Sodium and Angiotensin in Hypertension Induced by Long-term Nitric Oxide Blockade

Bernard Jover, Abderraouf Herizi, François Ventre, Madeleine Dupont, and Albert Mimran

The influence of dietary sodium restriction and angiotensin II blockade on hypertension induced by a 25-day period of administration of the inhibitor of nitric oxide synthesis NG-nitro-L-arginine-methyl ester (10 mg/kg, twice daily by gavage) was assessed in Wistar rats fed a normal or low sodium diet. In addition, the angiotensin II receptor blocker losartan (30 mg/kg, once daily by gavage) was administered before and during NG-nitro-L-arginine-methyl ester in rats fed the normal sodium diet. At the end of the studies, conscious systolic arterial pressure increased similarly in NG-nitro-L-arginine-methyl ester–treated groups maintained on normal or low sodium intake. Moreover, a 25% reduction in cardiac output due to a decrease in stroke volume was observed in both groups. A slight but significant cardiac hypertrophic response was observed in hypertensive rats irrespective of sodium intake. At the completion of studies, plasma renin activity was similar to corresponding controls in the hypertensive groups on normal or low sodium intake. Losartan totally prevented the development of hypertension as well as the decrease in stroke volume and cardiac hypertrophy associated with NG-nitro-L-arginine-methyl ester treatment in rats on normal sodium intake. In conclusion, hypertension resulting from long-term blockade of nitric oxide synthesis was not affected by dietary sodium restriction. A crucial role for the renin-angiotensin system was demonstrated in this new model of hypertension. (*Hypertension* 1993;21:944–948)

**KEY WORDS** • sodium, dietary • angiotensins • nitric oxide • arginine

**Methods**

Studies were conducted in five groups of eight male Wistar rats weighing 225–275 g (Iffa-Credo, L’Arbresle, France) housed in individual metabolic cages and fed either a normal sodium (NS) or a low sodium (LS) diet. The NS diet consisted of a low sodium rat chow (<5 mmol of sodium per kilogram of chow) and distilled water containing 77 mmol of sodium per liter as drinking fluid. In rats maintained on the LS diet, sodium was removed from the drinking fluid 10 days before the experimental period to allow the animals to reach a new sodium balance.

After a 3-day pretreatment period, L-NAME (Sigma Chemical Co., France) was administered by gavage for 25 days at the dose of 10 mg/kg twice daily. The vehicle of L-NAME (distilled water, 1 mL/kg) was given in vehicle-treated animals. In a fifth group of rats maintained on the NS diet, the angiotensin II receptor antagonist losartan (DuP 753, MK 954, Du Pont Merck Pharmaceutical Co., Wilmington, Del.) was administered by gavage 24 hours before and throughout L-NAME treatment at a single daily dose of 30 mg/kg. In preliminary studies, this dose of losartan was found to achieve total and prolonged (at least 24 hours) inhibition of the pressor effect of a bolus injection of exogenous angiotensin II (300 ng/kg) in conscious, instrumented rats.

Daily intake and excretion of water and electrolytes were measured throughout the experiments. Conscious...
Influence of dietary sodium restriction and angiotensin blockade on L-NAME-induced hypertension

In NS rats, L-NAME was associated with a rise in systolic arterial pressure from a basal value of 121 ± 4 to 188 ± 7 mm Hg on day 25 (Figure 1). This rise was progressive and significance was achieved on day 5 (142 ± 9 mm Hg) when compared with baseline. The

Analytical Methods and Statistical Analysis

Plasma renin activity was determined by radioimmunoassay (CEN-Oris, Saclay, France) of generated angiotensin I after incubation at pH 6.5 without addition of exogenous rat renin substrate. The detection limit was 10 pg, the coefficient of variation of duplicates was ±2%, and the between-assay reproducibility was ±5%. Urinary concentration of electrolytes was measured by flame photometry (Corning, France).

Results are expressed as mean ± SEM. Unpaired data were analyzed by one-factor analysis of variance (treatment effect) and means compared with the Bonferroni test. Paired data were analyzed by one-factor analysis of variance for repeated measures (time effect) and comparison of means made with Dunnett’s t test.

