Low-Dose Nitric Oxide Inhibition Produces a Negative Sodium Balance in Conscious Dogs

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Abstract. Nitric oxide modulates renal hemodynamics and salt and water handling. Studies on the latter have provided conflicting results, however. Electrolyte and water balances were therefore studied in 28 beagles for 4 d, to determine the various effects of nitric oxide synthase (NOS) inhibition on renal function. The dogs were chronically equipped with aortic occluders to reduce renal perfusion pressure (RPP), bladder catheters, and catheters for measurements of RPP and mean arterial BP. A swivel system allowed free movement within the kennels. In a first set of experiments, a nonpressor dose of L-\textsubscript{N}\textsuperscript{o}-nitroarginine (LN) (3 \textmu g/min per kg body wt) was administered, to prevent increases in mean arterial BP and thus pressure effects on renin release and natriuresis.Remarkably, the nonpressor dose of LN caused a negative sodium balance. The natriuretic effect may involve reduced plasma renin activity, reduced aldosterone concentrations, and increased atrial natriuretic peptide concentrations. Changes in aldosterone levels, however, were the only parameters to parallel the time course of sodium excretion. In a second set of experiments, a sodium-retaining challenge was elicited by reduction of RPP. Dogs without NOS inhibition escaped sodium retention during RPP reduction after 2 d ("pressure escape"). LN neither ameliorated nor aggravated the sodium-retaining effect of reduced RPP, nor did it compromise the accomplishment of pressure escape. In conclusion, inhibition of NOS with a low dose of LN results in a reduction of total-body sodium. This effect mainly relies on reduced aldosterone concentrations. Furthermore, LN does not change the regulatory response to long-term RPP reduction.

Since the first applications of nitric oxide (NO) synthase (NOS) inhibitors in experiments on kidney function, there has been agreement that NOS inhibition markedly reduces renal blood flow (1–4). The effects of NOS on volume and electrolyte excretion and renin release remain controversial, however. Several studies demonstrated an antinatriuretic effect of NOS blockade (4–6), whereas others observed either natriuresis (3,7,8), or no significant changes in sodium excretion (9–11). Similarly, some studies have demonstrated a stimulating effect of NO on renin release, whereas the opposite effect has also been described (12–15).

These discrepancies may be partly attributable to differences in protocols, such as the dosage of the NOS inhibitors, the use of anesthesia, and the length of the experiments (short-term versus long-term) (2,3,8). In long-term studies, mutual interference of controllers of sodium excretion and arterial pressure, as well as multiple regulatory feedback loops, must be taken into account. For instance, inhibition of NOS results, via general vasoconstriction, in increases in arterial pressure. However, sustained pressure elevation may increase sodium excretion, via both reduced renin release and the mechanism of pressure natriuresis. Prolonged natriuresis would result in a total-body sodium (TBS) deficit, which, in turn, would increase renin release and thus the activity of the renin-angiotensin-aldosterone system (RAAS).

The clarification of NO effects on sodium homeostasis remains of prime importance, because TBS content is well known to be a major determinant of long-term arterial pressure. The experiments presented here were performed in freely moving dogs over a period of 4 d. The objective was to elucidate the various effects of NOS inhibition on the control of sodium excretion. Sodium, water, and potassium balances were obtained, and the plasma renin activity (PRA), plasma aldosterone concentration (PAC), and atrial natriuretic peptide (ANP) concentration were frequently measured. A nonpressor dose of L-\textsubscript{N}\textsuperscript{o}-nitroarginine (LN) was deliberately chosen, to prevent pressure effects on renin release and natriuresis. NOS inhibition severely compromises gastric relaxation (16,17), thus reducing spontaneous food and water intake (18). To ensure accurate determination of balances, the intake of sodium, water, and potassium with food was strictly controlled in this study.

