Salt-Induced Increase in Arterial Pressure During Nitric Oxide Synthesis Inhibition

F. Javier Salazar, Antonio Alberola, José M. Pinilla, J. Carlos Romero, Tomás Quesada

The objective of this study was to determine in conscious dogs the role of endothelium-derived nitric oxide in mediating the arterial pressure and renal response to a prolonged increment of sodium intake. After a control period of 3 days, an inhibitor of nitric oxide synthesis, N\textsuperscript{G}-nitro-L-arginine-methyl ester, was infused intravenously during 5 consecutive days (0.1 µg/kg per minute). Sodium intake (80 mmol/d) did not change throughout the experiment in one group (n=4). In another group (n=6), 1 day after infusion of this inhibitor was started, sodium intake increased from 80 to 300 mmol/d during 4 consecutive days. Inhibition of nitric oxide synthesis in dogs with normal sodium intake induced a significant decrease in natriuresis and diuresis (P<.05) without changes in arterial pressure. However, in dogs treated with the nitric oxide synthesis inhibitor, mean arterial pressure increased from 95.2±3.3 to 106.2±4.0 mm Hg (P<.01) the first day that sodium intake was elevated and remained increased the following 3 days. In a different group of dogs (n=5), the increment of sodium intake during 4 days did not induce changes in arterial pressure when nitric oxide synthesis was not inhibited. Cumulative sodium balance was higher (P<.01) in dogs treated simultaneously with the nitric oxide synthesis inhibitor and high sodium intake (158±21 mmol sodium) than in those treated only with the nitric oxide synthesis inhibitor (82±19 mmol sodium) or with high sodium intake (36±13 mmol sodium). Our results demonstrated that dogs fail to handle appropriately a prolonged increase in sodium intake when nitric oxide synthesis is chronically inhibited. (Hypertension 1993;22:49-55)

KEY WORDS  • hypertension, sodium-dependent  • nitric oxide  • endothelium-derived relaxing factor  • sodium

It is well known that the increase in renal sodium excretion that occurs in response to an elevation in sodium intake is important for the maintenance of normal body fluid volumes and arterial pressure. A correlation has been found between renal excretory function and arterial pressure in several genetic models of hypertension, and, in some strains, a critical relation exists between sodium intake and the magnitude of hypertension.\textsuperscript{1,2} Different mechanisms have been proposed to be responsible for the altered renal response to elevations in sodium intake that occurs in several models of hypertension.\textsuperscript{3-5} One factor that may limit the ability of the kidney to respond normally to prolonged increments in sodium intake is a decrease in endothelium-derived nitric oxide (EDNO) synthesis.\textsuperscript{6,7} A recent study by our group\textsuperscript{7} in conscious chronically instrumented dogs demonstrated that inhibition of EDNO synthesis during 3 consecutive days induces a significant and sustained decrease in sodium and water excretion. In another study by our group,\textsuperscript{9} it was demonstrated that increases in natriuresis and diuresis in response to an acute extracellular volume expansion are inhibited by more than 25% when EDNO synthesis is inhibited. These studies support the concept that EDNO plays an important role in the long-term regulation of renal excretory function and in the regulation of the renal response to an acute increase of extracellular fluid volume. However, the role of EDNO in the regulation of the renal excretory response to prolonged increments in sodium intake has not been assessed.

We undertook the present study to determine the role of EDNO in the response of renal excretory function and arterial pressure to a prolonged increase in sodium intake. We hypothesized that endogenous EDNO may serve as a mediator in the renal response to an increase in sodium intake during several consecutive days and that arterial pressure may increase as a consequence of the sodium retention that may occur during simultaneous EDNO synthesis inhibition and increase in sodium intake. To test this hypothesis, we inhibited nitric oxide synthesis with an intravenous infusion of N\textsuperscript{G}-nitro-L-arginine-methyl ester (L-NAME) during 5 consecutive days at a dose (0.1 µg/kg per minute) previously shown in pilot studies not to produce changes in arterial pressure. In the present study, the response of renal
excretory function and arterial pressure was compared in four groups of conscious dogs in which (1) L-NAME was infused during 5 consecutive days without changes in sodium intake; (2) L-NAME was infused during 5 consecutive days, but sodium intake was increased from 80 to 300 mmol/d during 4 consecutive days 1 day after L-NAME infusion was started; (3) sodium intake was increased from 80 to 300 mmol/d during 4 consecutive days without the L-NAME infusion; and (4) L-arginine (L-Arg) (5 μg/kg per minute) was simultaneously infused with L-NAME (0.1 μg/kg per minute) during 5 consecutive days, and sodium intake was increased during 4 days 1 day after the administration of both substances was started.

