Reduction of Development of Left Ventricular Hypertrophy in Salt-Loaded Dahl Salt-Sensitive Rats by Angiotensin II Receptor Inhibition

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To determine the effect of the angiotensin II AT1 receptor antagonist losartan (DuP753) on echocardiographic left ventricular (LV) anatomy in Dahl rats on high sodium diet, 27 Dahl salt-sensitive (Dahl-S, 13 on drug and 14 receiving tap water) and 27 Dahl salt-resistant rats (Dahl-R, 13 on drug and 14 receiving tap water) were studied by M-mode echocardiography during 8 weeks of 8% NaCl diet. At the endpoint (after 8 weeks or the last echocardiogram for animals who died earlier), Dahl-S receiving losartan had lower LV mass (1.6 ± 0.4 g/kg0.59) than Dahl-S receiving tap water (2.2 ± 0.7 g/kg0.59; P < .005), although blood pressure was only partially reduced (167 ± 29 to 195 ± 52; P = .05). This difference was mainly due to lower LV wall thickness (P < .02), with a less consistent decrease in LV chamber size in Dahl-S receiving losartan. Blood pressure was normal in Dahl-R (tap water group = 116 ± 11 mm Hg; losartan group = 115 ± 13 mm Hg) and losartan had no effect on LV mass (1.6 ± 0.4 g/kg0.59 in both groups). In the majority of rats, echocardiographic measurements were compared between the end of second or third week and the last available study: LV mass increased in salt-loaded Dahl-S receiving tap water (1.6 ± 0.6 to 2.1 ± 0.7 g/kg0.59, P < .04) and was stable in Dahl-S receiving losartan (1.5 ± 0.1 to 1.5 ± 0.3 g/kg0.59), paralleling changes in LV chamber dimension. Thus, a high salt diet leads to hypertension and eccentric LV hypertrophy in Dahl-S but not in Dahl-R. Inhibition of angiotensin II AT1 receptors reduces the development of LV hypertrophy in Dahl-S rats despite lack of efficient control of blood pressure. Am J Hypertens 1996;9:216–222

KEY WORDS: Salt, left ventricular hypertrophy, losartan, hemodynamic overload, angiotensin II AT1 receptor, Dahl rat, DuP753.

Experimental, clinical, and epidemiological observations suggest that development of hypertensive left ventricular (LV) hypertrophy may be in part independent of the level of pressure overload. There is indeed evidence that the extent of volume load is as important as arterial pressure in the development of hypertensive cardiac hypertrophy. Recently, we have shown that a high salt diet produces
LV chamber enlargement and a degree of LV hypertrophy comparable with that caused by mild renovascular hypertension. The mechanism by which salt intake stimulates myocardial hypertrophy is probably volume-dependent, as suggested by previous studies in humans and animals.

Among the many biological substances that may contribute to the development of LV hypertrophy, angiotensin II might play a key role because of its ability to increase both pressure overload (through systemic vasoconstriction) and volume overload (through aldosterone-mediated and direct renal sodium and water retention). This role is also suggested by the observation of an especially consistent effect of angiotensin converting enzyme inhibitors in reducing LV hypertrophy. Whether the entire role of angiotensin II in the development of LV hypertrophy is hemodynamically mediated, or if it is in part direct, remains to be clarified. Little information is available on the effect of angiotensin II in influencing development of LV hypertrophy in volume-dependent forms of hypertension in which partial or even total suppression of the renin-angiotensin system may occur. Accordingly, to study the effect of angiotensin II activity blockade on development of LV hypertrophy in a form of sodium-stimulated arterial hypertension, LV anatomy was studied during high salt intake in Dahl salt-sensitive rats (Dahl-S), a model of volume-dependent hypertension, and in control salt-resistant animals (Dahl-R) with or without treatment with the angiotensin II AT1 receptor inhibitor losartan.