Results

Influence of Dietary Sodium Restriction and Angiotensin Blockade on L-NAME–Induced Hypertension

In NS rats, L-NAME was associated with a rise in systolic arterial pressure from a basal value of 121 ± 4 to 188 ± 7 mm Hg on day 25 (Figure 1). This rise was progressive and significance was achieved on day 5 (142 ± 9 mm Hg) when compared with baseline. The
increase in arterial pressure induced by L-NAME was not affected by dietary sodium restriction (from 125±7 to 189±3 mm Hg). No significant change in systolic arterial pressure was observed during the observation period in vehicle-treated NS and LS animals. Administration of losartan before and during L-NAME treatment prevented the development of hypertension in NS rats (127±6 mm Hg on losartan and before L-NAME, 119±5 mm Hg at the end of studies).

As depicted in Table 1, plasma renin activity measured at the end of the experiments was higher (p<0.01) in LS than NS rats receiving the vehicle. After 25 days of L-NAME administration, plasma renin activity was similar to corresponding untreated groups. It was higher (p<0.001) in animals concomitantly treated with L-NAME and losartan when compared with vehicle-treated and L-NAME-treated rats maintained on the NS diet.

**Effect of Long-term L-NAME on Electrolytes**

As shown in Figure 2, L-NAME treatment was associated with no significant change in natriuresis during either the initial phase (6 days) of L-NAME administration or the subsequent days in NS rats with or without losartan treatment. Of interest, no change in sodium intake was observed in either group. No modification in potassium intake and excretion occurred in response to L-NAME and losartan administration. In LS rats, urinary sodium excretion was 9±1 μmol per 24 hours before L-NAME and remained constant thereafter (7±3 μmol per 24 hours on day 24).

In addition, no change in serum electrolytes was observed in either group, whereas serum creatinine concentration was higher than the corresponding group in L-NAME-treated NS and LS rats.

**Systemic Hemodynamics**

In both NS and LS groups, long-term L-NAME was associated with a significant increase in total peripheral resistance, a reduction in cardiac output, and no change in heart rate (Table 1); as a consequence, stroke volume was lower in these groups when compared with corresponding untreated groups. These effects were prevented by the concomitant administration of losartan in rats on ad libitum sodium intake.

**Effect of Long-term L-NAME on Heart Weight**

At the end of the experiments, a similar and significant (p<0.05) increase in heart weight index (heart

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**TABLE 1. Systemic Hemodynamics and Serum Electrolyte and Renin Levels in Conscious Animals at the End of Experiments**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal sodium diet</th>
<th>Low sodium diet</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vehicle</strong></td>
<td>L-NAME</td>
<td>Los+L-NAME</td>
</tr>
<tr>
<td>BW (g)</td>
<td>326±12</td>
<td>323±12</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>123±5</td>
<td>155±4*</td>
</tr>
<tr>
<td>CI (mL/min per kilogram)</td>
<td>342±31</td>
<td>255±24*</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>386±12</td>
<td>427±21</td>
</tr>
<tr>
<td>SVI (mL per beat per kilogram)</td>
<td>0.88±0.06</td>
<td>0.62±0.06*</td>
</tr>
<tr>
<td>TPR (mm Hg·min·kg/mL)</td>
<td>0.39±0.05</td>
<td>0.65±0.07*</td>
</tr>
<tr>
<td>Serum Na⁺ (mmol/L)</td>
<td>136±2</td>
<td>138±2</td>
</tr>
<tr>
<td>Serum K⁺ (mmol/L)</td>
<td>3.4±0.1</td>
<td>3.6±0.1</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>0.53±0.05</td>
<td>0.86±0.12*</td>
</tr>
<tr>
<td>PRA (ng Ang I/mL per hour)</td>
<td>12±1</td>
<td>17±5</td>
</tr>
</tbody>
</table>

**L-NAME, N°-nitro-L-arginine-methyl ester; Los, losartan; BW, body weight; MAP, mean arterial pressure; CI, cardiac index; HR, heart rate; bpm, beats per minute; SVI, stroke volume index (SVI=CI/HR); TPR, total peripheral resistance (TPR=MAP/CI); PRA, plasma renin activity; Ang I, angiotensin I.**

*p<0.05 compared with vehicle-treated group on same diet.
weight/body weight) was observed in NS and LS groups treated with L-NAME (2.8±0.1 and 2.8±0.1 mg/g body wt, respectively) when compared with their normotensive counterparts (2.5±0.1 and 2.4±0.1 mg/g body wt, respectively) (Figure 3). Administration of losartan prevented the cardiac hypertrophic response to L-NAME (2.2±0.1 mg/g body wt). Similar observations were made when the ratio of left to right ventricular weight was considered. When data were pooled, a positive correlation between heart weight index and the systolic arterial pressure achieved at the end of the studies was obtained (r=0.67, p<0.0001).

