Vasopressin Does not Effect Hypertension Caused by Long-Term Nitric Oxide Inhibition

Cécile Loichot, Catherine Cazaubon, Michèle Grima, Wybren De Jong, Dino Nisato, Jean-Louis Imbs, Mariette Barthelmebs

Abstract—Nitric oxide attenuates both vasopressin-induced vasoconstriction and vasopressin release. We tested whether hypertension and renal dysfunction elicited by chronic inhibition of nitric oxide (NO) synthesis using \( \text{N}^6 \)-nitro-l-arginine (L-NNA) could be mediated in part by vasopressin \( \text{V}_1 \text{A} \) receptors. Male rats were treated orally for 6 weeks with L-NNA (15 mg/kg per day), a nonpeptide \( \text{V}_1 \text{A} \) receptor antagonist (SR 49059), or a combination of SR 49059 and L-NNA (same doses), or they received no treatment. Both drugs were added to the food. Measurements were performed in conscious rats (urine collection in metabolic cages, tail-cuff arterial pressure) and at the end of the study in anesthetized rats (clearance measurements). L-NNA produced sustained hypertension, decreased glomerular filtration rate, and increased renal vascular resistance, plasma renin activity, and urinary albumin excretion. SR 49059 had no effect per se on these parameters and also did not attenuate the hypertension and renal dysfunction induced by L-NNA. Surprisingly, SR 49059 potentiated L-NNA–induced hypertension at the end of the 6-week treatment. However, the blood pressure response and the renal and mesenteric vasoconstriction elicited by exogenous vasopressin were attenuated in rats treated with SR 49059. L-NNA did not change plasma vasopressin concentration or 24-hour urinary vasopressin excretion. Our findings suggest that activation of vasopressin \( \text{V}_1 \text{A} \) receptors does not contribute to the hypertension and renal dysfunction induced by chronic NO synthesis inhibition. They also demonstrate unchanged plasma vasopressin concentration in NO-deficient hypertension. (Hypertension. 2000;35:602-608.)

Key Words: hemodynamics \( \bullet \) hypertension, experimental \( \bullet \) \( \text{N}^6 \)-nitro-l-arginine \( \bullet \) receptors, vasopressin \( \bullet \) SR 49059

Nitric oxide (NO) is an important modulator of vascular tone.\(^1\) Chronic inhibition of NO synthase (NOS) with l-arginine analogues, such as \( \text{N}^6 \)-nitro-l-arginine (L-NNA) or \( \text{N}^7 \)-nitro-l-arginine methyl ester (L-NAME), results in systemic hypertension associated with a decrease in renal blood flow and glomerular filtration rate.\(^2\) The possible mechanisms involved in these effects have recently been discussed.\(^3\) The lack of tonic vasodilation contributes to the acute response to NOS inhibition, although the activation of the renin-angiotensin system (RAS)\(^4\) and of the sympathetic nervous system\(^2\) is prevailing in the chronic response. Vasopressin could also contribute to the systemic and renal hemodynamic effects elicited by chronic NOS inhibition, in view of the fact that NO attenuates both vasopressin-induced vasoconstriction and vasopressin release. Vasopressin is known to be a potent renal vasoconstrictor in vitro via stimulation of the vasopressin \( \text{V}_1 \text{A} \) receptor, and its constrictor effects are enhanced by NOS inhibition.\(^6,7\) In vivo, inhibition of NO synthesis potentiates the vasopressin-induced pressor effect\(^8\) as well as renal vasoconstriction.\(^9\) Increased NO release in response to activation of vasopressin \( \text{V}_1 \text{A} \) receptors has recently been detected in isolated rat kidneys by use of a chemiluminescence assay.\(^10\)

On the other hand, there is evidence that NO is involved in the control of vasopressin secretion. NOS is colocalized with vasopressin in several brain areas and in the nerve terminals of the posterior pituitary.\(^11\) NOS inhibition in rats enhances supraoptic neuronal activity in the hypothalamus\(^12\) and vasopressin release from the neural lobe of the pituitary gland in vitro.\(^13\) These data are in favor of an inhibitory control by NO of vasopressin release, although a stimulatory control has been reported by others.\(^14\) Moreover, low concentrations of vasopressin strongly potentiate adrenergic contractions in isolated arteries,\(^15\) an effect that could dominate after chronic NO synthesis inhibition. The contribution of vasopressin in the hemodynamic response to NOS inhibition, at least in the acute phase, has indeed been suggested because a vasopressin...
V₄ receptor antagonist has been found to attenuate the hypertension and the increase in renal vascular resistance elicited by the intravenous administration of L-NAME in dogs.¹⁶

