Cardiac Weight in Hypertension Induced by Nitric Oxide Synthase Blockade

Jean-François Arnal, Abdel-Ilah El Amrani, Gilles Chatellier, Joël Ménard, Jean-Baptiste Michel

Wistar rats given a nitric oxide synthase inhibitor, \( \text{NO}^- \)-nitro-L-arginine-methyl ester (L-NAME), for 4 weeks develop time- and dose-dependent hypertension without cardiac hypertrophy. This initial study of the relation between left ventricular weight and L-NAME-induced hypertension has now been extended by giving 50 mg/kg per day L-NAME to Wistar rats (n=30) for 8 weeks and comparing results with those from control rats (n=10) and two-kidney, one clip rats (n=14). Although L-NAME rats and two-kidney, one clip rats had increased systolic blood pressures during the last 3 weeks of the experiment (202±24 and 224±16 mm Hg, respectively), the ratio of left ventricular weight to body weight of L-NAME rats (2.12±0.32 mg/g) was not statistically different from that of control rats (1.93±0.13 mg/g), whereas that of two-kidney, one clip rats was increased (2.85±0.20 mg/g). The plasma renin activity of L-NAME rats was not significantly different from that of control rats. Two L-NAME rat subgroups were defined according to the presence of left ventricular hypertrophy (ratio of left ventricular weight to body weight > 2.19 mg/g, control mean + 2 SD) (6 of 25) or its absence (19 of 25). Systolic blood pressure, plasma renin activity, and cardiac angiotensin converting enzyme activity of L-NAME rats with left ventricular hypertrophy were significantly higher than those of the subgroup without. In a multiple regression analysis using the ratio of left ventricular weight to body weight as an independent variable and three dependent variables (L-NAME administration, plasma renin activity, and systolic blood pressure), we found that all of these three variables contributed to left ventricular weight independently of each other. Thus, even if the degree of left ventricular hypertrophy evolves in parallel with the duration and magnitude of a chronic rise in blood pressure, other factors, such as the renin-angiotensin system and nitric oxide production, influence this relation. (Hypertension. 1993;22:380-387.)

Key Words • guanosine cyclic monophosphate • heart hypertrophy • nitric oxide • renin

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A n inhibitor of nitric oxide synthase in vitro,1-4 \( \text{NO}^- \)-nitro-L-arginine-methyl ester (L-NAME) also inhibits the release of nitric oxide from endothelial cells and aortic rings. In vivo L-NAME administration to rodents is accompanied by a rise in blood pressure and a generalized decrease in peripheral blood flow, indicating that a continuous release of nitric oxide actively maintains vasodilator tone.2,5 Rats given L-NAME for 4 weeks show a time- and dose-dependent increase in blood pressure.6-7 Surprisingly, in our hypertensive rats, the total ratio of heart weight to body weight did not increase after 4 weeks of L-NAME administration (1 to 100 mg/kg per day).6 This absence of hypertrophy was in contrast to results obtained in the other models of experimental hypertension in which a chronic increase in blood pressure was accompanied by cardiac hypertrophy.8-12 This study of the relation between heart weight and L-NAME-induced hypertension has now been extended by giving Wistar rats 50 mg/kg per day L-NAME in their drinking water for 8 weeks. Blood pressure, left ventricular (LV) weight, and arterial wall cyclic GMP (cGMP) of these rats were measured, and the data were compared with those of control rats (given water only) and two-kidney, one clip (2K1C) rats. The consequences of long-term inhibition of nitric oxide generation on the renin-angiotensin system (plasma renin activity [PRA], plasma and cardiac angiotensin converting enzyme [ACE] activity) were assessed in parallel. The effects of an angiotensin II receptor antagonist (DuP 753, losartan) after 1 month of L-NAME-induced hypertension were studied to evaluate the dependence of this model on the renin-angiotensin system. The reversibility of the hypertension after 1 month of L-NAME was also studied.