In some studies, NOS inhibition alone did not affect sodium excretion but inhibition markedly reduced sodium excretion when combined with prostaglandin synthesis inhibition, which itself exerts a mild sodium-retaining effect (10,19,20). Therefore, in a second set of experiments, we instituted a sodium-
retaining challenge by reducing renal perfusion pressure (RPP) via a servo-control system. Normally, dogs escape this sodium-retaining stimulus after 1 or 2 d (“pressure escape”) (21,22). Low-dose LN infusion in these dogs served to demonstrate the extent to which the pressure escape phenomenon was dependent on NO formation.

Materials and Methods

Animals
Twenty-eight chronically instrumented, female beagle dogs (approximately 2 yr of age; body weight, 12 to 19 kg) were examined. After completion of the experimental period, implants were removed and the dogs were given to suitable private individuals. The study was approved by the Berlin government, according to the German Animal Protection Law.

Surgery and Maintenance
Each dog was equipped with a urinary bladder catheter, an inflatable occluder placed around the aorta above the renal arteries, and two femoral artery catheters. One catheter was advanced into the abdominal aorta directly below the renal arteries, whereas the tip of the other catheter was placed well above the renal arteries. All lines were exteriorized in the nape region. The dogs were allowed at least 3 wk for recovery. Catheter-related infections were prevented with a catheter-restricted antibiotic-lock technique (23). Daily assessments of general status, body temperature, body weight, and erythrocyte sedimentation rate ensured that only healthy dogs were studied. The dogs were housed individually in large kennels (9 m²), in a sound-protected, air-conditioned, animal room. For reasons of social well-being, at least one additional dog in an adjacent kennel accompanied the dog under investigation (24).

Experimental Protocol

Dietary Regimen. Beginning at least 5 d before the studies, food intake was controlled with respect to food composition, daily feeding time, and completeness of intake. The food provided 5.5 mmol/d per kg body wt Na⁺, 3.5 mmol/d per kg body wt K⁺, and 91 ml/d per kg body wt water (i.e., 82 mmol of Na⁺, 52 mmol of K⁺, and 1365 ml of water each day for a 15-kg dog). The dogs were offered the food once daily, at 8:30 a.m. If a dog did not finish its meal within 20 min, it was tube-fed with the remainder. Complete food and water intake was thus guaranteed. No additional feeding and no further access to water was allowed until the feeding the next day.

Experimental Configuration. During the study, the lines emerging from the nape region of each dog were connected to a swivel system, which allowed free movement within the limits of the 9-m² kennel (for details, see reference 24). From the swivel, the lines were led to an adjoining room (the laboratory) via a covering tube. All actions necessary to conduct the experiments could thus be performed from the laboratory without disturbing the dogs in the animal room, except for 1 h each day (8:00 to 9:00 a.m.). This 1 h was required for animal care and feeding and recalibration of the electronic equipment. With this configuration, each dog was studied for 4 d consecutively. Each 24-h period lasted from 8:00 a.m. to 8:00 a.m. the next day.

Experimental Groups and Interventions. The dogs were randomly assigned to one of four protocols (n = 7 dogs/protocol). In protocol 1 (control), dogs were studied without reduced RPP (rRPP) and with vehicle infusion only (DSW solution; infusion rate, 55 µl/min). In protocol 2 (LN), dogs were studied while LN (dissolved in vehicle; Sigma Chemical Co., St. Louis, MO) was continuously infused at a rate of 3 µg/min per kg body wt. In protocol 3 (rRPP), dogs were studied with vehicle infusion and continuous reduction of RPP to 75 to 80% of the individual dog’s mean arterial BP (MABP), as determined on control days. In protocol 4 (rRPP plus LN), dogs were studied while RPP was reduced as in protocol 3 and LN was infused as in protocol 2. All fluid administered via arterial lines was accounted for in the balances.

Servo-Controlled Reduction of RPP. Servo-control of RPP was achieved by infusion and deflation of the aortic occluder, driven by the output of a HSE Plugsys bus system (Hugo Sachs Elektronik, March, Germany), which continuously monitored RPP (21). The MABP (catheter above the aortic occluder) and RPP (catheter below the aortic occluder) were measured with pressure transducers integrated into the swivel. Reduction of RPP and infusions of LN or vehicle were initiated at 8:00 a.m. on day 1 and were never discontinued before the end of day 4.

