Isomyosin Transitions in Ventricles of Aldosterone-Salt Hypertensive Rats

ANNE F. MARTIN, RICHARD J. PAUL, AND ELLEN G. McMAHON

SUMMARY The isomyosin composition in left and right ventricles from aldosterone-salt-treated hypertensive rats and from vehicle-infused and aldosterone-infused normotensive control rats was compared. A significant incremental increase (20%) in the percentage of V2 isomyosin and parallel decrease in the percentage of V1 isomyosin occurred in both left and right ventricles from aldosterone-salt-treated animals compared with those in normotensive vehicle-infused controls. No change in the ventricular isomyosin distribution was observed in animals infused with aldosterone without salt, which indicates that aldosterone does not directly affect the ventricular isomyosin composition. The changes in left ventricular isomyosin composition were accompanied by significant left ventricular hypertrophy (38%; p < 0.05), whereas no hypertrophy was observed in the right ventricle. Plasma thyroxine levels were significantly lower in aldosterone-salt-treated rats (3.7 ± 0.6 μg/dl; p < 0.05) than in normotensive vehicle-infused (6.0 ± 0.7 μg/dl) or aldosterone-infused (6.7 ± 0.3 μg/dl) controls. These results indicate that factors such as alterations in thyroid status or a volume overload component of this hypertensive model, in addition to increased systolic blood pressure, may contribute to a biventricular shift in isomyosin composition in the aldosterone-salt model of hypertension. (Hypertension 8: 128–132, 1986)

KEY WORDS · hypertrophy · hypertension · heart · isomyosin · aldosterone

CARDIAC hypertrophy is commonly associated with the development of increased blood pressure in a number of experimental animal models of hypertension. However, the relationship between hypertension and cardiac enlargement is complicated in that vasodialator drugs such as hydralazine and minoxidil, which reduce systolic blood pressure, do not produce regression of cardiac hypertrophy. Thus, it has been suggested that other factors in addition to the pressure load may be involved in determining the degree of ventricular hypertrophy in experimental models of hypertension.

Cardiac hypertrophy produced by an increased pressure or volume load frequently is accompanied by changes in myosin adenosine triphosphatase (ATPase) activity and mechanical properties of the heart. A major factor in these changes has been shown to be alterations in the ventricular myosin composition.

Two myosin heavy chain genes are expressed in rat and rabbit ventricle, designated HCα and HCβ, and these result in three native isomyosins, V1 (HCαα), V2 (HCαβ), and V1 (HCββ). The individual isomyosins have different Ca2+-activated ATPase activities and can be separated electrophoretically. The ventricular myosin composition in rat and rabbit changes during development and can be modified by hormonal and nutritional factors in addition to changes in work load and exercise. Alterations in the ventricular myosin composition have been reported in spontaneously hypertensive rats and renal hypertensive rats. Regulation of the expression of the genes for myosin HCα and HCβ by thyroid hormone has been demonstrated in both rat and rabbit hearts.

In this study we attempted to characterize the development of cardiac hypertrophy and isomyosin composition in hearts from the aldosterone-salt hypertensive rat. This model of mineralocorticoid-dependent hypertension has the advantage that hypertension is produced readily in response to the administration of physiological doses of aldosterone. Furthermore, by replacing the saline drinking fluid with water, normotensive animals with elevated levels of aldosterone similar to those of hypertensive animals can be produced. These animals serve as controls for nonspecific effects of the aldosterone itself.
Materials and Methods

Male Sprague-Dawley rats, approximately 6 weeks old and weighing 150 g, were randomly assigned into three groups: control, aldosterone (without salt), and aldosterone-salt. The aldosterone-salt hypertensive rat model has been described in detail previously. Briefly, the rats in all groups were uninephrectomized. An osmotic minipump (Alza, Palo Alto, CA, USA), implanted subcutaneously, was used to administer d-aldosterone (Sigma Chemical, St. Louis, MO, USA), 0.25 μg/hr, to the animals for 4 weeks. Animals in the control group were implanted with a minipump containing only the vehicle, polyethylene glycol. Ether anesthesia was used in all surgical procedures. Control rats received 1% (wt/vol) NaCl in the drinking water, while the aldosterone-salt–treated group received 1% (wt/vol) NaCl supplemented with 0.3% (wt/vol) KCl to minimize weight loss. A third group of animals was treated with aldosterone but received no salt in the drinking water. Systolic blood pressure was measured using the tail cuff technique.