METHODS

Animal Models Twenty-seven male Dahl-S and 27 male Dahl-R rats (Harlan Sprague-Dawley, Indianapolis, IN) were studied between 1990 and 1991 under a protocol approved by the Institutional Animal Care and Use Committee of Cornell University Medical College. All rats were 6 to 7 weeks old at the beginning of the study and were housed individually for 5 weeks in metabolic cages under controlled conditions of light, temperature, and humidity. All the animals were fed 8% NaCl chow (Zeigier Bros., Inc., Gardners, PA) and were allowed to drink tap water ad libitum. A nonpeptide angiotensin II receptor inhibitor, losartan (DuP723; 2-n-butyl-4-chloro-5-hydroxymethyl-1[2′-(1H-tetrazol-5-yl)biphenyl-4-yl]methylimidazole; DuPont Merck Pharmaceutical Co., Wilmington, DE) was given as a potassium salt to 13 Dahl-S and 13 Dahl-R at the dose of 30 mg/kg body weight/day dissolved in part of the drinking water. The other 14 Dahl-S and 14 Dahl-R rats served as controls. Systolic blood pressure was measured weekly in awake animals by tail cuff sphygmomanometry (PE-300, Narco Bio-System, Houston, TX), the same day as recording of body weight.

Experimental Procedures Echocardiograms were performed in all animals at the end of the second or third week on the experimental diet, and again at the fifth, sixth, seventh, and eighth week of salt loading. The echocardiograms were done on the day after the weekly blood pressure determination, under light anesthesia with intramuscular ketamine (50 mg/kg body weight), according to the procedure of our laboratory. With this anesthesia animals slept for the 10 to 20 min required to perform echocardiograms. Forty-eight hours after the last echocardiogram, at the end of the eighth week of salt loading, the surviving rats were decapitated and blood was collected for creatinine, plasma renin activity (PRA), atrial natriuretic factor (ANF), and microhematocrit determinations. PRA and plasma ANF immunoreactivity were determined by radioimmunoassay as previously reported in detail.

The day before killing, urinary volume and urinary protein and creatinine excretion were determined, allowing calculation of creatinine clearance. Hematocrit, plasma and urinary creatinine, and urinary protein were measured by standard analytical techniques.

Echocardiographic Method A Hewlett-Packard (Andover, MA) 770720 echocardiographic system was used, equipped with a 3 MHz, shallow focus, 21211A phased-array transducer, placed on the left hemithorax with the rat in the partial left decubitus position. Two-dimensional targeted M-mode echocardiograms obtained from short axis views of the left ventricle at or just below the tip of the mitral valve leaflets were recorded on strip-chart paper at 100 mm/sec.

For end study evaluation, tracings performed at the eighth week of diet in surviving animals or the last tracings available before spontaneous death were used. Tracings performed after 2 or 3 weeks of high-salt diet in the majority of rats were compared to end-study tracings. All tracings were numerically coded and interpreted at the end of the study by two observers blinded to knowledge of type of rat, age, weight and presence of losartan in drinking water. Rats that died before the fifth week of salt loading were not considered in the present study.

To measure LV end-diastolic dimension (EDD) and interventricular septal and posterior wall thicknesses (IVS and PWT), interfaces were marked by the leading edge method at the time of maximum diastolic dimension by two independent observers on at least three cardiac cycles. Tracings were subsequently measured using a graphic tablet and a digitizing pen interfaced with a PC computer. Systolic shortening of LV minor axis measured at endocardium was used as an index of LV function.

LV mass (LVM) was calculated by an anatomically validated model, based on the assumption of an elliptical LV shape with long-axis measurement constant:
LVM = 1.04 \times 4/3 \pi \left( \frac{(EDD/2) + (PWT + IVS)/2}{12.28} \right)^2 
\times \left( \frac{EDD/2}{12.28} \right)^3/10^3

where 12.28 is a constant derived by a nonlinear regression analysis in a learning series of rats and validated in a test series\(^{21}\) and \(10^{-3}\) converts milligrams to grams. Cross-sectional area was also computed as a measure of LV weight independent of geometric assumptions.\(^6\) Diastolic relative wall thickness, calculated as \((PWT + IVS)/EDD\), was used as an additional index of LV geometry.\(^8\)

Echocardiographic LV mass and cross-sectional area were closely related to necropsy weight \((R = 0.90, P < .0001)\) in sequential learning and test series of rats.\(^7,21\)

Normalization for body size was obtained using body weight to the appropriate power to linearize its relations with LV mass \((\text{exponent} = 0.59)\), cross-sectional area \((\text{exponent} = 0.53)\), and LV diastolic dimension \((\text{exponent} = 0.21)\), as previously reported in detail.\(^5\)

For convenience LV mass/body weight\(^{6,29}\) will be called LV mass index.