Measurements and Analyses

MABP, RPP, and Heart Rate Measurements. MABP, RPP, and heart rate (HR) were measured continuously on all days and stored on a computer as 1-min averages (1440 values/d per dog).

Urine Collection and Analyses. Urine collections were performed throughout the study period. The urinary bladder was emptied every 20 min by means of a computerized urine collection system (for details, see reference 25). The urine collected during each 24-h period was analyzed for sodium, potassium, and creatinine levels. The urine volume was measured gravimetrically.

Blood Collection and Analyses. Every 4 h (i.e., at 9:00 a.m., 1:00 p.m., 5:00 p.m., 9:00 p.m., 1:00 a.m., and 5:00 a.m.), blood samples were obtained via a line otherwise used to continuously flush the arterial catheters. The withdrawn blood was always replaced by an equal amount of stored blood, which had been collected from the respective dog approximately 2 wk before the experiments. For each blood sample (10 ml), the plasma sodium, potassium, and protein concentrations, plasma osmolality, ANP concentration, PAC, and PRA were analyzed. The GFR was assessed by measuring the exogenous creatinine clearance (for details, see reference 21).

Calculations

Calculation of Mean 24-h Values. For each dog, daily mean values for plasma parameters (calculated from the values for the six daily plasma samples) and MABP, HR, and RPP (calculated from the 1440 daily 1-min values) were determined. To obtain mean 24-h values for each protocol day, the individual daily means for the seven dogs in the protocol were averaged. Accordingly, the individual daily sodium and potassium excretion rates and urine volumes were averaged.

Calculation of Cumulative Balances. The difference between 24-h intake and 24-h urinary excretion of water, sodium, and potassium in the control group was considered extrarenal loss. Daily extrarenal losses were considered constant throughout the study period, because the room temperature was kept constant and the body temperature and physical activity of the dogs did not change markedly during the observation period. Therefore, individual 24-h balances were calculated as the differences between the excretion in the control group and the excretion by individual dogs on each day for protocols 2, 3, and 4. The cumulative balances for individual dogs were calculated by summing the 24-h balance values for the consecutive 4 d of the study. The mean cumulative balances were obtained by averaging the individual cumulative balances for each protocol. Changes in
cumulative balances reflect changes in TBS, total-body potassium (TBP), and total-body water (TBW).

Statistical Analyses

Statistical comparisons were made using Number Cruncher statistical software (NCSS 6.0, J. L. Hintze, Kaysville, UT). Differences in the mean 24-h values between the groups were assessed by using the unpaired t test with Bonferroni’s adjustment for multiple comparisons, with a significance level of $P < 0.05/m$, where $m$ is the number of comparisons between groups. Differences between mean 24-h values for different days within each protocol were assessed by general linear model ANOVA for repeated measures, followed by Duncan’s multiple-comparison test, with a significance level of $P < 0.05$. Data are presented as means ± SEM.

Results

During NOS inhibition (LN and rRPP plus LN protocols), most of the dogs refused to eat spontaneously; therefore, tube feeding was often necessary to guarantee complete sodium, potassium, and water intake. In addition, experiments with numerous dogs could not be completed (results not included here) because NOS inhibition led to vomiting, probably via inhibition of gastric relaxation (16,17).

Hemodynamic Parameters

**Mean 24-h MABP.** For control animals, MABP averaged approximately 120 mmHg (Figure 1). MABP was not significantly altered by LN, compared with control values, nor did MABP change throughout the LN study. MABP increased during the course of day 1 of the rRPP protocol, reaching a 24-h mean of approximately 132 mmHg. MABP increased gradually on the subsequent days, reaching approximately 142 mmHg on day 4. Similarly, MABP increased during the course of day 1 of the rRPP plus LN protocol (24-h mean, approximately 131 mmHg) and continued to increase to approximately 148 mmHg on day 4, a level significantly higher than that observed with rRPP alone.