Methods

Experimental Procedures

Experiments were performed in female mongrel dogs (14 to 22 kg). All experimental procedures were designed according to the Recommendations From the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals approved by the Council of the American Physiological Society. Surgery was performed under aseptic conditions and with dogs under pentobarbital sodium (30 mg/kg iv) anesthesia for implantation of Tygon catheters into the femoral artery and vein. The catheters were placed in the aorta, distal to the origin of the renal arteries, and in the vena cava, respectively. The catheters were tunneled subcutaneously, exteriorized between the scapulae, and placed in neck collars. The dogs were allowed to recover from surgery for at least 2 weeks, during which time antibiotics were given to prevent postoperative infections.

After recovery from surgery and 7 days before the experiments were started, the dogs were housed in individual metabolic cages and fitted with harnesses that contained blood pressure transducers mounted at heart level and connected to an amplifier (model 12-4615-71, Gould Instruments, Cleveland, Ohio). Mean arterial pressure (MAP) was recorded continuously with a Gould recorder (Thermal writing recorder, model 2400S). MAP readings were obtained every 30 minutes from the paper recorder; the single daily value was the average of the readings obtained each 30-minute period over a 20- to 22-hour period. The femoral vein catheter was connected to a roller pump (model 410, Watson-Marlow, Falmouth, UK) that was used to infuse solutions continuously throughout the study. All solutions were infused through a disposable filter (Cathivex, Millipore Corp, Bedford, Mass) to prevent minute air bubbles and possible contaminants from entering the venous system. The filters were changed frequently throughout the study. The infusion tubing and transducers were protected by a vacuum hose attached to the harness. Dogs were restrained in such a way that they could not turn more than 180°, to prevent twisting and consequent damage to the infusion lines or transducer cable.

During the control, experimental, and recovery periods of the study, all dogs were fed a sodium-deficient diet (H/D, Hill's Pet Products, Topeka, Kan) that provided 5 to 7 mmol sodium and 70 mmol potassium per day. Dogs were allowed free access to tap water throughout the experiment. Isotonic saline was continuously infused via the femoral vein at a rate of approximately 500 mL/d to maintain the total sodium intake constant at 80 mmol/d, including the sodium provided in the food.

Experimental Groups

Group 1 (n=4): Effects of L-NAME infusion. After a 3-day control period, L-NAME (Sigma Chemical Co, London, UK) was infused intravenously for 5 days at a continuous rate of 0.1 μg/kg per minute. Recovery measurements were obtained for 3 days after L-NAME infusion was stopped. In preliminary experiments, it was found that this dose of L-NAME does not modify arterial pressure.

Group 2 (n=6): Effects of sustained increase of sodium intake during L-NAME infusion. A similar protocol to that of group 1 was performed, the difference being that after the first day of L-NAME infusion, sodium intake was increased from 80 to 300 mmol/d during 4 consecutive days by the intravenous infusion of isotonic saline at a rate of 1920 mL/d. After L-NAME infusion was stopped, sodium intake decreased to 80 mmol/d, and a recovery period of 3 days was then allowed.

Group 3 (n=6): Effects of sustained increase of sodium intake. After a 3-day control period, sodium intake was increased from 80 to 300 mmol/d during 4 consecutive days by the intravenous infusion of isotonic saline at a rate of 1920 mL/d. Sodium intake was then decreased to 80 mmol/d during the following 3 days.