On the morning of the experiment, the animals were decapitated and the blood was collected for plasma thyroxine (T₄) measurements. The heart was rapidly excised, the atria and extraneous tissue removed, and the left and right ventricles dissected. The left ventricular free wall plus septum and the right ventricle were weighed separately and frozen immediately in liquid nitrogen. Frozen tissue was pulverized in a stainless steel percussion mortar, precooled in liquid nitrogen. Frozen tissue was stored at -80°C.

Myosin was extracted from approximately 100 mg of the frozen pulverized tissue in 1 ml of 0.1 M Na₃P₂O₇, 5 mM ethylene glycol bis(β-aminoethyl ether)N,N,N′,N′-tetraacetic acid (EGTA), 5 mM dithiothreitol, and leupeptin, 5 μg/ml, pH 8.6, as described by Hoh et al.

Isomyosins were separated by electrophoresis on polyacrylamide gels, under nondissociating conditions as described by Hoh et al. The gels were fixed in a solution of 10% trichloracetic acid and 50% methanol before staining with sodium anazolene (Coomassie blue R; 0.03% in 25% isopropanol and 10% acetic acid). Following destaining in 7.5% acetic acid, the gels were scanned under safelight conditions. Isomyosin bands were quantified by measuring the density of each band using a commercial imaging system (Immmorphase; Corning Medical and Scientific, Medfield, MA, USA). The data were expressed as means ± standard error of the mean (SEM).

Results

Infusion of aldosterone into rats for 4 weeks in conjunction with salt-supplemented drinking water produced a significant increase in systolic blood pressure compared with that in both control and aldosterone-infused rats given no salt in their drinking water (Table 1). The rate of aldosterone infusion used (0.25 μg/hr) has previously been found to produce a consistent level of hypertension at plasma levels of aldosterone within the “stressed” physiological range. Animals treated with aldosterone without salt had slightly higher systolic blood pressures but no change in body weights compared with controls (see Table 1). The blood pressure in aldosterone-treated animals was within the range of values reported for blood pressures in normal control rats. Body weights in aldosterone-salt–treated animals were significantly lower than in controls, although all animals continued to gain weight throughout the study. Left and right ventricular weights were unchanged in both the aldosterone-treated and aldosterone-salt–treated rats compared with those in the control animals.

To assess the presence of left or right ventricular hypertrophy in the hypertensive aldosterone-salt–treated animals, we determined the normal relationship between left ventricular weight and body weight (Figure 1) and right ventricular weight and body weight were estimated from the area under the peaks as previously described; the relative amount of the V₁ isomyosin peak was distributed equally between V₁ and V₃. Each sample was run on three separate gels, and the results from each gel scan were combined to obtain a final estimate of the proportions of V₁ and V₃ isomyosins present.

Blood plasma levels of thyroxine (T₄) were measured using a commercial radioimmunoassay system (Immmorphase; Corning Medical and Scientific, Medfield, MA, USA). The data are expressed as means ± standard error of the mean (SEM). Intergroup comparisons, when appropriate, were performed using one-way analysis of variance. The significance of differences between control, aldosterone-treated, and aldosterone-salt–treated groups was tested using the Bonferroni approach for three comparisons. A p value less than 0.05 was considered statistically significant.