Statistical Analysis Data are presented as mean ± standard deviation. Distributions of variables used in this study were examined by the Shapiro-Wilk Test and found to deviate from normality. Thus, logarithmic transformation was used for analysis of variance. Two-way ANOVA with post hoc computation of simple main effects was used to detect the effect of treatment in either strain of rats at the end of the study. Analysis of variance for repeated measurements was used to compare changes over time between the second or third week and final echocardiograms among the groups of experimental animals. The null hypothesis was rejected at a two-tailed \(P < .05\).

RESULTS

Among the rats studied, 4 Dahl-S receiving tap water (29%), 9 Dahl-S receiving losartan (69%), 12 Dahl-R receiving tap water (86%), and 11 Dahl-R receiving losartan (85%) survived through the end of the study. The other animals were found dead in their cages.

At the time of last echocardiogram, body weight was lower \((P < .0001)\) in Dahl-S \((311 ± 47 \text{~g} \text{ receiving tap water and } 352 ± 28 \text{~g} \text{ receiving losartan, } P < .002)\) than in Dahl-R \((374 ± 29 \text{~g} \text{ receiving tap water and } 388 ± 27 \text{~g} \text{ receiving losartan, } P = \text{NS})\), partially due to the fact that the endpoint echocardiogram occurred at the end of the fifth, sixth, or seventh week in 10 of 14 Dahl-S receiving tap water. Blood pressure was significantly higher in Dahl-S than in Dahl-R groups \((P < .0001)\). Blood pressure in Dahl-S receiving losartan was slightly lower than in Dahl-S receiving tap water \((167 ± 29 \text{~mm} \text{ Hg} \text{ vs } 195 ± 57 \text{~mm} \text{ Hg}; P = .05)\); no difference was noted in Dahl-R \((116 ± 11 \text{~mm} \text{ Hg} \text{ receiving tap water; } 115 ± 13 \text{~mm} \text{ Hg receiving losartan})\).

Table 1 shows blood tests of animals that survived until being killed at the eighth week of salt loading: in Dahl-S receiving tap water, plasma renin activity was very high \((P < .0003 \text{ vs Dahl-R receiving tap water})\), hematocrit was reduced \((P < .01 \text{ vs the other groups})\), and clearance of creatinine was low \((P < .005 \text{ vs the other groups})\). Urinary protein excretion was higher in both groups of Dahl-S than in Dahl-R, independent of therapy \((P < .0002)\). ANF tended to be higher in both Dahl-S and Dahl-R treated with losartan, but differences did not attain statistical significance, because of both the high within-group variance and the small number of animals in each cell.

Patterns of Left Ventricular Geometry in Experimental Animals Table 2 shows that after 5 to 8 weeks

| TABLE 1. HORMONAL PROFILE AND RENAL FUNCTION IN DAHL SALT-SENSITIVE AND SALT-RESISTANT RATS WITH OR WITHOUT LOSARTAN TREATMENT THAT SURVIVED AFTER 8 WEEKS OF 8% NaCl DIET |
|---------------------------------|-----------------|-----------------|-----------------|
|                                | Water (n = 4/14) | Losartan (n = 9/13) | Water (n = 12/14) | Losartan (n = 10/13) |
| Body weight (g)                | 306 ± 10‡       | 349 ± 29         | 374 ± 31         | 397 ± 24          |
| Plasma renin activity (ng/mL/h) | 11.1 ± 12.7     | 2.83 ± 2.79      | 0.85 ± 1.01      | 2.69 ± 4.04       |
| Hematocrit (%)                 | 35.5 ± 4.4*     | 44.0 ± 5.6       | 44.3 ± 2.7       | 44.2 ± 2.6        |
| Creatinine clearance (mL/h)    | 1.52 ± 0.46‡    | 2.56 ± 0.46      | 2.62 ± 0.47      | 2.70 ± 0.41       |
| Urinary Na excretion (mmol/day) | 13.5 ± 11.4     | 21.4 ± 4.8       | 29.3 ± 6.6       | 30.3 ± 5.2        |
| Urinary protein excretion (mg/day) | 90.3 ± 39.2‡    | 124.4 ± 49.1‡    | 18.5 ± 9.0       | 16.0 ± 8.2        |
| Atrial natriuretic factor (pmol/mL) | 24.3 ± 0.8     | 50.2 ± 22.5      | 37.8 ± 29.2      | 42.7 ± 26.0       |