The aim of the present study was to elucidate the role of vasopressin and of the activation of vasopressin V₁A receptors in the cardiovascular and renal effects elicited by chronic NOS inhibition in rats. For this purpose, we used (2S)-1-(2R,3S)-5-chloro-3-(2-chlorophenyl)-1-(3,4-dimethoxy-benzene-sulfonyl)-3-hydroxy-2,3-dihydro-1H-indole-2-carbonyl]-pyrrolidine-2-carboxamide (SR 49059), a potent, nonpeptide, and orally active specific V₁A receptor antagonist.¹⁷ The effects of SR 49059 were evaluated on L-NNA–induced hypertension and renal dysfunction. Part of the present study has been published as an abstract.¹⁸

Methods

Animals and Treatments

Six-week-old male Sprague-Dawley rats (Ifa-Credo, L’Arbresle, France) were kept at 20°C with a light/dark cycle of 12 hours (light on at 6:00 AM) and fed with standard rat food (AO4 powder, UAR) or standard food supplemented with SR 49059 (Sanofi Recherche) and/or L-NNA (Sigma). Food intake was measured individually in rats put in metabolic cages every 1 or 2 weeks. The concentrations of drugs in food were progressively adjusted in order to maintain the rats put in metabolic cages every 1 or 2 weeks. The concentrations of drugs in food were progressively adjusted in order to maintain the daily treatment with SR 49059 and L-NNA at ≈30 mg/kg and 15 mg/kg. Experiments were performed in accordance with guidelines of the European Community and the French government concerning the use of animals.

Experimental Protocols

Three protocols were performed as follows. Protocol 1 examined the effects of SR 49059 on systemic and renal responses to 6-week NOS inhibition with L-NNA. We compared 4 groups of rats receiving either no treatment (control), SR 49059 or L-NNA alone (SR 49059 group, L-NNA group), or SR 49059 and L-NNA in combination (SR 49059+L-NNA group). Systolic blood pressure (SBP), heart rate (HR), and renal excretions were measured before and every 2 weeks after treatment began. At the end of the 6-week treatment, renal hemodynamics were assessed from clearance measurements in anesthetized rats. Protocol 2 examined the effects of a 4-week treatment with L-NNA on plasma vasopressin concentration. At the end of this treatment, measurements of SBP, HR, and renal excretions were performed as in protocol 1, and then plasma vasopressin was measured with rats in a conscious unrestrained state. Protocol 3 examined the effects of a 3-week treatment with SR 49059 (30 mg/kg daily dose) on the cardiovascular response to intravenously administered vasopressin. Mean blood pressure and regional vasoconstrictor responses to exogenous vasopressin were measured in anesthetized rats. This assessment was also performed in some rats at the end of protocol 1.

Experimental Measurements

SBP and HR were measured in the morning in trained conscious rats by indirect tail-cuff sphygmomanometry (Narco-System, Roucaire). Rats were thereafter placed in individual metabolic cages (Ifa Credo) with free access to tap water and food. After a 24-hour period to accustom the rats, urine was collected over another 24-hour period. Protease inhibitors (200 µL of a phenylmethylsulfonyl fluoride solution [100 mmol/L] and 200 µL of an azide sodium solution [1%]) were added to the urine collection vial; urinary albumin and total protein had already been determined. Also, urinary volume, urinary electrolytes, γ-glutamyltranspeptidase (GGT), and lactate dehydrogenase (LDH) were measured. Water and food intakes were also assessed. Vasopressin was measured on an aliquot of urine preserved on boric acid (10 mg/mL urine). Nitrite/nitrate (NOx) measurements were performed on an additional 5-hour urine sample collected on antibiotics/antimycotics.¹⁹

Clearance study was performed in rats anesthetized with thionbutabarbital (Inactin, 100 mg/kg IP, Byk Gulden) as previously described.²⁰ Glomerular filtration rate and renal plasma flow were evaluated from the clearance of polyfructosan and p-aminohippuric acid, respectively. At the end of the study, arterial blood was collected on Na₂-EDTA for plasma renin activity (PRA), urea nitrogen, and creatinine measurements. Rats were killed by anesthetic overdose before the kidneys and the heart were excised, blotted, and weighed.

