Sodium Intake Modulates the Development of Cardiac Hypertrophy in Two-Kidney, One Clip Rats

Alberto Gallo, Carlos M. Taquini, Maria Fontan, Roxana Campissi, Ivanna Kuraja, Hernan Gómez Llambi, and Alberto C. Taquini

Sodium homeostasis exerts a powerful influence on the cardiovascular system in normotensive and hypertensive animals. Previous studies indicate that factors other than blood pressure can influence cardiac hypertrophy. In the present experiments, we evaluated the effects of different sodium diets in the two-kidney, one clip hypertension model in the rat. After the renal artery had been clipped, the rats received a normal sodium (177 meq/kg), high sodium (517 meq/kg), and low sodium (7 meq/kg) diet during 4 weeks. The final blood pressure was almost the same in the three groups (normal sodium 170 ±12 mm Hg; low sodium 168 ±4 mm Hg; and high sodium 162 ±7 mm Hg). Sodium restriction significantly reduced the development of cardiac hypertrophy as compared with rats on normal or high sodium diets. Thus, ventricular weight and ventricular weight/body weight ratio were significantly higher in rats subjected to a normal or high sodium diet (p<0.01). The hypertrophied hearts of rats on normal and high sodium diets showed a larger increase in the number of cardiac β-adrenergic receptors than those observed in hearts from low sodium diet, clipped rats. These results show that sodium modulates the development of cardiac hypertrophy in two-kidney, one clip hypertensive rats. Similarly, the cardiac β-adrenergic receptors appear to be influenced by dietary sodium intake. A possible role of the sympathetic nervous system is suggested. (Hypertension 1990;15(suppl I):I-157-I-160)
Methods

Experiments were performed in male Wistar rats (n=84) weighing 280–300 g. Rats were anesthetized with ether. In half of them, a silver clip (0.22 mm gap) was placed in the left renal artery, and the right kidney was untouched (2K1C rats). A sham operation was performed in the other half of the rats. Subsequently, the rats were housed in metabolic cages and subjected to three different diets during 4 weeks. Groups C1 and S1 continued to receive regular rat chow (Purina, Cabeca, Buenos Aires, Argentina) (sodium, 177 meq/kg). Groups C2 and S2 received a sodium-deficient diet (sodium, 7 meq/kg); and groups C3 and S3 were fed with a high sodium diet (sodium, 517 meq/kg). The diet was prepared according to specifications from the Department of Bromatology, University of Buenos Aires. All rats received tap water ad libitum. To control the effectiveness of the diet, the volume of urinary sodium excretion (UNaV) was measured weekly in all animals (meq/kg/24 hr). Systolic blood pressure (SBP) (mm Hg) was measured twice weekly by the tail-cuff method in unanesthetized restrained rats, artificially warmed to 35° C for 10 minutes. Body weight (g) was also monitored weekly.

Four weeks after clipping and under ether anesthesia, the rats were killed, and the heart was rapidly excised. The connective tissue, atria, and aorta were removed, and the ventricles were blotted dry and rapidly weighed in a precision balance. The ventricles from four animals of each group were frozen in dry acetone and kept at -70° C until the moment of β-adrenergic receptor determinations. The rest of the ventricles were desiccated by repeated lyophilization until minimal weights remained constant on successive determinations. Ventricular dry weight (g) was also measured, and tissue water content was calculated by using the formula: 100−(dry wt/wet wt)×100.

Cardiac β-Adrenergic Receptors Studies

Protein was determined by the method of Lowry et al. β-Adrenergic receptors were measured according to the method of Williams and Lefkowitz. The membrane fragments were prepared, and the binding assay was done on the same day. The membrane suspension was incubated for 30 minutes at 25° C with varying concentrations of [3H]dihydroalprenolol (0.1–6 mmol/l) in the presence or absence of 10−5 mol/l propanolol. The incubation was ended by the addition of 5 ml Tris buffer, 50 nmol at pH 8. The suspension was immediately subjected to rapid filtration through a Whatman GF/B filter (Whatman Lab Sales, Inc., Hillsboro, Oregon), and the filters were washed three times with 5 ml buffer. Membrane-bound radioactivity was retained on the filters, which were dried and counted by liquid scintillation, and specific binding was determined by the total binding of [3H]dihydroalprenolol minus the binding that occurred in the presence of propanolol. The maximum number of receptor sites (Bmax) and dissociation constant (Kd) were calculated by Scatchard analysis and expressed as femtomoles per milligram membrane protein. The total ventricular receptors (fmol/ventricle) were calculated.

Statistical Analysis

The results are expressed as mean±SEM. Statistical analysis was done with linear regression analysis for Scatchard plots and by analysis of variance followed by a Newman-Keuls test to determine differences among individual groups. The significance of differences in SBP and body weight before and after treatment was determined by Student’s t test for paired groups. A 5% confidence (p value) level was used to define statistical significance.