Methods
Experimental Design and Animal Preparation
The procedure followed in the care and euthanasia of the study animals was in accordance with the European Community Standards on the care and use of laboratory animals (Ministère de l'Agriculture, France; authorization No. 00577, April 30, 1989). Seven groups of young male Wistar rats (Iffa Credo, Lyon, France) weighing 120 to 130 g were used: (1) L-NAME group. Thirty rats were given L-NAME (50 mg/100 mL, Sigma Chemical Co, St Louis, Mo) in the drinking water for 8 weeks. At this concentration, the daily intake of L-NAME was approximately 50 mg/kg. This dose previously had caused sustained hypertension for 4 weeks and a maximal blockade of aortic nitric oxide synthase.6 (2) Control group. Ten rats were given tap drinking water alone for 8 weeks. (3) Losartan (Control_c) group. Ten rats were given tap drinking water for 4
weeks and losartan (10 mg/100 mL DuP 753, Du Pont Merck Pharmaceutical Co, Wilmington, Del) in the drinking water for the last 4 weeks. Their average daily intake of losartan was 10 mg/kg per day. (4) L-NAME plus losartan (L-NAME+LO) group. Fifteen rats were given 50 mg L-NAME per kilogram per day in the drinking water for 8 weeks, and 10 mg/100 mL losartan was added to their drinking water for the last 4 weeks. (5) L-NAME-4 group. Ten rats were given 50 mg L-NAME per kilogram per day in the drinking water for 4 weeks only and had tap water for the last 4 weeks. (6) 2K1C rats. Thirty rats were anesthetized with ether. The left renal artery was clipped (0.2 mm diameter), and the right kidney was not disturbed. The six rats that failed to develop hypertension (ie, systolic blood pressure greater than 160 mm Hg) all had infarction of the clipped kidney. Because their blood pressure, heart weight, and PRA at the end of the experimental period did not differ from those of the sham-operated rats, these rats could not be considered as 2K1C rats and were eliminated from the study. The remaining rats were followed up. (7) Sham-operated control rats. Twenty rats underwent sham operation, an incision being made in the flank without clipping.

Ten 2K1C rats and 10 sham-operated rats were killed after 4 weeks; the remaining rats were killed after 8 weeks.

Systolic blood pressure was measured weekly by the tail-cuff method (W+W electronic recorder 8005, Aplelab, Bagneux, France). An average of the systolic blood pressures at 6, 7, and 8 weeks (mean systolic blood pressure) was calculated and used for statistical analysis. At the end of the experimental protocol, all rats were killed by decapitation. At the time of decapitation, the homogenates were centrifuged at 15 000g, and the result-
in supernatant was collected and centrifuged at 16 000g for 30 minutes, the residue being lyophilized in a Speed-Vac and dissolved in 0.1 mol/L phosphate buffer, pH 7.4, 0.1% Triton, and 0.1% trifluoroacetic acid. Plasma atrial natriuretic factor was determined by nonequilibrium radioimmunoassay using a double antibody to separate the free and bound fractions. 14 Anti-atril natriuretic factor antibody-
Aliquots were sonicated for 2 x 10 seconds at position 3 using a model W-10 sonicator (Electro-mech Instrument Co, Perkasie, Pa).

ACE activity was estimated by measuring the hydrolysis of 100 μmol/L [glycine-1-14C] hippuryl-L-histidyl-L-leucine (3 μCi/mmol, Amersham) in the presence or absence of 10−7 mol/L captopril. Incubations (final volume, 100 μL) were performed at 37°C under conditions of initial velocity measurement in 0.1 mol/L phosphate buffer, pH 8.0, containing 0.3 mol/L NaCl and 10 μmol/L ZnCl2 and were stopped after 5 hours by adding 50 μL 0.3N HCl, as described by Cushman and Cheung.20 14C metabolites were separated from the substrate by adding 0.5 mL ethyl acetate to the acidified enzyme reaction mixture and shaking for 1 minute. The mixture was centrifuged at 18 000g for 7 minutes, and a 0.3-mL aliquot of the organic layer containing 14C metabolites was counted by scintillation spectrometry (Pico-Fluor 15, Packard Instrument Co). Contamination by the substrate was less than 3%, and the metabolite recovery was more than 90%.