**Mean 24-h rRPP.** During the rRPP and rRPP plus LN protocols, RPP was set to 75 to 80% of the dog’s MABP measured on individual control days. During the rRPP protocol, mean 24-h RPP values were 88 ± 3 mmHg on day 1, 87 ± 3 mmHg on day 2, 88 ± 3 mmHg on day 3, and 89 ± 3 mmHg on day 4. During the rRPP plus LN protocol, mean 24-h RPP values were 88 ± 2, 87 ± 2, 87 ± 2, and 88 ± 2 mmHg, respectively.

**Mean 24-h HR.** For LN-treated dogs, HR decreased strikingly during the course of day 1 and remained at the lower level (approximately 70% of control HR) throughout the observation period (Figure 1). During the rRPP protocol, HR also decreased (to approximately 85 to 90% of control HR). During the rRPP plus LN protocol, HR was even lower than during the LN protocol (approximately 65% of control HR).

**Mean 24-h GFR.** The mean 24-h GFR remained unchanged during the LN protocol, as well as during the rRPP plus LN protocol (Table 1). The GFR was decreased only on day 4 of the rRPP protocol.

Urinary Excretion Values and Cumulative Balances

**Sodium Excretion and Balance.** On control days, the mean 24-h urinary sodium excretion was approximately 90% of daily intake (Figure 2, top). During the LN protocol, urinary sodium excretion was significantly increased on days 2 and 3 (approximately 109% and approximately 107% of intake, respectively). Accordingly, TBS decreased during these LN days, amounting to an average total sodium loss of 1.9 mmol/kg body wt by the end of day 4 (Figure 3, top). During rRPP day 1, sodium excretion was reduced (approximately 30% of intake); therefore, TBS increased by approximately 3.4 mmol/kg body wt. On the subsequent rRPP days, sodium excretion was in the range of control values. Therefore, TBS remained stable, at the elevated level, on days 2 to 4 of the rRPP protocol. During the rRPP plus LN protocol, sodium excretion was reduced on day 1 (approximately 44% of intake) and remained slightly reduced on day 2 (approximately 80% of intake). Sodium excretion reached control levels on day 3 of the rRPP plus LN protocol and increased even above control.
levels on day 4 (approximately 103% of intake). Changes in TBS on days 1 to 3 of the rRPP plus LN protocol did not differ significantly from those observed during the rRPP protocol. Because of the increase in sodium excretion on day 4 of the rRPP plus LN protocol, the TBS surplus at the end of day 4 was slightly less (approximately 2.4 mmol/kg body wt) than that observed with rRPP alone (approximately 3.8 mmol/kg body wt).

**Potassium Excretion and Balance.** On control days, the mean 24-h urinary potassium excretion was approximately 83% of intake (Figure 2, middle). In LN-treated dogs, urinary potassium excretion was significantly reduced on day 1 but returned to control levels on the subsequent days. Accordingly, TBP increased, with the greatest surplus amounting to approximately 0.8 mmol/kg body wt at the end of day 2 of the LN protocol (Figure 3, middle). Urinary potassium excretion increased and TBP transiently decreased on rRPP day 1, but TBP returned to control levels thereafter. During the rRPP plus LN protocol, urinary potassium excretion was reduced on days 1 and 2 but control levels were attained thereafter. TBP remained elevated throughout the study, amounting to a maximum of approximately 1.2 mmol/kg body wt on rRPP plus LN day 2.