Plasma vasopressin was measured in conscious unrestrained rats instrumented previously with an intra-aortic catheter under pentobarbital anesthesia (50 mg/kg IP, Sanofi Santé Nutrition Animale). After 24 hours, aortic blood (3 mL) was withdrawn by use of Na₂-EDTA in ice-chilled tubes for plasma vasopressin determination. Control experiments had revealed that at this time after the operation plasma vasopressin had returned to the basal values.

Systemic and regional hemodynamic responses to exogenous vasopressin were evaluated in anesthetized rats as previously described.²¹ Left renal and superior mesenteric blood flows (flows; electro-magnetic flow probes, Skalar) were continuously measured, together with mean blood pressure, which enabled regional vascular resistance calculation. Vasopressin (arginine vasopressin, UCB Pharma) was injected as a bolus (10 to 300 ng/kg IV). Responses were expressed as percent change of basal value.

Analytical Methods

Electrolytes were measured with ion-selective electrodes (EL-Ise, Beckman); GGT and LDH activities, by enzymatic kinetics (Beckman autoanalyzer); arterial hematocrit, by a micromethod using glass capillaries; and albumin, by radial immunodiffusion with rabbit anti-rat albumin–specific antibodies (Tebu). Creatinine and urea nitrogen concentrations (Beckman autoanalyzer), together with concentrations of proteins,²² p-aminohippuric acid, and polyfructosan,²³ were determined by use of colorimetric methods. PRA was assessed by radioimmunoassay²³ as was determined by plasma vasopressin concentration after ethanol extraction (Bühlmann Laboratories AG). NOx content in urine was measured by Griess reaction.

Statistical Analysis

Data are mean±SEM. Results at any time were analyzed by unpaired Student t test, 1-way ANOVA, or 2-way ANOVA. Equality of variance between groups was verified by the Levene test. Logarithmic transformation of data was used when necessary. In case of significant interaction in 2-way ANOVA, the effect of L-NNA alone was tested. Survival was compared by a χ² test. Dose-response curves for vasopressin were analyzed by ANOVA with repeated measurements and Greenhouse-Geisser adjustments. Statistics were run with BMDP Statistical Software (Statistical Software Ltd), and values of P<0.05 were considered significant.

Results

Effects of 6-Week Treatment With L-NNA and/or SR 49059

Rats were treated with L-NNA at a mean daily dose of 14.4±0.3 and 14.3±0.2 mg/kg for 6 weeks (L-NNA and L-NNA+SR 49059 groups, respectively) and/or with SR 49059 at a mean daily dose of 29.1±0.4 and 28.7±0.3 mg/kg (SR 49059 and L-NNA+SR 49059 groups, respectively).

SBP and HR

As expected, L-NNA–treated rats became hypertensive, with a significant increase in SBP from 2 weeks after beginning treatment (Figure 1). Hypertension developed further with the duration of treatment and was associated with a decrease in HR. SR 49059 had no effect per se on SBP and HR. At the
end of the 6-week treatment, the effect of L-NNA on blood pressure was potentiated in the group receiving combined treatment with SR 49059 (significant interaction, \(P<0.001\)).

**Urinary Excretions and Neurohormonal Activation**

L-NNA–induced hypertension was not associated with changes in body weight, diuresis, natriuresis, GGT, or LDH excretion in urine (Table 1) or food and water intake (data not shown). However, L-NNA induced a 3-fold increase in albumin excretion from the fourth week of treatment (Figure 1). This increase was not observed for total protein excretion (Table 1). SR 49059 had no effect per se and also did not modify L-NNA–induced effects. The decrease in glomerular filtration rate was accompanied by an increase in plasma creatinine and urea nitrogen (Table 2). L-NNA had no effect on kidney weight (data not shown) but increased the heart weight (1.40±0.04 versus 1.27±0.05 g in control group, \(P<0.05\)) and the heart weight/body weight ratio (0.32±0.01×10^{-2} versus 0.29±0.01×10^{-2}, \(P<0.01\)). SR 49059 had no effect per se on the weight parameters and also did not modify L-NNA–induced changes.