Response of Arterial Pressure to Vasoactive Substances

As shown in Table 2, complete blockade of the pressor response to exogenous angiotensin II was achieved in losartan-treated animals. The arterial pressure response to acetylcholine was unaltered, whereas the response to bradykinin was blunted by 46% in L-NAME–treated rats.

Discussion

In the present studies, it was observed that long-term administration of L-NAME was associated with the

<table>
<thead>
<tr>
<th>Vasoactive agent</th>
<th>Normal sodium diet</th>
<th>Low sodium diet</th>
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<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>L-NAME</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetylcholine (10 μg/kg)</td>
<td>−25.5±2.1</td>
<td>−26.7±1.7*</td>
</tr>
<tr>
<td>Bradykinin (10 μg/kg)</td>
<td>−28.8±5.2</td>
<td>−11.9±1.7*</td>
</tr>
<tr>
<td>Angiotensin II (300 ng/kg)</td>
<td>39.2±2.4</td>
<td>36.7±2.5</td>
</tr>
</tbody>
</table>

L-NAME, N°-nitro-L-arginine-methyl ester; Los, losartan. Changes in mean arterial pressure are expressed in millimeters of mercury.

*p<0.05 compared with vehicle-treated group on same diet.
development of hypertension. The level of arterial pressure achieved at the end of the studies was not affected by dietary sodium restriction. In addition, L-NAME hypertension was entirely prevented by treatment with the angiotensin II receptor antagonist losartan given before and during L-NAME, thus suggesting that the renin-angiotensin system plays a crucial role in this model of experimental hypertension.

Long-term L-NAME administration was associated with no change in natriuresis or sodium intake during the duration of the studies. This suggests that sodium retention may not be an important factor in this model; this was confirmed by the lack of influence of dietary sodium restriction on the progression of hypertension. However, because plasma renin activity was higher probably before and at the end of L-NAME treatment in sodium-restricted than sodium-replete rats, the possibility remains that activation of the renin system prevented any effect of dietary sodium restriction.

In acute studies, it was observed that pressor doses of nitric oxide synthase inhibitors resulted in a natriuretic response that was prevented when renal perfusion pressure was not allowed to increase by an aortic clamp. In contrast, nonpressor doses of inhibitors were associated with a decrease in urinary sodium excretion. In addition, in dogs it was observed that a 5-day infusion of L-NAME did not have an effect on arterial pressure, but dogs became slightly hypertensive when sodium intake was increased. It is possible that the elevation in arterial pressure observed in the present studies prevented eventual changes in sodium balance during L-NAME administration.

Despite the finding of identical levels of plasma renin activity in L-NAME–treated and untreated groups, losartan prevented the development of hypertension. Acute administration of L-NAME at pressor doses was shown to be associated with a fall in plasma renin activity, whereas an increase was observed after nonpressor doses of inhibitor. Activation of the renin-angiotensin system during the initial phase of L-NAME treatment cannot be excluded, thus explaining the favorable effect of losartan. Unfortunately, arterial pressure was not measured before day 5 of L-NAME in the present studies.

In addition, the efficacy of losartan may be related to enhancement by L-NAME of the pressor effect of endogenous vasoconstrictors, including angiotensin II. Hypertension may also result from blunting of nitric oxide–dependent vasodilator influences. Such a possibility is suggested by the decrease in blood pressure–lowering effect of exogenous bradykinin observed in the present and other studies and the lack of effect of bradykinin antagonist on arterial pressure and regional circulation after acute administration of nitric oxide synthase inhibitors. As found by others, the vasodepressor response to acetylcholine was not affected by L-NAME. This is in contrast with in vitro observation and remains to be elucidated.

Strong evidence that blockade of nitric oxide synthase is achieved in the present studies is provided through the demonstration that a similar dose of L-NAME resulted in a marked fall in aortic wall cyclic GMP, the second messenger of endothelium-derived nitric oxide.

L-NAME hypertension was maintained by an increase in systemic vascular resistance; however, a decrease in cardiac output and stroke volume was observed similar to that in acute studies. Such changes can be related to increased afterload and possibly decreased myocardial perfusion (preliminary studies showed that coronary blood flow was reduced by approximately 55% in L-NAME–treated rats) as well as an alteration of left ventricular contractility. Additional studies on anatomic myocardial changes are needed.

In contrast with others, a slight (approximately 12%) increase in heart weight index was observed, and a positive correlation between systolic arterial pressure and heart weight index was obtained. Such a cardiac response to hypertension appears insufficient when compared with that observed in renovascular hypertension and deserves additional attention.

References

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