**Urine Volume and Volume Balance.** On control days, the mean 24-h urine volume was approximately 87% of water intake (Figure 2, bottom). During the LN protocol, urine volume was in the range of control values on days 1, 2, and 4; it was increased only on day 3. No significant changes in TBW were observed (Figure 3, bottom). During the rRPP protocol, urine volume was markedly reduced on day 1, slightly reduced on days 2 and 4, and in the range of control values on day 3. Accordingly, TBW was increased by approximately 26 ml/kg body wt on day 1 and reached approximately 39 ml/kg body wt at the end of day 4. During the rRPP plus LN protocol, the time course of urine volume changes was similar to that for the rRPP protocol, except for a greater excretion rate on day 4 (approximately 92% of intake). Changes in TBW were not

### Table 1. Plasma concentrations of sodium, potassium, and protein; plasma osmolality; and GFR

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma sodium concentration (mM)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>146.1 ± 1.3</td>
<td>146.8 ± 1.1</td>
<td>147.1 ± 0.9</td>
<td>146.4 ± 1.1</td>
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<tr>
<td>LN</td>
<td>142.4 ± 1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>142.0 ± 1.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>142.9 ± 1.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>142.0 ± 1.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>rRPP</td>
<td>143.8 ± 1.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>144.1 ± 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>144.5 ± 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>144.7 ± 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>rRPP + LN</td>
<td>144.4 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>145.6 ± 0.3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>145.4 ± 1.0&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>145.0 ± 0.5&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Plasma potassium concentration (mM)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>3.84 ± 0.06</td>
<td>3.92 ± 0.04</td>
<td>3.88 ± 0.05</td>
<td>3.94 ± 0.03</td>
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<tr>
<td>LN</td>
<td>3.91 ± 0.07</td>
<td>4.02 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.11 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.05 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>rRPP</td>
<td>4.00 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.97 ± 0.06</td>
<td>4.02 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.11 ± 0.06&lt;sup&gt;b,e,f&lt;/sup&gt;</td>
</tr>
<tr>
<td>rRPP + LN</td>
<td>3.96 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.18 ± 0.05&lt;sup&gt;b,c,d,e&lt;/sup&gt;</td>
<td>4.20 ± 0.07&lt;sup&gt;b,c,d,e&lt;/sup&gt;</td>
<td>4.37 ± 0.06&lt;sup&gt;b,c,d,e,f,g&lt;/sup&gt;</td>
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<tr>
<td><strong>Plasma osmolality (mosM)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>302.4 ± 1.0</td>
<td>302.2 ± 0.9</td>
<td>301.7 ± 0.6</td>
<td>302.9 ± 1.1</td>
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<tr>
<td>LN</td>
<td>302.1 ± 0.4</td>
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<td>302.8 ± 0.9</td>
<td>303.8 ± 1.0</td>
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<tr>
<td>rRPP</td>
<td>299.2 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>298.9 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>298.4 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>300.0 ± 1.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>rRPP + LN</td>
<td>302.8 ± 0.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>302.7 ± 0.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>301.9 ± 1.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>301.4 ± 0.9&lt;sup&gt;b,c,d&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>GFR (ml/min per kg body wt)</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>control</td>
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<td>3.80 ± 0.13</td>
<td>3.82 ± 0.22</td>
<td>3.92 ± 0.37</td>
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<td>LN</td>
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<td>3.92 ± 0.27</td>
<td>4.01 ± 0.30</td>
</tr>
<tr>
<td>rRPP</td>
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<td>3.79 ± 0.24</td>
<td>3.62 ± 0.23</td>
<td>3.47 ± 0.17&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>rRPP + LN</td>
<td>3.78 ± 0.17</td>
<td>3.80 ± 0.21</td>
<td>3.76 ± 0.32</td>
<td>3.84 ± 0.25</td>
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</table>

<sup>a</sup> Mean 24-h values were calculated for 4 d of control conditions (Control), 4 d of continuous infusion of L-<sup>N</sup>-nitroarginine (LN), 4 d of reduced renal perfusion pressure (rRPP), and 4 d of rRPP plus LN infusion (rRPP + LN). Values are means ± SEM (n = 7 dogs/protocol).

<sup>b</sup> Significant versus control.

<sup>c</sup> Significant versus LN.

<sup>d</sup> Significant versus rRPP (unpaired t test with Bonferroni’s adjustment for multiple comparisons).

<sup>e</sup> Significant versus day 1.

<sup>f</sup> Significant versus day 2.