Group 4 (n=4): Effects of a sustained increase of sodium intake during simultaneous infusion of L-NAME and L-Arg. The experimental protocol was similar to that of group 2, with the exception that L-Arg (Sigma) (5 μg/kg per minute) was simultaneously infused with L-NAME (0.1 μg/kg per minute). L-Arg infusion was started 2 hours before L-NAME administration. L-Arg was infused to determine whether the renal response to the L-NAME infusion was secondary to the inhibition of EDNO synthesis. The L-Arg dose was larger than the L-NAME dose because it has been demonstrated that L-Arg excess reverses the systemic effects of L-NAME by competitive binding to the nitric oxide synthetase. In preliminary experiments (n=2), it was demonstrated that the administration of L-Arg alone during 5 consecutive days (5 μg/kg per minute) did not induce significant changes in arterial pressure or renal hemodynamic and excretory function.

The L-NAME and L-Arg solutions were prepared daily. Twenty-four-hour urine samples, infusion volume, and water intake were measured between 9 and 10 AM each day for the assessment of daily electrolyte and water balances. Blood samples for measurements of glomerular filtration rate (GFR) (24-hour endogenous creatinine clearance) and plasma sodium, potassium, and osmolality were drawn daily 20 to 22 hours after the last feeding. Sodium balance was determined by the difference between the total sodium intake and urinary sodium excretion (UNaV). In preliminary experiments, it was demonstrated that fecal sodium excretion is less than 1 mEq/d.

Analytical Methods

Sodium and potassium concentrations in urine and plasma were measured by flame photometry (model...
Results

Group 1 (n=4): Effects of Sustained L-NAME Infusion

Fig 1 illustrates changes in MAP, GFR, UNaV, and urine flow rate (UV). It can be observed that L-NAME infusion during 5 consecutive days failed to induce significant changes in MAP. GFR decreased (P<.05) only on days 1 (55.5±9.7 mL/min) and 5 (50.9±6.4 mL/min) of L-NAME infusion. However, GFR was found within control values (65.6±6.2 mL/min) on all other days of the experiment. The L-NAME infusion induced a significant decrease in UNaV (P<.05), with a cumulative sodium balance of 19.6±6.0, 33.6±9.5, 65.4±14.3, and 58.0±10.5 mmol of sodium on days 1, 2, 3, and 4, respectively, of nitric oxide synthesis inhibition.

During the recovery period, sodium excretion increased to levels that were slightly but not significantly higher than those during the control period (Fig 1). Changes in UV exhibited directional trends similar to those in UNaV, but the decrement was maintained (P<.05) during the 5 days of L-NAME infusion. Both urine and plasma osmolality and potassium excretion did not change throughout the experiment.

Group 2 (n=6): Effects of Sustained Increase in Sodium Intake During L-NAME Infusion

The effects of the increase in sodium intake during L-NAME infusion are shown in Fig 2. It can be observed that MAP did not change during the first day of EDNO synthesis inhibition and increased from 95.7±3.3 to 106.2±3.9 mm Hg (P<.05) when sodium intake was enhanced. This increment in MAP remained significant (P<.05) during the following 3 days that sodium intake was increased and then returned toward control levels during the 3-day recovery period. GFR decreased from 57.8±5.8 to 41.7±4.0 mL/min (P<.05) during the first day of L-NAME infusion and returned to control levels thereafter (Fig 2). UNaV decreased (81.2±5.8 to 60.5±2.8 mmol/d) on day 1 of L-NAME infusion and returned to control levels thereafter (Fig 2). UNaV decreased (81.2±5.8 to 60.5±2.8 mmol/d) on day 1 of L-NAME infusion and returned to control levels thereafter (Fig 2). UNaV decreased (81.2±5.8 to 60.5±2.8 mmol/d) on day 1 of L-NAME infusion and returned to control levels thereafter (Fig 2). UNaV decreased (81.2±5.8 to 60.5±2.8 mmol/d) on day 1 of L-NAME infusion and returned to control levels thereafter (Fig 2).
sodium intake was enhanced. There were no statistical differences between the cumulative sodium balances during the first 3 days that sodium intake was elevated. However, cumulative sodium balance was significantly higher during the fourth day than during the first 3 days that sodium intake was enhanced in this group. Changes in UV were similar to those found in UNaV (Fig 2). There were no significant changes in potassium excretion and plasma osmolality throughout the experiment. Urine osmolality decreased significantly when sodium intake changed to 300 mmol/d and increased to control levels during the recovery period.