**Determination of Left Ventricular Myosin Isoenzyme Profile**

Crude tissue obtained from 50-mg samples was extracted by the method of Mercadier et al.21 Polyacrylamide gel electrophoresis under nondissociating conditions was performed according to Hoh et al22 as modified by Mercadier et al.21 Gels (3.68% acrylamide [wt/vol], 0.12% N,N'-methylene-bis-acrylamide [wt/vol]) were loaded with 30 μL crude extract (diluted 40-fold). The electrophoresis buffer was maintained at 2°C to 4°C, and gels were run at a constant voltage of 14 V/cm for 20 hours. Densitometric tracings of the gels (stained with Coomassie brilliant blue) were made, and V1, V2, and V3 were expressed as the percentage of the total densitometer peak height (V1 + V2 + V3).

**Statistical Methods**

Results are expressed as mean±1 SD. One-way analysis of variance (ANOVA) or two-way ANOVA with a repeated factor (time) was used to test the differences between groups. When the F test allowed us to reject the null hypothesis of no difference between groups, paired comparisons were performed with the Scheffé procedure to avoid the false-positive risk due to multiple comparisons. Regression curves were obtained by the least-squares method. The relation between two variables was tested with the Pearson correlation coefficients. The two-sided comparison of two estimated correlation coefficients was made according to the method of Sachs.23 When the relation between two variables was graphically nonlinear, we tried to obtain a better fit with a second-order regression (y=ax2+bx+c). The relation of a dependent variable with several correlated independent variables was assessed by multiple linear regression. For both linear regression and ANOVA, non-normally distributed variables were log transformed. The blood pressure and LV weight of rats that died during the trial are reported, but these rats were not included in the statistical analyses. A value of P<.05 was considered significant.

![Line graph shows weekly systolic blood pressure (mean±SEM) of Wistar rats given water (Control, n=10, ○), 10 mg/kg per day losartan during last 4 weeks (Control Los., n=10, □), 50 mg/kg per day NΩ-nitro-L-arginine-methyl ester (L-NAME, n=25, ●), or 50 mg/kg per day L-NAME plus 10 mg/kg per day losartan for last 4 weeks (L-NAME los., n=15, ▲). Also shown are sham-operated Wistar rats (S, n=20 during 1st month, n=10 during 2nd month; dotted line) and two-kidney, one clip rats (2K-1C, n=18 during 1st month, n=8 during 2nd month; □).](http://hyper.ahajournals.org/)

**Results**

**Mortality Rate, Blood Pressure, and Body Weight**

A total of 11 rats died before the end of the study: 5 of 30 L-NAME-treated rats (dates of death: 32nd, 44th, 46th, 50th, and 51st day) and 6 of 30 2K1C rats (dates of death: 18th, 20th, 24th, 29th, 32nd, and 40th day). All these rats had a systolic blood pressure greater than or equal to 220 mm Hg at least once before death.

Eight weeks of oral L-NAME treatment induced a time-dependent increase in blood pressure (Fig 1). The mean systolic blood pressure (of the 6th, 7th, and 8th weeks) was 202±24 versus 130±4 mm Hg in control rats (Table 1). Rats given L-NAME plus losartan in the last 4 weeks (group L-NAME los.) had a mean systolic blood pressure 18% lower than that of rats given L-NAME alone (−36 mm Hg). Rats given losartan alone in the last 4 weeks (group Control Los.) had a mean systolic blood pressure 8% lower than that of the controls (−11 mm Hg).

2K1C rats also showed a time-dependent increase in blood pressure (Fig 1). Compared with L-NAME rats, the integrated systolic blood pressure (from the 1st to the 8th week) of 2K1C rats was significantly higher (223 vs 187 mm Hg, P<.0001), whereas the mean systolic blood pressure (of the 6th, 7th, and 8th weeks) was not (224±16 vs 202±24 mm Hg, F=1.49, NS).

L-NAME reduced the rate of body weight increase compared with controls (Table 1). This smaller weight gain averaged 45 g (only 10% of the body weight of these hypertensive rats). Similarly, the body weight of the 2K1C rats was 8% lower than that of the sham-operated rats.

The last blood pressure measurements of the five L-NAME and six 2K1C rats that died were 237±7 and 243±6 mm Hg, respectively. Their body weights were, respectively, 27% and 31% lower than those of their controls at the same time.