Results

Body Weight

All animals showed a normal pattern of growth and no differences among dietary groups occurred during the experiments. At the time of killing, however, body weight was slightly but significantly lower in rats from group C3 (Table 1).
**Table 1. Effects of Different Sodium Diets in Two-Kidney, One Clip Rats and Controls on Systolic Blood Pressure, Body Weight, Absolute and Relative Heart Weight, Ventricular Dry Weight, Total Protein Content, and Cardiac \( \beta \)-Adrenergic Receptor Number**

<table>
<thead>
<tr>
<th>Group</th>
<th>SBP (mm Hg)</th>
<th>BW (g)</th>
<th>HW (g)</th>
<th>HW/BW ratio</th>
<th>VDW (g)</th>
<th>PRO (mg)</th>
<th>cBr (F mol/ventrical)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>170±12*</td>
<td>348±5</td>
<td>0.96±0.6**</td>
<td>2.73±0.17**</td>
<td>0.27±0.0**</td>
<td>206±7*</td>
<td>1,856±150**</td>
</tr>
<tr>
<td>C2</td>
<td>168±4*</td>
<td>337±9</td>
<td>0.84±0.2*</td>
<td>2.59±0.07*</td>
<td>0.24±0.0*</td>
<td>191±7*</td>
<td>958±76*</td>
</tr>
<tr>
<td>C3</td>
<td>162±7*</td>
<td>322±8</td>
<td>0.97±0.4**</td>
<td>3.09±0.14**</td>
<td>0.27±0.0**</td>
<td>195±12*</td>
<td>2,072±280**</td>
</tr>
<tr>
<td>S1</td>
<td>115±3</td>
<td>358±8</td>
<td>0.81±0.01</td>
<td>2.24±0.01</td>
<td>0.20±0.0</td>
<td>154±7</td>
<td>674±50</td>
</tr>
<tr>
<td>S2</td>
<td>118±2</td>
<td>347±12</td>
<td>0.76±0.01</td>
<td>2.18±0.05</td>
<td>0.21±0.0</td>
<td>143±18</td>
<td>695±59</td>
</tr>
<tr>
<td>S3</td>
<td>118±2</td>
<td>344±9</td>
<td>0.77±0.00</td>
<td>2.24±0.04</td>
<td>0.22±0.0</td>
<td>163±7</td>
<td>948±29</td>
</tr>
</tbody>
</table>

SBP, systolic blood pressure; BW, body weight; HW, heart weight; VDW, ventricular dry weight; PRO, total protein content; cBr, cardiac \( \beta \)-adrenergic receptor number; C1, clip-normal sodium; C2, clip-low sodium; C3, clip-high sodium; S1, sham-normal sodium; S2, sham-low sodium; S3, sham-high sodium.

*\( p<0.01 \) vs. S1, S2, and S3.
†\( p<0.01 \) vs. C2.

**Urinary Sodium Determinations**

Rats receiving the low sodium diet showed suppression of 24-hour sodium excretion to extremely low levels when compared with rats on a normal diet (0.32±0.03 vs. 4.54±0.73 meq/kg/24 hr, respectively, \( p<0.01 \)). Rats from the high sodium group excreted threefold the sodium of rats on the normal sodium diet (\( p<0.01 \)).

**Systolic Blood Pressure**

Seven days after surgery, the clipped rats showed an increase in SBP (C1, 115.5±1.3 to 155.5±3.0 mm Hg; C2, 119.5±4.0 to 150.2±3.0 mm Hg; and C3, 122.1±2.0 to 150.3±3.0 mm Hg; \( p<0.05 \)). A progressive increase up to the end of the fourth week was observed (C1, 170±12.0 mm Hg, \( p<0.05 \); C2, 168±4.0 mm Hg, \( p<0.05 \); and C3, 162±7.0 mm Hg, \( p<0.05 \)). All the sham-operated rats remained normotensive throughout all the experiments, without differences in values among the three groups.

**Heart Weight**

At killing, a significant degree of cardiac hypertrophy was found in groups C1, C2, and C3 when compared with the sham groups. Despite the similar unaltered and marked hypertension, however, the rats that received the low sodium diet (C2) showed a significantly lower heart weight and heart weight/body weight ratio than rats from groups C1 and C3 (\( p<0.05 \)). The heart weight and heart weight/body weight ratio were similar in sham rats on different diets.

**Tissue Water Contents**

The tissue water content was similar between the clipped and sham rats despite the diet (C1, 72%; C2, 72%; and C3, 72%) (S1, 75%; S2, 73%; and S3, 71%).

**Cardiac \( \beta \)-Adrenergic Receptors and Cardiac Protein Content**

The number of cardiac \( \beta \)-adrenergic receptors increased during ventricular hypertrophy; however, this increase was significantly lower in the low sodium group (\( p<0.05 \)). No changes in \( K_d \) and \( B_{max} \) were observed. Total proteins were increased in clipped rats.