* \(P < .01\); ‡ \(P < .005\) vs Dahl-S on losartan; † \(P < .01\) vs Dahl-R.
of salt loading, echocardiographic LV mass and cross-
sectional area were markedly higher in Dahl-S receiving
tap water than in Dahl-S on losartan, as either absolute
values or normalized for body size (P < .005 vs Dahl-S on
losartan). No difference was observed between the two Dahl-R
groups. Difference in LV mass between the two Dahl-
S groups was due predominately to higher wall thick-
ness in Dahl-S receiving tap water, a difference that
reached statistical significance for the posterior wall (P
< .05, Table 2) and for the sum of posterior wall and
septum (P < .02). Because LV chamber dimension was
also slightly greater in Dahl-S receiving tap water than
in Dahl-S receiving losartan, relative wall thickness was
not significantly elevated in the former group of rats
(Table 2). Endocardial fractional shortening was higher
in pooled Dahl-S than in pooled Dahl-R (P < .05), but
no significant drug effect was detected in either strain
(Table 2).

**Longitudinal Changes in Left Ventricular Geometry**

Within-group comparison was made between the eco-
cardiographic studies at the end of second or third week
of high-salt diet and at the end-study in 10 Dahl-S re-
ceiving tap water, 11 Dahl-S receiving losartan, 12 Dahl-
R receiving tap water, and 9 Dahl-R receiving losartan.

At the time of the first available echocardiogram, 
blood pressure was statistically indistinguishable in all
groups (Table 3). Blood pressure increased over time
in all four groups (.0001 < P < .04), but this increase
was more pronounced in Dahl-S than in Dahl-R on ei-
ther treatment (P < .02 with tap water and P = .08
with losartan).

Figure 1 shows that LV mass index increased over
time in Dahl-S receiving tap water, but was stable in
Dahl-S receiving losartan. The difference in LV mass
between the Dahl-S groups was insignificant at the time
of first echocardiogram, but became highly significant
at the end of the study (P < .001), due to a greater
increase in LV chamber dimension in Dahl-S receiving
tap water than in Dahl-S receiving losartan (between-
group P < .02, Figure 2). Relative wall thickness did
not change significantly.

**LV mass index did not change significantly over time
in either group of Dahl-R rats (1.67 ± 0.24 vs 1.62 ± 0.36
g/kg\textsuperscript{0.59} receiving tap water and 1.62 ± 0.31 vs 1.45 ±
0.27 g/kg\textsuperscript{0.59} receiving losartan).**

**DISCUSSION**

This study was designed to assess changes in LV geo-
metry in a model of sodium-stimulated arterial hyperten-
sion, without primary dependence on renin-angioten-
sin system activity, to determine whether or not develop-
ment of LV hypertrophy can be influenced by
blocking the action of angiotensin II. The strain of Dahl

**TABLE 3. BLOOD PRESSURE AT THE TIME OF
FIRST ECHOCARDIOGRAM AND AT END STUDY
IN DAHL-S AND DAHL-R ON LOSARTAN
OR ON TAP WATER**

<table>
<thead>
<tr>
<th></th>
<th>Baseline Blood Pressure (mm Hg)</th>
<th>End-Study Blood Pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dahl-S on losartan (n = 11)</td>
<td>111 ± 16</td>
<td>165 ± 26†</td>
</tr>
<tr>
<td>Dahl-S on tap water (n = 10)</td>
<td>115 ± 25</td>
<td>183 ± 51†</td>
</tr>
<tr>
<td>Dahl-R on losartan (n = 9)</td>
<td>99 ± 11</td>
<td>118 ± 13*</td>
</tr>
<tr>
<td>Dahl-R on tap water (n = 12)</td>
<td>96 ± 14</td>
<td>16 ± 12*</td>
</tr>
</tbody>
</table>

* P < .04; † P < .0001.
hypertension-prone rats represents a valuable model, because it develops a volume-dependent form of severe hypertension, suppressing plasma renin activity in the first 4 weeks of salt loading. After 4 weeks, however, renin activity increases and reaches high levels by the eighth week. In our surviving animals, high plasma renin activity was found in untreated salt-sensitive rats and was associated with kidney dysfunction. No statistical group difference was found in ANF, because of the great variability of the values associated with the small number of surviving animals.