**Left Ventricular Weight**

The LV weight and ratio of LV weight to body weight (LV index) of rats given L-NAME were not statistically
different from those of controls, despite the increase in blood pressure (Table 1). In the L-NAME plus losartan group (L-NAMElo; n=15), the LV weight was lower than that of controls, but the LV index was not statistically different. No rat in the latter group had LV hypertrophy.

The LV weights and LV indexes of 2K1C rats at 1 and 2 months were significantly increased compared with control groups, but the ratio of RV weight to body weight (RV index) was not different from their respective sham-operated groups. The RV index values were not different from those of the respective sham-operated groups.

Thus, L-NAME slowed entire body and RV growth. The LV index appeared the most appropriate parameter for defining LV hypertrophy, taking into account the potential general effect of L-NAME on growth and thereby allowing subsequent comparisons.

Fig 2 represents the relation between the systolic blood pressure (mean of the last 3 weeks) and the LV index in the L-NAME group, the 2K1C groups, and their respective controls. It shows that 19 of 25 of the L-NAME rats had an LV index less than 2.19 mg/g (ie, control mean+2 SD) and therefore that only 6 of 25 of these rats had LV hypertrophy.

The LV index was also determined in the L-NAME and 2K1C rats that died before the end of the experiment (the body and LV weights were measured within 12 hours of death). All these rats had LV hypertrophy, with an LV index averaging 2.92±0.18 mg/g in the five L-NAME rats and 3.37±0.15 mg/g in the six 2K1C rats.

**Table 1. Blood Pressure and Heart Weight of Control and Experimental Rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>SBP (mm Hg)</th>
<th>BW (g)</th>
<th>LVW (g)</th>
<th>LVW/BW (mg/g)</th>
<th>RVW (g)</th>
<th>RVW/BW (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=10)</td>
<td>130±4</td>
<td>459±35</td>
<td>886±65</td>
<td>1.93±0.13</td>
<td>245±25</td>
<td>0.53±0.03</td>
</tr>
<tr>
<td>ControlLo (n=10)</td>
<td>119±4</td>
<td>441±30</td>
<td>767±60</td>
<td>1.68±0.06</td>
<td>239±21</td>
<td>0.52±0.03</td>
</tr>
<tr>
<td>L-NAME (n=25)</td>
<td>202±24*</td>
<td>414±44*</td>
<td>874±60</td>
<td>2.12±0.32</td>
<td>219±16*</td>
<td>0.54±0.06</td>
</tr>
<tr>
<td>L-NAMELo (n=15)</td>
<td>166±18*</td>
<td>405±37*</td>
<td>767±65*</td>
<td>1.90±0.12</td>
<td>195±11*</td>
<td>0.49±0.04</td>
</tr>
</tbody>
</table>

*P<.05 vs respective control group (control or sham).

SBP, average of systolic blood pressure at 6, 7, and 8 weeks; BW, body weight; LVW, left ventricular weight; LVW/BW, ratio of LV weight to body weight; RVW, right ventricular weight; RVW/BW, ratio of RV weight to body weight; Los, losartan; L-NAME, N°-nitro-L-arginine-methyl ester; 2K1C, two-kidney, one clip rats. Data are mean±SD; P value of one-way ANOVA.

**Plasma Renin-Angiotensin System and Atrial Natriuretic Factor**

Table 2 shows plasma and tissue parameters of the various rat groups. The PRA of L-NAME rats was not significantly different from that of controls. The PRA of losartan-treated rats was elevated in both L-NAME and control rats. The PRA of 2K1C rats was elevated at 1 month but did not differ from that of sham-operated rats after 2 months. The plasma angiotensinogen levels of L-NAME and control rats were comparable, but they were significantly decreased in rats given losartan. The plasma angiotensinogen levels of 2K1C rats were significantly decreased at 1 month compared with sham-operated rats, but the difference was no longer significant at 2 months. The plasma ACE activity did not change in any group of rats.

Plasma atrial natriuretic factor was nonsignificantly elevated in L-NAME and 2K1C rats compared with control and sham-operated rats, respectively.