**Discussion**

It is accepted that cardiac hypertrophy is not the result of a single alteration. Differences between hypertrophy because of differing pressure loads and other causes have been demonstrated but the variations found among different types of pressure load are less well known.

Cardiac hypertrophy in systemic hypertension can be induced and maintained by factors other than blood pressure. Development of ventricular hypertrophy in spontaneously hypertensive rats and its reversal during medical treatment does not depend on blood pressure alone.\(^6\) Hemodynamic disturbances (e.g., heart rate and cardiac output), cardiac adrenergic drive, and the renin-angiotensin system, might be involved in the development-regression mechanisms.\(^5\)-\(^13\) Moreover, certain vasodilating agents that significantly reduce arterial pressure and total peripheral resistance do not cause regression of hypertrophy, whereas other agents diminish ventricular mass despite a smaller reduction in blood pressure and resistance.\(^13\)

In 2K1C hypertension, a positive correlation between blood pressure and the weight of the ventricles during both development and regression of cardiac hypertrophy has been observed;\(^7\) ventricular weight was reduced significantly when arterial pressure was normalized by surgical reversal of renal stenosis, by medical treatment,\(^7\),\(^14\) or by both. Recently, however, a similar dissociation between blood pressure and cardiac hypertrophy in the 2K1C rat was demonstrated, that is, sodium restriction resulted in reversal of cardiac hypertrophy but did not lower blood pressure.\(^8\)

Our results show that neither a low sodium diet nor a high sodium diet given during 4 weeks resulted in any significant change in blood pressure in sham-operated rats. Similarly, in the clipped rats, the same elevation of SBP was observed independently of the given diet. This result corresponds with our previous studies of this rat in which the development of hypertension remained unaffected.
by the institution of a sodium-deficient or high sodium diet. Moreover, in the present study, we were able to demonstrate that sodium restriction modulated the development of cardiac hypertrophy in 2K1C renal hypertension.

Our data do not allow us to pinpoint the mechanism by which sodium restriction modulated the development of cardiac hypertrophy. It is well known that sodium homeostasis can directly influence the cardiac cell ionically or indirectly through the modulation of neurohumoral systems such as the renin-angiotensin system, vasopressin, prostaglandins, and the atrial natriuretic factor, all of which have cardiovascular effects.

On the other hand, the sympathetic nervous system and catecholamines have important roles in the development and regression of cardiac hypertrophy. In fact, suppressor doses of norepinephrine or synthetic catecholamines will similarly produce ventricular hypertrophy. Conversely, centrally acting antiadrenergic agents produce a regression of left ventricular mass even when arterial pressure is not completely controlled. Because sodium homeostasis interferes with the reactivity of the sympathetic nervous system, dietary salt intake can play a pathogenetic role in the process of left ventricular hypertrophy. Thus, in renal hypertensive rats, dietary sodium restriction led to a significant reduction in heart weight as compared with rats on a normal diet although hypertension was not reversed. Furthermore, in these rats, sodium restriction restored tissue catecholamine content. Differences in cardiac β-adrenergic receptor number have been observed during cardiac hypertrophy in 2K1C hypertension. In agreement with previous results, we found an increase in the number of cardiac β-adrenergic receptors 4 weeks after clipping in rats subjected to normal, low, and high sodium diets, however, the increase was significantly smaller in low sodium hypertensive rats. Similarly, sham-operated rats on a high sodium diet have a significant increase in β-adrenergic receptor numbers. Recent studies suggest that dietary sodium alters the catecholamine levels within the physiological range, and these changes can influence the number of β-adrenergic receptors. In fact, Fraser et al showed that the white cells of normal subjects on the high sodium diet had 50% more β-adrenergic receptors than those white cells of subjects consuming a low sodium diet. This increase in sodium intake reduced catecholamine levels and, by reducing down-regulation, allowed the β-adrenergic receptor numbers to increase.

On the other hand, an increase in tissue norepinephrine, epinephrine, and dopamine has been observed in 2K1C hypertensive rats on the low sodium diet when compared with animals on the normal diet. Thus, it is possible that sodium intake can modify the neurotransmitter release and that the changes in β-adrenergic receptors observed by us could be an expression of down-regulation of the heart’s β-adrenergic receptors. The results of this study indicate that a low sodium diet can influence the degree of cardiac hypertrophy secondary to hypertension and that β-adrenergic receptor number is similarly modulated by a sodium diet. These observations raise the possibility of sympathetic nervous system involvement in the severity of cardiac hypertrophy under these conditions. The exact mechanism, however, is now unknown, and in future studies, it might be important to correlate cardiac hypertrophy with parameters for afterload more sensitive than SBP. Comparison of right and left ventricular characteristics might more clearly delineate the effects of systemic hypertension and subsequent treatment.

Key Words • blood pressure • renovascular hypertension • β-adrenergic receptors • cardiac hypertrophy

References

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