**Effect of Salt Loading on Left Ventricular Mass in Dahl Rats** In Dahl-R, which, as expected, did not develop arterial hypertension, salt loading was associated with a lower level of LV mass in relation to body size than we had previously detected in sham-operated Wistar rats (2.15 ± 0.32 g/kg) in one-kidney shams.
and 2.21 g/kg.4-6 in two-kidney shams) on a less extremely (4% ± 8%) high salt diet. This difference cannot be due to the different methods used to calculate LV mass in the present and in the previous study,4,6 because validation studies17,21 have shown that both approaches yield very similar values of LV mass. Thus, Dahl-R show partial protection against hemodynamically-mediated cardiac changes induced by salt loading, possibly associated with their exceptional capacity to increase their glomerular filtration rate. Conversely, a marked increase in LV mass occurred in untreated hypertensive Dahl-S rats, confirming that the combination of both pressure and volume overload dramatically stimulates development of LV hypertrophy.5,10 This increase in LV mass was completely inhibited in rats treated with losartan, although the drug was not as effective in reducing blood pressure as has been previously reported with diuretic therapy.23 Because relative wall thickness remained normal in our untreated Dahl-S that developed LV hypertrophy, whereas LV chamber dimension was increased, the increased LV mass resulted in eccentric LV hypertrophy, a geometric pattern associated with volume overload.5,6

At lower dietary sodium concentration (4%), no effect has been reported for losartan on LV geometry after 7 weeks of salt loading in Dahl-S, even at higher doses of losartan.24 In that study, the necropsy mean LV mass index in rats receiving tap water was 2.59 g/kg, corresponding to about 1.53 g/kg.5,6, a value markedly lower than the average value detected in our Dahl-S on 8% sodium diet. In addition to the different levels of hemodynamic overload produced by the two different sodium concentrations, in our study the potential survivor effect (i.e., rats with less severe cardiac involvement) was avoided by using the last echocardiogram available for the study before spontaneous death. In our study, indeed, LV mass index was much lower in the four Dahl-S that survived to the end of the trial (1.53 ± 0.25 g/kg) than in animals who died before the eighth week (2.38 ± 0.75 g/kg).6,9

Mechanisms Underlying the Effect of Angiotensin II Receptor Inhibition An important consideration is whether angiotensin II receptor inhibition directly affected development of LV hypertrophy, as has been previously suggested.25 Long-term losartan treatment did not induce any significant difference in measures of LV mass or chamber size in Dahl-R on high salt diet compared to tap water-treated Dahl-R. The substantially lower LV mass in losartan-treated Dahl-S compared to tap water-treated animals (29% reduction) is in line with that expected for a 7% lower indexed LV dimension (Table 2) and an 14% lower systolic blood pressure, suggesting it may have been due to parallel decreases in both LV volume and pressure loads. This interpretation is indirectly supported by evidence from previous studies on human essential26 and renovascular27 hypertension, as well as experimental renovascular hypertension28 in which activation of the renin-angiotensin system did not appear to add to the level of hemodynamic load as a stimulus to LV hypertrophy. However, in the present study, losartan only partially blocked the tendency for Dahl-S to increase LV chamber size during serial echocardiograms (Figure 2) and losartan's effect on LV mass in Dahl-S appeared to be due mostly to a reduction in wall thickness, a decrease that exceeded that expected for the measured difference in arterial pressure.

CONCLUSIONS

High salt intake stimulates development of eccentric LV hypertrophy in Dahl-S rats. Inhibition of angiotensin II AT1 receptors blunts the development of LV hypertrophy in Dahl-S rats, despite only partial control of blood pressure, possibly due to control of concomitant volume overload or to direct nonhemodynamic effects.

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REFERENCES