**Tissue Parameters**

The cGMP content of the arterial wall was dramatically altered in L-NAME–treated rats (Table 2). The aortic cGMP content was decreased approximately 10-fold in rats given L-NAME. Losartan did not influence aortic cGMP content in control or L-NAME rats. The aortic cGMP content was not significantly influenced by 2K1C hypertension.
TABLE 2. Plasma and Tissue Parameters of Control and Experimental Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>PRA (log Ang I/mL/h)</th>
<th>Plasma AG (ng Ang l/mL)</th>
<th>Plasma ACE ([nmol/mL]/min)</th>
<th>Plasma ANF (pg/mL)</th>
<th>Cardiac ACE activity ([pmol/mg/min])</th>
<th>Aortic cGMP content (fmol/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=10)</td>
<td>5.3±1.5</td>
<td>1284±180</td>
<td>13.4±2.1</td>
<td>281±98</td>
<td>1.55±0.30</td>
<td>2278±443</td>
</tr>
<tr>
<td>Control (n=10)</td>
<td>37.7±11.7*</td>
<td>759±160*</td>
<td>13.6±2.4</td>
<td>245±52</td>
<td>1.58±0.38</td>
<td>2369±769</td>
</tr>
<tr>
<td>L-NAME(n=25)</td>
<td>7.4±3.4</td>
<td>1153±161</td>
<td>13.1±2.0</td>
<td>359±155</td>
<td>1.22±0.52</td>
<td>206±74*</td>
</tr>
<tr>
<td>L-NAME (n=15)</td>
<td>39.2±5.9*</td>
<td>742±202*</td>
<td>12.8±1.3</td>
<td>276±141</td>
<td>1.66±0.35</td>
<td>172±59*</td>
</tr>
<tr>
<td>P (ANOVA)</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>NS</td>
<td>NS</td>
<td>.02</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Sham1 month(n=10)</td>
<td>5.5±1.2</td>
<td>1307±179</td>
<td>13.7±2.0</td>
<td>224±79</td>
<td>1.70±0.30</td>
<td>2047±521</td>
</tr>
<tr>
<td>Sham1 month (n=10)</td>
<td>4.6±2.0</td>
<td>1273±131</td>
<td>13.2±1.5</td>
<td>220±66</td>
<td>1.60±0.22</td>
<td>2131±696</td>
</tr>
<tr>
<td>2K1C 1 month (n=10)</td>
<td>35.6±20.1*</td>
<td>905±170*</td>
<td>12.5±1.6</td>
<td>302±108</td>
<td>2.64±0.50*</td>
<td>1627±612</td>
</tr>
<tr>
<td>2K1C 2 months (n=10)</td>
<td>9.5±4.4</td>
<td>1083±155</td>
<td>13.0±2.0</td>
<td>321±135</td>
<td>1.99±0.23</td>
<td>1892±463</td>
</tr>
<tr>
<td>P (ANOVA)</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>NS</td>
<td>NS</td>
<td>&lt;.0001</td>
<td>NS</td>
</tr>
</tbody>
</table>

PRA, plasma renin activity; Ang I, angiotensin I; AG, angiotensinogen; ACE, angiotensin converting enzyme; ANF, atrial natriuretic factor; cGMP, cyclic guanosine monophosphate; Los, losartan; L-NAME, N\textsuperscript{\textdagger} -nitro-L-arginine-methyl ester; 2K1C, two-kidney, one clip rats. Data are mean±SD; P value of one-way ANOVA.

*P<.05 vs respective control group (control or sham).

The cardiac ACE activity was not significantly different in L-NAME rats compared with control rats; it was significantly increased in 2K1C rats at 1 month but not at 2 months.