Table 1. Blood Pressure, Body Weight, and Ventricular Weights in Control, Aldosterone-Treated, and Aldosterone-Salt-Treated Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood pressure (mm Hg)</th>
<th>Body weight (g)</th>
<th>LV weight (g)</th>
<th>LV weight calculated* (g)</th>
<th>RV weight (g)</th>
<th>RV weight calculated* (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 9)</td>
<td>116 ± 5</td>
<td>320 ± 9</td>
<td>0.68 ± 0.02</td>
<td>0.66 ± 0.02</td>
<td>0.17 ± 0.01</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>Aldosterone (n = 6)</td>
<td>139 ± 3</td>
<td>353 ± 13</td>
<td>0.75 ± 0.03</td>
<td>0.71 ± 0.02</td>
<td>0.18 ± 0.01</td>
<td>0.19 ± 0.01</td>
</tr>
<tr>
<td>Aldosterone-salt (n = 9)</td>
<td>205 ± 7†</td>
<td>256 ± 15</td>
<td>0.78 ± 0.04</td>
<td>0.55 ± 0.02</td>
<td>0.16 ± 0.01</td>
<td>0.16 ± 0.01</td>
</tr>
</tbody>
</table>

Data are means ± SEM. LV = left ventricular; RV = right ventricular.
*Calculated ventricular weight was obtained from regression lines in Figures 1 and 2.
†p < 0.01, compared with values in control or aldosterone-treated groups; ‡p < 0.01, compared with calculated LV weight.
FIGURE 1. Relation between left ventricular weight and body weight. Data were analyzed by least-squares regression: intercept = 0.1264; slope = 1.658 × 10^-3; r = 0.95 in 47 normal rats (O). Mean values ± SEM (bars) for 9 control rats (•), 6 aldosterone-treated rats (A), and 9 aldosterone-salt-treated rats (■) are also shown.

FIGURE 2. Relation between right ventricular weight and body weight. Data were analyzed by least-squares regression: intercept = 0.0606; slope = 0.3680 × 10^-3; r = 0.85 in 47 normal rats (O). Mean values ± SEM (bars) for 9 control rats (•), 6 aldosterone-treated rats (A), and 9 aldosterone-salt-treated rats (■) are also shown.

(Figure 2) using data from a pool of 47 normal Sprague-Dawley rats. The normal ventricular weight for a particular body weight, given in Table 1, was calculated from the parameters of the least-squares regression lines (see Figures 1 and 2) as follows: calculated left ventricular weight = (body weight × 1.658 × 10^-3) + 0.1264; calculated right ventricular weight = (body weight × 0.3680 × 10^-3) + 0.0606. There were no significant differences between the actual and calculated left or right ventricular weights in the control and aldosterone-treated animals. The left ventricular weight in the aldosterone-salt-treated group was significantly higher than the calculated left ventricular weight. The right ventricular weight in the hypertensive animals was not different from the calculated weight. We have based our definition of cardiac hypertrophy in the aldosterone-salt model of hypertension on the assumption that an increase in the heart weight to body weight ratio compared with that of weight-matched normal animals represents cardiac hypertrophy. It is possible, however, that differential growth of the heart compared with that of other organs may occur under conditions of slower weight gain in the hypertensive animals.

The isomyosin composition of representative samples from each group of animals is illustrated by densitometric gel scans presented in Figure 3. The relative proportions of V₁ (V₁ + V₂) and V₃ (V₃ + ½ V₂) isomyosins are given in Table 2. A significant increase in the percentage of V₁ isomyosin and a corresponding decrease in the percentage of V₃ isomyosin occurred in both left and right ventricles of aldosterone-salt–treated animals compared with those in controls and aldosterone-treated animals.

Although a shift in isomyosin composition in the left ventricle is consistent with an increased pressure overload and left ventricular hypertrophy, the alteration in the right ventricular isomyosin composition was unexpected. Therefore, we evaluated the potential involvement of alterations in the thyroid status of these animals on the ventricular isomyosin distribution. We
found that the change in ventricular isomyosin composition in aldosterone-salt-treated animals was associated with a significant decrease in plasma thyroxine levels (see Table 2), which were unchanged in the aldosterone-treated animals compared with those in controls. A shift in isomyosins in both left and right ventricles would be consistent with the response of the heart to changes in thyroid status of the animals.

