in DS rats from 139±7.6 to 167±3.9 mg (p<0.01).

After 4 weeks on the high salt diet, total protein of DR and DS rats was still significantly higher than respective low salt controls, but the absolute level of protein in DR rats decreased from 155±4.8 mg after 2 weeks to 147±5.3 mg after 4 weeks of the high salt diet. Similarly, total protein decreased in DS rats from 167±3.9 after 2 weeks to 155±7.7 mg after 4 weeks of the high salt diet.

Total renal DNA, also shown in Figure 2, was increased in DS but not DR rats after 1 week of the high salt diet (p<0.01). Total DNA was increased 16% in DR rats (p<0.01) and 29% in DS rats (p<0.01) after 2 weeks of the high salt diet when compared with DR and DS rats on the low salt diet. After 4 weeks of the high salt diet, the total DNA content of DR rats was increased by 13% (p<0.01 vs. low salt group) whereas DNA content of DS rats was increased by 35% (p<0.01 vs. low salt group).

Protein/DNA ratio shown in Figure 3 remained unchanged in DR rats after ingestion of the high salt diet. On the other hand, the DS rats displayed a significant decrease in protein/DNA ratio as the length of time on the high salt diet increased.

The calculated number of nuclei (number of cells) in the kidneys of DR and DS rats is shown in Figure 4. High salt diet increased the number of cells in the kidney of DS when compared with DR.
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Discussion

Increased renal weight may be due to an increase in the number of cells (hyperplasia), enlargement of existing cells (hypertrophy), or an increase in interstitial tissue, water, or fat. 10,11 One previous study in normal rats showed only that a high salt diet increased renal weight. 6 In the present study, the ability of a high sodium intake to increase renal weight was also demonstrated in Dahl rats. In addition, salt-induced renal growth was shown to be due to increased renal protein and DNA content in both DR and DS rats.

In DS rats, increments in renal index and DNA were greater by 13% and 25%, respectively, after 4 weeks of a high salt diet compared with those in DR rats. In addition, a high salt diet decreased the protein/DNA ratio of DS, but not DR rats. In DS rats, therefore, growth occurred due to hyperplasia with an increased number of smaller cells. In DR rats, hyperplasia also occurs, but the resulting cell size is not smaller. Therefore, hyperplasia appears dominant as a mechanism for increasing renal mass in the adult Dahl rat on a high salt diet, whereas hypertrophy has been described as the major component of compensatory renal growth. 12 These findings support the hypothesis that hypertrophy and hyperplasia are separate processes. 1

Conclusions concerning the mechanism of renal enlargement based on the protein/DNA ratio were confirmed in the present study by calculation of the number of diploid nuclei per kidney and weight per nucleus (Figure 4). These calculations assume constancy of DNA per nucleus, 13-18 diploid renal cells, 19-21 and a diploid nucleus containing 6.2 pg of DNA. 10 Although hepatic growth may involve the development of mononucleate and binucleate cells and polyploid nuclei, these events probably do not occur during renal growth. 10

It is possible that renal enlargement observed in DS rats could be due to compensatory hypertrophy of nephrons not damaged by salt consumption. In the event that damage and loss of nephrons occurred with hypertrophy of remaining nephrons, it would result in no change or a decrease in total DNA with an increase in the protein/DNA ratio. However, the present study gave opposite results, which argues for the predominance of hyperplasia. In addition, several histological studies 22-25 have only indicated the presence of an occasional dilated tubule in DS rats at all time points studied. The number of cells in the kidneys of DS rats on the high salt diet increased after 1, 2, and 4 weeks compared with DS rats on the low salt diet, whereas cell number in DR rats was increased only after 2 or 4 weeks of the high salt diet. The average weight per nucleus (cell size) is also displayed in Figure 4. After 1 and 4 weeks of the high salt diet, cell size was smaller in DS compared with DR rats. Those DR rats on the high salt diet for 2 and 4 weeks had significantly smaller cells than DR rats on the low salt diet, whereas DS rats on the high salt diet for 1, 2, and 4 weeks showed smaller cells compared with DS rats on the low salt diet.

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rats on a high salt diet. Morphometric studies are needed to confirm whether these observations are specific evidence of hypertrophy.

Increased workload in the form of elevated glomerular filtration rate subsequent to reduction of renal mass has been cited as a possible cause of compensatory renal growth. Glomerular hyperfiltration that results from elevated blood pressure cannot be excluded as a factor contributing to the renal growth observed in the Dahl rats consuming a high salt diet. However, Azar et al found that, despite the presence of increased superficial single nephron glomerular filtration rate only in DS when compared with DR rats on a high salt diet, there were no interstrain differences in kidney weight. In addition, renal enlargement occurred in both normotensive DR rats as well as DS rats in the present study, which argues for a dissociation of blood pressure and renal enlargement. Previous studies have not conclusively shown an effect of blood pressure on renal enlargement. We were also concerned about the effect of blood pressure on renal enlargement; we therefore examined intact, normotensive Sprague-Dawley rats after ingestion of a high salt diet for 1, 3, or 5 weeks and compared them with respective Sprague-Dawley rats on a low salt diet. The normotensive Sprague-Dawley rats on a high salt diet demonstrated renal enlargement. Therefore, hypertension or hypertension-induced hyperfiltration may not be major determinants of renal size in this model.

Sodium chloride may initiate renal growth via other mechanisms, including changes in circulating renal growth factors. These growth factors might alter membrane phospholipid metabolism or cyclic nucleotide concentration. We have recently demonstrated genetic differences in cardiac phospholipid metabolism in DR and DS rats on a high salt diet, differences that may also exist in the kidney and contribute to the variance observed in interstrain renal growth.

Genetic differences in renal sodium handling exist between DR and DS rats. Tobian et al demonstrated that the isolated, blood-perfused kidneys of DS rats excreted less sodium than those of the DR rats at the same perfusion pressure. Similar studies in isolated, perfused kidneys by Roman reached the same conclusions. These differences in renal sodium handling between DR and DS rats might be related to the differences in renal growth in response to a high sodium intake, although the mechanism by which this might occur is not apparent.

In conclusion, a high salt diet induces renal growth in DR and DS rats. However, the extent of enlargement and its mechanism differs between strains. These differences could be an important marker, reflecting genetic differences between salt-sensitive and salt-resistant individuals. Further investigation at the cellular level into changes that occur during growth, including histological examination and measurement of hormones, growth factors, and enzymes, is required to determine the pathophysiological significance of the differing renal growth response to a high salt diet in this model.

Acknowledgment

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**KEY WORDS** • salt • renal growth • Dahl rats
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