**Survival**

One rat died in the L-NNA group on day 40, with a high SBP (210 mm Hg). Four rats died in the SR 49059+L-NNA group after 4 weeks (n=1) or 6 weeks of treatment (n=2) or after anesthesia (n=1); all exhibited high SBP (190 to 210 mm Hg). Survival analysis showed no significant difference between groups.

**Effects of 4-Week Treatment With L-NNA**

In this experiment, the rats developed cardiovascular and renal function changes similar to those reported in protocol 1. SBP increased to 159±4 mm Hg (119±3 mm Hg in control group, n=7, \(P<0.001\)), and urinary albumin excretion increased to 1.03±0.38 mg/dl (0.39±0.08 mg/dl in control group, \(P<0.05\)). However, urinary excretion of vasopressin did not change (3.14±0.35 and 2.99±0.39 ng/dl in treated and control group, respectively), as reported above, for the 6-week treatment. L-NNA treatment also left plasma vasopressin concentration unchanged (1.12±0.34 and 1.24±0.20 pg/ml). Rats were treated with L-NNA at a mean daily dose of 13.6±0.2 mg/kg over the 4 weeks.

**Effects of 3-Week Treatment With SR 49059**

Intravenous bolus injections of vasopressin dose-dependently increased mean blood pressure, with a parallel decrease in both renal and mesenteric blood flows (Figure 3). The 3-week treatment by SR 49059 antagonized the hemodynamic effects induced by exogenous vasopressin, by whatever parameter was considered (\(P<0.001\), Figure 3). Hemodynamic responses to the lowest doses of vasopressin were completely abolished. This inhibition was maintained at the end of the 6-week treatment with SR 49059, as assessed in 2 or 3 rats per group at the end of the clearance study (data not shown). Rats were treated with SR 49059 at a mean daily dose of 29.6±0.5 mg/kg over the 3 weeks.

**Renal Hemodynamics, Kidney, and Heart Weights**

L-NNA treatment for 6 weeks resulted in a 30% decrease in renal plasma flow and glomerular filtration rate and a 3-fold increase in renal vascular resistance (Figure 2). SR 49059 had no effect per se and also did not modify L-NNA–induced effects. The decrease in glomerular filtration rate was accompanied by an increase in plasma creatinine and urea nitrogen (Table 2). L-NNA had no effect on kidney weight (data not shown) but increased the heart weight (1.40±0.04 versus 1.27±0.05 g in control group, \(P<0.05\)) and the heart weight/body weight ratio (0.32±0.01×10^{-2} versus 0.29±0.01×10^{-2}, \(P<0.01\)). SR 49059 had no effect per se on the weight parameters and also did not modify L-NNA–induced changes.

**Discussion**

In the present study, NOS inhibition with L-NNA for 6 weeks induced a marked increase in systemic blood pressure and decreases in renal blood flow and glomerular filtration rate. These results confirm earlier reported observations for both L-NAME and L-NNA.\(^3\)\(^,\) Using an orally active nonpeptide vasopressin antagonist, SR 49059, we were able to investigate for the first time the contribution of vasopressin V_1_ receptors to the hemodynamic changes elicited by chronic inhibition of NO synthesis. Blockade of the V_1_ receptors failed to attenuate the elevation in blood pressure and the alterations in renal functions induced by L-NNA. However, the treatment with
SR 49059 completely abolished the hemodynamic responses to low doses of exogenous vasopressin, without a decrease in the effectiveness of vasopressin V1A receptor inhibition during the study.

Our findings with chronic NOS inhibition differ from those of Manning et al., who investigated the acute effects of L-NAME in dogs. These authors reported that the increase in blood pressure and renal vascular resistance elicited by L-NAME was attenuated by the peptide V1 receptor antagonist d(CH2)5Tyr(Me)vasopressin. A possible explanation may be that potentiation of vasoconstrictor responses to vasopressin did not persist after long-term inhibition of NO synthesis. Indeed, although acute NOS inhibition increased vasoconstrictor responses to vasopressin of various vascular beds, sensitivity to vasopressin was left unchanged in mesenteric resistance arteries taken from rats treated for 2 weeks by L-NAME.