Determinants of Left Ventricular Weight

LV index was highly correlated with the log of PRA in both the L-NAME group (L-NAME+control,

![Figure 3](http://hyper.ahajournals.org/)

**FIG 3.** Panel A: Plot shows relation (y=0.699x+1.583, r²=.62, P<.0001) between plasma renin activity and ratio of left ventricular (LV) weight to body weight of Wistar rats given water (control group, n=10, ○) or 50 mg/kg per day N\textsuperscript{\textdagger} -nitro-L-arginine-methyl ester (L-NAME group, n=25, ●). Panel B: Plot shows relation (y=1.250x+1.139, r²=.86, P<.0001) between plasma renin activity and ratio of LV weight to body weight of 1-month sham-operated rats (n=10, ○) and 1-month two-kidney, one clip rats (n=10, ◆). Semi-logarithmic transformation was used because of the large dispersion of plasma renin activity values (from 1.7 to 69 ng angiotensin I [AI] per milliliter per hour).

n=35, r=.78, P<.0001) and the 2K1C group (RV\textsubscript{1 month}+Sham\textsubscript{2 month}, n=20, r=.93, P<.0001) (Fig 3). The correlation coefficients between LV index and mean systolic blood pressure differed significantly from zero in both groups, but the correlation coefficient was significantly higher in the 2K1C group than in the L-NAME group (0.94 vs 0.63, P<.001).

We performed multiple linear regression to assess the separate effects of renin and blood pressure on LV index. The model using the log of LV index as the dependent variable and the independent variables of presence or absence of L-NAME administration, log of PRA, and mean systolic blood pressure gave the best fit (r²=.954, P<.0001, n=35). Each of the three independent variables was significantly associated with LV index, indicating that both renin and blood pressure contributed to LV weight independently of each other.

To further analyze the L-NAME group, we defined a posteriori two subgroups of L-NAME rats according to the presence of LV hypertrophy (LV index >2.19 mg/g, group L-NAME\textsubscript{a}, 6 of 25) or its absence (LV index ≤2.19 mg/g, group L-NAME\textsubscript{b}, 19 of 25). Table 3 compares the characteristics of these subgroups with those of the control group. The LV weight of group L-NAME\textsubscript{a} was significantly higher than that of group L-NAME\textsubscript{b} but not different from controls. Mean systolic blood pressure, PRA, and cardiac ACE activity of group L-NAME\textsubscript{a} were significantly higher than those of group L-NAME\textsubscript{b}. The cGMP content of the arterial wall and the plasma atrial natriuretic factor level were comparable in the two subgroups.

Myosin isoenzyme profile was determined in six left ventricles in each group (Fig 4 and Table 3). The left ventricles of control and group L-NAME\textsubscript{a} rats contained only the V1 form, whereas those of group L-NAME\textsubscript{b} had a shift from the V1 to the V3 form. These results were similar to those found in 2K1C rats (V1=63±3, V3=19±2, V5=18±2, n=6).

Reversibility of 1-Month L-NAME Hypertension

After L-NAME was stopped, systolic blood pressure was measured every 2 days by the tail-cuff method and averaged 189, 180, 158, 144, 137, and 135 mm Hg at 0, 2,
TABLE 3. Blood Pressure, Heart Weight, and Plasma and Tissue Parameters of Control Group and L-NAME Groups Without and With Left Ventricular Hypertrophy

<table>
<thead>
<tr>
<th>Group</th>
<th>SBP (mm Hg)</th>
<th>BW (g)</th>
<th>LVW (mg)</th>
<th>LVW/BW (mg/g)</th>
<th>RVW (mg)</th>
<th>RVW/BW (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=10)</td>
<td>130±4</td>
<td>459±35</td>
<td>866±65</td>
<td>1.93±0.13</td>
<td>245±26</td>
<td>0.53±0.03</td>
</tr>
<tr>
<td>L-NAMEa (n=19)</td>
<td>192±17*</td>
<td>433±30</td>
<td>852±47</td>
<td>1.96±0.10</td>
<td>218±17*</td>
<td>0.51±0.04</td>
</tr>
<tr>
<td>L-NAMEb (n=6)</td>
<td>234±11†</td>
<td>356±26†</td>
<td>934±49†</td>
<td>2.61±0.23†</td>
<td>223±15</td>
<td>0.63±0.04†</td>
</tr>
<tr>
<td>$P$ (ANOVA)</td>
<td>&lt;.0001</td>
<td>.0001</td>
<td>.008</td>
<td>&lt;.0001</td>
<td>.005</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

SBP, average of systolic blood pressure at 6, 7, and 8 weeks; BW, body weight; LVW, left ventricular weight; LVW/BW, ratio of LV weight to body weight; RVW, right ventricular weight; RVW/BW, ratio of RV weight to body weight; L-NAMEa, rats treated with Nω-nitro-L-arginine-methyl ester without LV hypertrophy; L-NAMEb, L-NAME rats with LV hypertrophy; PRA, plasma renin activity; Ang I, angiotensin I; cGMP, cyclic guanosine monophosphate; ACE, angiotensin converting enzyme. Data are mean±SD; $P$ value of one-way ANOVA.