<sup>g</sup> Significant versus day 3 within the respective protocol (general linear model ANOVA for repeated measures with Duncan’s multiple-comparisons test).
significantly different between the rRPP plus LN and rRPP protocols. The surplus of TBW amounted to 29 ml/kg body wt at the end of day 4 of the rRPP plus LN protocol.

**Plasma Parameters**

**Mean 24-h PRA.** During the control protocol, mean 24-h PRA was 3.2 ng angiotensin I (AngI)/ml per h (Figure 4, top). During the LN protocol, PRA was strikingly reduced, with the lowest level being 0.5 ng AngI/ml per h on day 3. During the rRPP protocol, PRA was increased, with the highest level being 5.7 ng AngI/ml per h on day 4. During the rRPP plus LN protocol, PRA was observed to be at or below control levels, i.e., it was higher than during LN administration but lower than with rRPP alone.

**Mean 24-h PAC.** During the control protocol, the mean 24-h PAC was approximately 80 pg/ml (Figure 4, middle). During the LN protocol, PAC was decreased on days 2 and 3, with the lowest level being 52 pg/ml on day 3. During the rRPP protocol, PAC was increased on day 1 (130 pg/ml) but decreased to or even below control levels on the subsequent days. During the rRPP plus LN protocol, changes similar to those for the rRPP protocol were observed; after elevation of PAC on day 1 (105 pg/ml), PAC decreased to or even below control levels on the subsequent days.

**Mean 24-h ANP Concentrations.** During the control protocol, the mean 24-h ANP concentration was approximately 40 pg/ml; during the LN protocol, ANP concentrations were elevated throughout the observation period, with the highest level being 84 pg/ml on day 2 (Figure 4, bottom). During the rRPP protocol, ANP concentrations increased strikingly on days 1 and 2 and reached 151 pg/ml on day 4. During the rRPP plus LN protocol, changes similar to those for the rRPP protocol...
were observed; the ANP concentration reached 154 pg/ml on day 4.

**Other Parameters.** The mean 24-h plasma sodium concentration was reduced during the LN, rRPP, and rRPP plus LN protocols, compared with the control protocol (Table 1). The mean 24-h plasma potassium concentration was increased during the LN, rRPP, and rRPP plus LN protocols, compared with the control protocol; the highest plasma potassium concentration was observed with the rRPP plus LN protocol (Table 1). The mean 24-h plasma osmolality was considerably reduced during the rRPP protocol and was slightly reduced during the rRPP plus LN protocol, compared with the control protocol (Table 1). The mean 24-h plasma protein concentration was reduced during the LN, rRPP, and rRPP plus LN protocols, compared with the control protocol; the lowest plasma protein concentration was observed with the rRPP plus LN protocol (Table 1).

Extrarenal sodium losses were assessed from control measurements. The amount (approximately 0.5 mmol/kg body wt per d) and fraction (approximately 9% of sodium intake) of extrarenal losses were relatively small. Therefore, possible changes in extrarenal losses were neglected in calculations of 24-h balances for protocols 1 to 3. Because dogs have no sweat glands, extrarenal sodium losses are predominantly attributable to fecal loss. Aldosterone is known to stimulate intestinal sodium reabsorption. Decreased PAC, as observed on LN protocol days 2 and 3, may increase fecal sodium loss, in addition to its natriuretic effect. The total amount of sodium loss, *i.e.*, the actual decrease in TBS, would thus be underestimated. With elevated PAC on day 1 of the rRPP and rRPP plus LN protocols, the total amount of sodium retained would be underestimated. However, because extrarenal losses are small, the changes in extrarenal losses should be even smaller and these underestimations should thus be minimal.

**Discussion**

NO seems to be an important factor in the interdependence of BP and fluid and electrolyte homeostasis. NO relaxes vascular smooth muscle cells and may thus reduce BP. Arterial pressure, however, affects sodium and fluid excretion via pressure-dependent renin release and pressure natriuresis. Remarkably, NO contributes to pressure-dependent renin release (15) and was also supposed to mediate pressure natriuresis (26). When these multiple interactions are considered, it is not surprising that the isolated effects of NO on water and electrolyte balances have not been previously unraveled.