TABLE 1. Effects of 6-wk Treatment With L-NNA and/or SR 49059 on Urinary Excretion in Conscious Rats

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Body Weight, g</th>
<th>Volume, mL/d</th>
<th>Sodium, mmol/d</th>
<th>GGT, IU/d</th>
<th>LDH, IU/d</th>
<th>Protein, mg/d</th>
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<tbody>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Control (9)</td>
<td>201 ± 5</td>
<td>6.1 ± 0.7</td>
<td>1.4 ± 0.1</td>
<td>3.8 ± 0.4</td>
<td>0.8 ± 0.1</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>L-NNA (12)</td>
<td>210 ± 9</td>
<td>8.5 ± 0.8</td>
<td>1.5 ± 0.1</td>
<td>3.3 ± 0.3</td>
<td>1.0 ± 0.1</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>SR 49059 (9)</td>
<td>202 ± 3</td>
<td>6.2 ± 0.5</td>
<td>1.4 ± 0.1</td>
<td>4.4 ± 0.5</td>
<td>1.0 ± 0.1</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>L-NNA+SR 49059 (12)</td>
<td>208 ± 9</td>
<td>7.5 ± 0.5</td>
<td>1.5 ± 0.1</td>
<td>4.1 ± 0.3</td>
<td>1.0 ± 0.1</td>
<td>0.9 ± 0.1</td>
</tr>
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2-wk treatment

<table>
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<tr>
<th>Experimental Group</th>
<th>Body Weight, g</th>
<th>Volume, mL/d</th>
<th>Sodium, mmol/d</th>
<th>GGT, IU/d</th>
<th>LDH, IU/d</th>
<th>Protein, mg/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (9)</td>
<td>310 ± 9</td>
<td>8.9 ± 1.2</td>
<td>1.6 ± 0.1</td>
<td>12.5 ± 1.0</td>
<td>1.2 ± 0.1</td>
<td>11.5 ± 0.9</td>
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<tr>
<td>L-NNA (12)</td>
<td>311 ± 8</td>
<td>9.9 ± 1.1</td>
<td>1.5 ± 0.1</td>
<td>11.2 ± 1.1</td>
<td>1.2 ± 0.1</td>
<td>10.5 ± 1.5</td>
</tr>
<tr>
<td>SR 49059 (9)</td>
<td>315 ± 4</td>
<td>7.7 ± 0.7</td>
<td>1.6 ± 0.1</td>
<td>12.9 ± 1.3</td>
<td>1.2 ± 0.1</td>
<td>9.8 ± 1.7</td>
</tr>
<tr>
<td>L-NNA+SR 49059 (12)</td>
<td>304 ± 4</td>
<td>9.7 ± 1.4</td>
<td>1.5 ± 0.1</td>
<td>11.3 ± 1.0</td>
<td>1.2 ± 0.2</td>
<td>8.8 ± 0.9</td>
</tr>
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4-wk treatment

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<tr>
<th>Experimental Group</th>
<th>Body Weight, g</th>
<th>Volume, mL/d</th>
<th>Sodium, mmol/d</th>
<th>GGT, IU/d</th>
<th>LDH, IU/d</th>
<th>Protein, mg/d</th>
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</thead>
<tbody>
<tr>
<td>Control (9)</td>
<td>374 ± 14</td>
<td>10.7 ± 1.6</td>
<td>1.9 ± 0.2</td>
<td>12.5 ± 1.0</td>
<td>1.3 ± 0.2</td>
<td>17.3 ± 1.8</td>
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<tr>
<td>L-NNA (12)</td>
<td>379 ± 12</td>
<td>14.7 ± 1.8</td>
<td>2.1 ± 0.1</td>
<td>14.5 ± 1.5</td>
<td>1.7 ± 0.3</td>
<td>18.4 ± 1.1</td>
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<tr>
<td>SR 49059 (9)</td>
<td>381 ± 8</td>
<td>11.2 ± 1.1</td>
<td>1.9 ± 0.1</td>
<td>14.1 ± 2.5</td>
<td>1.7 ± 0.4</td>
<td>14.7 ± 1.2</td>
</tr>
<tr>
<td>L-NNA+SR 49059 (11)</td>
<td>367 ± 11</td>
<td>12.6 ± 1.2</td>
<td>1.9 ± 0.1</td>
<td>14.7 ± 1.5</td>
<td>1.5 ± 0.2</td>
<td>16.0 ± 1.4</td>
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6-wk treatment