* $P<.05$ vs control.
† $P<.05$ L-NAMEa vs L-NAMEb.

4, 6, 8, and 10 days, respectively. The rats were killed 1 month after L-NAME was stopped. The LV index, PRA, plasma atrial natriuretic factor, cardiac ACE activity, and aortic cGMP were not different from those of control rats, demonstrating a complete reversibility of the effects of L-NAME, including the slower rise in body weight.

Discussion

Nitric oxide released from L-arginine by endothelial cells activates vascular smooth muscle soluble guanylate cyclase, resulting in cGMP generation and vascular relaxation.\textsuperscript{1,4,24} Long-term oral administration of L-NAME is a novel experimental model producing hypertension.\textsuperscript{5,7} The present study confirms that the arterial wall of rats given 50 mg/kg per day L-NAME has a massive decrease of its cGMP content (one tenth normal). This holds true for all groups of L-NAME-treated rats, and the aortic wall cGMP is not influenced by the status of the renin-angiotensin system, which was stimulated in group L-NAMEa, normal in group L-NAMEb, and inhibited in the L-NAME rats treated with the angiotensin II antagonist losartan (group L-NAMEa). The hypertension due to nitric oxide synthase blockade is relatively sensitive to the angiotensin II antagonist losartan, as previously described,\textsuperscript{7} but it is interesting to observe that none of the treated rats achieved, at this losartan dose (10 mg/kg per day), the same blood pressure levels as those measured in the control rats. Furthermore, the blood pressure of control rats decreased by 8% with losartan treatment and is therefore still 46 mm Hg lower than that of L-NAME rats treated with losartan. Thus, the effects of L-NAME and losartan seem to be close to additive.

LV hypertension represents, besides age, the most powerful risk factor for cardiovascular morbidity and mortality.\textsuperscript{25} Arterial blood pressure is an important determinant of LV hypertension in most rat models of experimental hypertension.\textsuperscript{8-12} LV hypertrophy is thus considered to be a universal mechanism for adapting to cardiac overload. This was confirmed in the present 2K1C rats, the LV index being increased by 54% 4 weeks after induction of the hypertension and by 59% after 8 weeks. On the contrary, blockade of nitric oxide synthase for 8 weeks induced hypertension without LV hypertrophy (19 of 25, group L-NAMEa), confirming the observations after only 4 weeks of treatment.\textsuperscript{6} However, in 6 of 25 surviving rats (group L-NAMEa) and in the 5 rats that died, L-NAME induced LV hypertension (increases of 35% and 45%, respectively, of the control value). Mean systolic blood pressure was much higher in the rats with LV hypertrophy (L-NAMEa, 234±11 mm Hg) than in those without (L-NAMEa, 192±17 mm Hg). The level of hypertension was not the same in L-NAME rats as in 2K1C rats, which furthermore had a more rapid hypertension onset. These different profiles may influence differently the development of LV hypertrophy. Fig 2 indicates a possible rightward shift of the curve relating LV index to systolic blood pressure in L-NAME rats compared with 2K1C rats, suggesting that higher pressures are required to produce a unit increase in mass in L-NAME rats.