From the data presented here, it can be concluded that NO indeed has a pressure-independent sodium-retaining effect; the low dose of the NOS inhibitor LN, *i.e.*, a nonpressor dose that was sufficiently high to induce severe bradycardia, produced a negative sodium balance on days 2 and 3 of the study period (Figures 2 and 3). Because GFR was unchanged by LN, the natriuresis observed is apparently related to reduced tubular sodium reabsorption. Studies on the direct effects of NO on tubular transport processes have produced controversial results. For instance, a micropuncture study (27) suggested that increased NO levels decreased proximal fluid reabsorption. However, a study using renal clearance and *in situ* microperfusion techniques (28) reported that NO enhanced tubular sodium reabsorption via increased cGMP levels. Reduced tubular sodium reabsorption during the LN protocol, however, does not necessarily rely on a direct NO action on tubular transport processes. The RAAS may mediate this effect. Indeed, PRA was markedly reduced by LN (Figure 4). Therefore, decreased sodium reabsorption could be evoked by decreased AngII levels. Moreover, aldosterone levels were decreased in the LN-treated dogs (Figure 4). The suppression of aldosterone was significant only on days 2 and 3. Remarkably, this was the time period during which natriuresis occurred (Figure 2). Therefore, the decrease in aldosterone levels may be the pivotal mechanism underlying LN natriuresis.

In short-term studies in conscious dogs (15), NOS inhibition
was demonstrated to reduce renin release. Studies with prolonged NOS inhibition, however, have yielded conflicting results (6,10,29). As concluded by Qiu et al. (3) from a long-term study in conscious rats, these discrepancies may be mainly related to infrequent plasma sampling on different days of NOS inhibition, because prolonged NOS inhibition may result in a certain time course of PRA. Initial suppression of renin release, with increased arterial pressure or without pressure changes (as in this study), would result in natriuresis and reduced TBS. TBS, however, is a major determinant of renin release (30).

Because of this feedback loop, renin release can be expected to again increase, and the time frame may depend on the duration and magnitude of the initial natriuresis. The same may apply to aldosterone levels, and the reincrease in aldosterone levels on day 4 of the LN protocol (Figure 4) supports this hypothesis.

ANP levels increased in response to the nonpressor infusion of LN, possibly because of atrial distension attributable to increased central venous pressure caused by LN-induced venoconstriction. However, ANP levels were also elevated on days when normal sodium excretion was observed, i.e., LN days 1 and 4. Therefore, ANP may play a minor role in the observed natriuresis.

A sodium-eliminating effect after NOS inhibition seems to be in contrast to the results of early studies that focused on the renal effects of NO (6,26,31,32). Various mechanisms by which NOS blockade may induce antinatriuresis have been suggested (27,33–36). One hypothesis has attracted particular interest, namely that NOS inhibition may, via its effect on endothelium-derived NO, disturb the mechanisms of pressure natriuresis and/or renal adaptation to high sodium intake. This hypothesis seems to be supported by several findings. The short-term relationship between RPP and sodium excretion was found to be blunted by NOS inhibition in anesthetized dogs (26) and anesthetized rats (37), and acute increases in RPP led to similar changes in renal medullary NO activity (35). Long-term renal medullary NOS inhibition in uninephrectomized rats decreased sodium and water excretion (34). Recent long-term balance studies in conscious dogs, however, brought into question the importance of NO in pressure natriuresis and renal adaptation to increased sodium intake. Granger et al. (38) demonstrated that NOS inhibition did not alter the escape from mineralocorticoid-induced sodium retention, which is known to depend mainly on pressure natriuresis (39). Manning et al. (29) observed that NOS inhibition did not compromise the renal adaptation to increased sodium intake, nor did it increase the sodium sensitivity of long-term arterial pressure. Finally, several recent studies demonstrated either no effect of NOS inhibition on sodium excretion or a natriuretic effect (3,10,29,38,40). These results support our finding that a non-pressor dose of a NOS inhibitor induces a negative sodium balance.