<table>
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<tr>
<th>Experimental Group</th>
<th>Body Weight, g</th>
<th>Volume, mL/d</th>
<th>Sodium, mmol/d</th>
<th>GGT, IU/d</th>
<th>LDH, IU/d</th>
<th>Protein, mg/d</th>
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</thead>
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<tr>
<td>Control (9)</td>
<td>421 ± 6</td>
<td>11.0 ± 1.5</td>
<td>1.8 ± 0.2</td>
<td>11.4 ± 0.9</td>
<td>1.6 ± 0.3</td>
<td>17.0 ± 1.2</td>
</tr>
<tr>
<td>L-NNA (11)</td>
<td>430 ± 14</td>
<td>14.0 ± 1.5</td>
<td>1.9 ± 0.1</td>
<td>12.6 ± 1.2</td>
<td>1.9 ± 0.2</td>
<td>23.2 ± 2.4</td>
</tr>
<tr>
<td>SR 49059 (9)</td>
<td>432 ± 9</td>
<td>12.7 ± 0.8</td>
<td>2.0 ± 0.1</td>
<td>13.6 ± 1.5</td>
<td>1.5 ± 0.1</td>
<td>17.6 ± 1.4</td>
</tr>
<tr>
<td>L-NNA+SR 49059 (9)</td>
<td>425 ± 11</td>
<td>16.2 ± 2.4</td>
<td>2.0 ± 0.1</td>
<td>14.1 ± 1.9</td>
<td>2.3 ± 0.6</td>
<td>21.0 ± 4.4</td>
</tr>
</tbody>
</table>

Values are mean ± SEM and are analyzed by 1-way ANOVA (baseline) or 2-way ANOVA (for the different treatment durations). Whatever the parameter considered, ANOVA analyses were not significantly different. The number of rats per group is given in parentheses.

TABLE 2. Effects of 6-wk Treatment With L-NNA and/or SR 49059 on Urinary Excretions of NOx, and Vasopressin, Plasma Urea Nitrogen and Creatinine Levels, and PRA

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>NOx, μmol/5h</th>
<th>Vasopressin, ng/d</th>
<th>Plasma Urea, mmol/L</th>
<th>Plasma Creatinine, μmol/L</th>
<th>PRA, ng Ang II/(mL · h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (9)</td>
<td>0.90 ± 0.14</td>
<td>3.7 ± 0.4</td>
<td>6.3 ± 0.6</td>
<td>52 ± 2</td>
<td>20.4 ± 4.3</td>
</tr>
<tr>
<td>L-NNA (11)</td>
<td>0.24 ± 0.03</td>
<td>3.3 ± 0.4</td>
<td>7.4 ± 0.6</td>
<td>58 ± 3</td>
<td>49.4 ± 14</td>
</tr>
<tr>
<td>SR 49059 (9)</td>
<td>0.67 ± 0.14</td>
<td>4.0 ± 0.6</td>
<td>5.7 ± 0.4</td>
<td>43 ± 2</td>
<td>23.8 ± 5.8</td>
</tr>
<tr>
<td>L-NNA+SR 49059 (8)</td>
<td>0.35 ± 0.07</td>
<td>3.9 ± 0.7</td>
<td>7.4 ± 0.7</td>
<td>57 ± 4</td>
<td>43.5 ± 11.1</td>
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</table>

2-way ANOVA

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>NOx, μmol/5h</th>
<th>Vasopressin, ng/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-NNA</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>SR 49059</td>
<td>...</td>
<td>NS</td>
</tr>
<tr>
<td>SR 49059×L-NNA</td>
<td>&lt;0.05</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean ± SEM and are analyzed by 2-way ANOVA. NS indicates nonsignificant. The number of rats per group is given in parentheses. PRA was determined in 3 to 6 animals in each group. Urinary excretions of NOx and vasopressin were measured respectively in a 5-h and a 24-h urine sample collected in metabolic cages at the end of the 6-wk treatment. Plasma urea nitrogen, creatinine, and renin activity were measured in plasma samples collected at the end of the clearance study of anesthetized rats.
Compensatory mechanisms, linked to an upregulation of vasodilatory systems such as endothelium-derived relaxing factor or prostanoids, may have occurred during chronic NOS inhibition.