**Discussion**

The response of the heart to an increased systolic blood pressure, produced by infusion of aldosterone and increased salt intake, was similar to that seen in other experimental models of hypertension in that significant left ventricular hypertrophy occurred. The usual index of ventricular hypertrophy, namely the ventricular weight to body weight ratio, indicated hypertrophy of both left and right ventricles in response to aldosterone-salt treatment. However, because of the slower growth rate of animals exposed to aldosterone-salt, their body weight was significantly less than that of the age-matched control group. Under these circumstances, the ratio of ventricular weight to body weight can be a misleading index of the degree of ventricular hypertrophy. Comparison of the ventricular weight to body weight ratios of the experimental animals to that of weight-matched normal animals indicated that the left, but not the right, ventricular weight of the aldosterone-salt-treated animals increased significantly (38%).

Aldosterone-salt treatment produced both left ventricular hypertrophy and alterations in isomyosin composition. These changes are consistent with those seen in other models of pressure overload hypertrophy such as renal hypertension and coarctation of the aorta. However, a shift in the right ventricular isomyosin pattern of the same magnitude and direction in the absence of hypertrophy was unexpected. The data suggest that other factors in addition to pressure overload are involved in the adaptive response of the heart to aldosterone-salt hypertension.

There are at least two possible explanations for this right ventricular response. A significant volume expansion has been shown to occur in rats treated with deoxycorticosterone acetate-salt during the first 2 weeks of treatment, and this may also occur in the aldosterone-salt model. This volume expansion disappears in the third and fourth week when the systolic blood pressure rises, but the right ventricular effects may be initiated during the early period of volume overload. The presence of left ventricular failure resulting in an increase in pulmonary vascular resistance could also produce changes in the isomyosin composition of the right ventricle, although we observed no overt signs of cardiac failure in the hypertensive animals. A second possibility is the involvement of thyroid hormone.

In the present study, circulating thyroxine levels were significantly reduced in aldosterone-salt-treated rats compared with those in control and aldosterone-treated animals. Since both left and right ventricular isomyosin composition can be manipulated by changing plasma thyroxine levels, it is possible that the lower levels of thyroxine in the aldosterone-salt-treated animals were responsible for the shift to $V_3$ isomyosin in the right ventricle. We have previously found that reduction of plasma thyroxine levels in the presence of a left ventricular pressure overload affects the right ventricular isomyosin composition and produces an additional shift in the isomyosin composition of the left ventricle.

It is not clear what causes the reduction in plasma thyroxine levels in aldosterone-salt–treated rats. Since rats infused with aldosterone and receiving no salt showed normal levels of plasma thyroxine, it is probably not due to a direct effect of aldosterone on thyroid hormone production or metabolism. We found that the slower growth rate in the aldosterone-salt–treated animals compared with that in control animals was associated with a 20 to 40% reduction in food intake during the third and fourth weeks of treatment. Dillmann et al. reported that a 50% reduction of food intake in rats for 8 weeks lowered plasma thyroxine levels. Thus, reduction in the amount of food consumed by the hypertensive rats may be responsible for the reduction in plasma thyroxine levels seen in this group of animals. However, the animals studied by Dillmann et al. lost 42% of their original body weight, whereas the hypertensive rats in our study continued to gain weight.

Alpert and Mulieri postulated that a shift to the $V_3$ isoform of myosin in the hypertrophied myocardium represents an adaptation to a more energy efficient form of myosin. Several reports show a relation between isomyosin composition and mechanical function. However, the functional consequences of parallel isomyosin transitions in both left and right ventricles compared with alterations in only one ventricle is unclear. Left and right ventricular hypertrophy and adaptive changes in isomyosin composition in both ventricles occur in the cardiac enlargement resulting from increased left ventricular pressure overload in 21-day-old rats. The enlarged hearts from these animals maintain normal function. In contrast, a similar pressure overload in adult animals produced cardiac enlargement with impaired hemodynamic function. Significant changes in isomyosin composition are seen only in the left ventricle of these hypertrophied adult
hearts. Tarazi et al. have found that the hemodynamic characteristics of hypertensive patients with primary aldosteronism are significantly different from those of patients with essential hypertension. Thus, the biventricular response of the heart to aldosterone-induced hypertension may be of functional significance and characteristic for this model of hypertension.

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