In this study, we also investigated the role of NO on pressure escape. Pressure escape describes the re-establishment of sodium balance in the presence of chronically decreased RPP (21). Reduction of RPP leads to transient sodium and water retention for 1 or 2 d. Thereafter, sodium and water intake/output balances again become equilibrated (Figure 3). LN infusion did not either ameliorate or aggravate the sodium- and water-retaining effects observed with rRPP alone. In particular, LN neither hastened nor delayed the achievement of equilibrated intake/output balances (Figures 2 and 3). Investigating conscious dogs during chronic aldosterone excess, Granger et al. (38) also observed that the initial sodium retention and the time course of “mineralocorticoid escape” were unchanged by NOS inhibition. Although that study supports the findings presented here, it was not fully comparable to our experimental design. Because RPP was not controlled in the study by Granger et al. (38), the finding of an unchanged sodium balance may be partly accounted for by the increased RPP during NOS inhibition.

Our earlier studies (22,24) clarified the role of the RAAS during long-term reduction of rRPP in dogs without NOS inhibition. Initial sodium and water retention occurs via pressure-dependent renin release and thus increased aldosterone concentrations (24). Pressure escape, i.e., re-equilibration of intake/output balances, is mainly accomplished by aldosterone suppression despite increased PRA levels (22). As shown in Figure 4, pressure-dependent renin release was decreased by NOS inhibition but PRA was still markedly increased during the rRPP plus LN protocol, compared with LN alone. However, PRA levels reached control levels only. It was thus very surprising to observe that the PAC on day 1 of the rRPP plus LN protocol was far above control levels. Accordingly, the aldosterone/PRA ratio increased dramatically; the same finding was noted with the LN protocol. We therefore hypothesize that NOS inhibition amplifies the stimulatory action of AngII on adrenal aldosterone secretion. We can only speculate on the mechanisms underlying this intriguing finding. In vitro studies suggest that NO impairs steroidogenesis by direct interaction with the heme group of the cytochrome P450 enzymes (41). Studies using cultured adrenal zona glomerulosa cells suggest that endothelium-derived NO may inhibit basal and AngII-induced aldosterone biosynthesis (41,42). In rats, the number of adrenal angiotensin AT1 receptors was observed to be increased after prolonged NOS inhibition; however, it was not determined whether that effect was attributable to a direct action of NOS inhibition or occurred secondary to changes in TBS (43). Finally, another in vitro study suggests that NO inhibits the angiotensin-convertase enzyme (44).

On day 1 of the rRPP plus LN protocol, the increased PAC probably led to sodium retention, as has been demonstrated for sodium retention during rRPP without NOS inhibition (24). Similar to pressure escape without NOS inhibition, pressure escape during NOS inhibition apparently relies on the suppression of aldosterone levels. In addition, the relatively low PRA values, which probably resulted in low AngII levels, may have contributed to the escape.

In summary, a low dose of LN (which does not increase arterial BP but is sufficiently high to induce bradycardia and attenuation of renin release) results in a negative sodium balance. This effect mainly relies on reduced aldosterone levels. NOS inhibition during 4 d of rRPP did not change the time course of pressure escape, nor did it change the magnitude of sodium and water retention. Pressure escape is accomplished in
conjunction with a decrease in aldosterone levels, with or without NOS inhibition. NOS inhibition, however, dramatically increases the aldosterone/PRA ratio, via unknown mechanisms. The results presented here emphasize that the response to long-term NOS inhibition consists of primary changes and secondary feedback regulation. For instance, primary suppression within the RAAS results in initial sodium loss. This deficit of TBS then triggers compensatory mechanisms (e.g., the reincrease in aldosterone levels), which counteract further sodium loss. These long-term effects of the feedback control of TBS should be kept in mind when NO inhibitors or donors are used in clinical practice.

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