No change in plasma vasopressin was found in the present study after chronic NOS inhibition by L-NNA. This was the case when plasma vasopressin concentration was measured directly in conscious rats, but there was also no alteration in urinary vasopressin excretion assessed after 4 or 6 weeks of treatment with the NOS inhibitor. Vasopressin is filtered at the level of the glomerulus without tubular reabsorption or secretion. Urinary excretion of vasopressin over a time period may thus be a marker for the mean plasma level of the peptide over that time. A number of studies reported that NO might be involved in an inhibitory control of vasopressin secretion. Indeed, in several in vitro experiments investigating vasopressin release from the hypothalamus or the neurohypophysis, different NO donors or L-arginine inhibited vasopressin release, whereas NOS inhibitors had opposite effects. A 2-fold increase in plasma vasopressin has also been reported after intravenous infusion of L-NAME in conscious rabbits. Alternatively, the possible involvement of NO as a stimulator of vasopressin release has also been reported. Intracerebroventricular injections of NO donors or L-arginine enhanced plasma vasopressin level, whereas a NOS inhibitor had opposite effects. However, whether an increase or a decrease in vasopressin release was elicited by acute NOS inhibition, these responses lasted only a few minutes. Such transient changes might explain why we observed no change in plasma or urinary vasopressin after chronic NOS inhibition. The present results agree with the recent observation of Manning et al, who also found unchanged plasma vasopressin level after a 5-day treatment with L-NAME in dogs. Moreover, vasopressin does not seem to be an essential actor in NO-deficient hypertension, in view of the fact that NOS inhibition was able to elicit sustained increases in blood pressure in homozygous Brattleboro rats.

Surprisingly, rats receiving combined treatment with L-NNA and SR 49059 developed a more pronounced hypertension after 6 weeks of treatment. A similar observation was previously reported by Pucci et al in an acute study in which the L-NNA–induced pressor effect was enhanced in rats pretreated with a peptide V1 receptor antagonist. In the present study, this interaction was not likely linked to the RAS because the activation of this system by L-NNA, as shown by the increase in PRA, was identical in the absence or the presence of SR 49059. A further activation of this system could have been expected.
because the activation of V₁ receptors has been shown to inhibit renin release.³⁴ Vasopressin V₂ receptor–related water and sodium retention are also unlikely to play a role because intake and renal excretion of both water and sodium remained unchanged. Therefore, the present study does not allow us to delineate the precise mechanism of the elevation of SBP resulting from the interaction between NOS blockade and vasopressin V₁A receptor inhibition.

The present study provides insight into the contribution of the kidney to NO-deficient hypertension. Renal failure, blunted pressure natriuresis, and stimulation of the RAS may all contribute to hypertension induced by NOS inhibition. In fact, renal vasoconstriction and glomerular alteration resulted in renal failure, as was obvious in the present study by the decrease in glomerular filtration rate, the increase in plasma creatinine and urea nitrogen levels, and leakage of albumin in urine. Urinary excretions of LDH and GGT, respective decrease in glomerular filtration rate, the increase in plasma creatinine and urea nitrogen levels, and leakage of albumin in urine. Urinary excretions of LDH and GGT, respectively, markers of epithelial cell lysis and proximal tubule brush border disruption, were not affected. These results agree with histological data showing predominant glomerular and arteriolar damage after chronic NOS inhibition.³⁵ Daily sodium excretion remained stable over the 6-week treatment with L-NNA despite the increase in SBP, an observation consistent with an alteration in the pressure-natriuresis relation as previously reported.³ Finally, we found an increase in PRA after the 6-week treatment with L-NNA, although variable changes in PRA have been reported during chronic NOS inhibition.³ The activation of the RAS has been correlated with the occurrence of necrotic lesions of renal arterioles and left ventricular hypertrophy.³⁶ Consistent with that report, we also observed cardiac hypertrophy in L-NNA–treated rats. However, the increase in the heart weight/body weight ratio remained small (≈10%) compared with that in deoxycorticosterone acetate–salt hypertension at similar blood pressure levels.³⁷

In conclusion, inhibition of vasopressin V₁A receptors was unable to inhibit the development of hypertension and the decrease in renal blood flow and glomerular filtration rate observed after chronic inhibition of NOS activity with L-NNA in rats. Chronic treatment with L-NNA did not change plasma vasopressin concentration. The activation of V₁A receptors does not seem to participate in NO-deficient hypertension and the concomitant alteration of renal functions.

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