The renin-angiotensin-aldosterone system has been strongly implicated in the pathophysiology of 2K1C hypertension.\textsuperscript{8,10,26} At 1 month, the 2K1C rats had high PRA, which tended to return to baseline at 2 months.
Hypertensive rats given L-NNAME for 4 weeks had a PRA slightly below that of controls. This decrease in renin secretion could be attributed either to an increase in renal perfusion pressure or to the suppression of a direct stimulation of renin release by nitric oxide. PRA remained normal or low in rats treated with L-NNAME for 8 weeks without LV hypertrophy (group L-NNAME<sub>c</sub>). However, the L-NNAME rats with LV hypertrophy and the most severe hypertension had elevated PRA (group L-NNAME<sub>s</sub>). This secondary rise in PRA is probably due to the development of the renal lesions of nephroangiosclerosis, as previously described in rats treated with L-NNAME for 2 months. However, as demonstrated by ANOVA using multiple regression, two partially independent phenomena that may influence the development of LV hypertrophy have occurred in parallel: the rise in blood pressure and the activation of the renin-angiotensin system, in addition to L-NNAME administration. None of the L-NNAME rats given losartan during the second month (group L-NNAME<sub>los</sub>) developed LV hypertrophy. In these animals, the efficacy of the renin-angiotensin blockade is shown by a concomitant rise in renin and decrease in angiotensinogen, and the blood pressure was decreased to a level between that of the control rats and untreated L-NNAME rats.

ACE is an endothelial ectoenzyme involved in the generation of angiotensin II from Ang I and in the catabolism of other vasoactive peptides, such as bradykinin. Plasma ACE activity was normal in all groups. Cardiac ACE activity was normal in group L-NNAME<sub>c</sub> but increased in the hypertrophied left ventricle of both L-NNAME rats (group L-NNAME<sub>s</sub>) and 2K1C rats. Increased ACE activity has already been reported in association with cardiac hypertrophy caused by aortic coarctation and myocardial infarction. In L-NNAME hypertension as well as in 2K1C hypertension, increased cardiac ACE activity appears to be closely associated with cardiac hypertrophy and is absent when there is no LV hypertrophy. Another parameter that is similarly modified in the hypertrophied hearts of 2K1C and L-NNAME rats is the myosin isoenzyme pattern, independently of the mechanism of the hypertrophy. The myosin isoenzyme shift may precede LV hypertrophy and can occur in its absence. The absence of both myosin isoenzyme shift and LV hypertrophy in most of the L-NNAME rats is meaningful. Finally, release of atrial natriuretic factor in response to pressure overload was not significantly increased in the two models of hypertension and did not influence the arterial wall cGMP level.

The reason why L-NNAME hypertension did not induce LV hypertrophy, except if severe and accompanied by stimulation of the renin-angiotensin system, remains unknown. The occurrence of LV hypertrophy in this rat strain seems to be dependent on a certain blood pressure threshold below which no hypertrophy is observed. It is therefore likely that the absence of cardiac hypertrophy during L-NNAME administration is related to a level of blood pressure that is not sufficient to trigger renin release by a damaged kidney vasculature. However, even when blood pressure tended to be high and the renin-angiotensin system stimulated, LV hypertrophy was still less marked in the L-NNAME rats (35% of control value) than in the 2K1C rats (58%). It is possible that endothelium influences cardiomyocyte functions.

Endothelial cells, in particular endocardium, regulate cardiac contractility. Cardiac myocytes have a soluble guanylate cyclase, and cGMP is known to alter both cardiac contraction and relaxation. Elevation of cGMP induces a negative inotropic effect in ferret papillary muscle. Thus, the complete blockade of nitric oxide synthase by L-NNAME (as demonstrated by the 10-fold drop in arterial cGMP) could induce a positive inotropic effect, a functional adaptation that could replace the structural adaptation of LV hypertrophy. However, we did not test this hypothesis and this still remains speculative. Thus, endothelium-derived nitric oxide and the renin-angiotensin system probably influence LV hypertrophy in association with the increase in afterload.

The present study emphasizes the importance of nitric oxide and its second messenger cGMP in blood pressure regulation. Long-term administration of L-NNAME induces an increase in blood pressure and decreases aortic cGMP. Blockade of nitric oxide synthase for 8 weeks induces hypertension without LV hypertrophy in most rats (19 of 30) but also severe hypertension associated with LV hypertrophy (11 of 30). This hypertrophy is less marked than in 2K1C rats and is associated with an increased PRA, a shift in the isomyosin pattern, and an increased cardiac ACE activity. This study shows that, even if the degree of LV hypertrophy evolves in parallel with the duration and magnitude of a chronic rise in blood pressure, other factors, such as the renin-angiotensin system and nitric oxide production, influence this relation.

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