**Group 3 (n=6): Effects of Sustained Increase in Sodium Intake**

Fig 3 illustrates changes in MAP, GFR, UNaV, and UV when sodium intake was first increased from 80 to 300 mmol/d and then decreased to 80 mmol/d. MAP and GFR were not significantly altered throughout the experiment. GFR averaged 75.2±7.5 mL/min at sodium intake of 80 mmol/d, 79.7±2.5 mL/min at 300 mmol/d, and 69.8±1.9 mL/min when sodium intake decreased to 80 mmol/d. As expected, UNaV and UV increased significantly when sodium intake changed from 80 to 300 mmol/d and decreased gradually to control levels when

<table>
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<tr>
<th>Cumulative Sodium Balance</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>44±19</td>
<td>57±18</td>
<td>33±18</td>
<td>36±13</td>
</tr>
<tr>
<td>L-NAME*</td>
<td>105±14†</td>
<td>116±15†</td>
<td>125±22†</td>
<td>158±21†</td>
</tr>
<tr>
<td>L-NAME+L-Arg</td>
<td>38±11</td>
<td>57±5</td>
<td>44±15</td>
<td>58±19</td>
</tr>
</tbody>
</table>

L-NAME, Nω-nitro-L-arginine-methyl ester; L-Arg, L-arginine. Cumulative sodium balance (millimoles per day) is shown during the 4 consecutive days that sodium intake was increased from 80 to 300 mmol/d in conscious dogs treated with L-NAME, L-NAME+L-Arg, or vehicle (Control). Administration of L-NAME and L-Arg started 1 day before sodium intake increased to 300 mmol/d. Values are mean±SEM.

*Includes sodium retained the first day of nitric oxide synthesis inhibition.
†P<.05 vs groups treated with L-NAME+L-Arg or vehicle.
FIG 3. Line graphs show changes in mean arterial pressure (MAP), glomerular filtration rate (GFR), urinary sodium excretion (UNaV), and urine flow rate (UV) in response to an increase in total sodium intake from 80 to 300 mEq/d during 4 consecutive days and during recovery period (days 5, 6, and 7). *P<.01 vs day 0.

sodium intake returned to 80 mmol/d. It should be noted (Table) that cumulative sodium balance was significantly higher (P<.05) when sodium intake increased during the simultaneous administration of L-NAME than when sodium intake increased in the absence of nitric oxide synthesis inhibition.

Group 4 (n=4): Effects of Sustained Increase in Sodium Intake During Simultaneous Infusion of L-NAME and L-Arg

No significant changes in MAP and GFR were observed when sodium intake was increased from 80 to 300 mmol/d during the simultaneous administration of L-NAME and L-Arg. MAP averaged 94.6±2.7 mm Hg during the control period, 97.1±4.5 mm Hg during the first 24 hours that L-NAME and L-Arg were simultaneously infused, 94.3±2.5 mm Hg when sodium intake increased to 300 mmol/d, and 94.7±2.9 mm Hg during the 3-day recovery period. UNaV and UV did not change significantly during the first day of simultaneous infusion of L-NAME and L-Arg and then increased (P<.05) when sodium intake was enhanced from 80 to 300 mmol/d. Both parameters returned gradually to control levels during the 3-day recovery period. Cumulative sodium balance increased (P<.05) when sodium intake was enhanced up to 300 mmol/d during the simultaneous administration of L-NAME and L-Arg, but the increment was significantly lower than that found in group 2 (Table).

Discussion

The present study provides new evidence for a role of EDNO in the regulation of sodium excretion and blood pressure during a prolonged increase in sodium intake. It has been observed that the elevation of sodium intake during 4 consecutive days induces a significant increase in arterial pressure when nitric oxide synthesis is inhibited. This elevation of arterial pressure seems to be secondary to a significant sodium retention, because the increment of cumulative sodium balance was larger than that observed in the control groups. The L-NAME dose used in this study did not induce changes in arterial pressure when sodium intake was not altered. These findings may have important physiological and pathological implications, because it has been demonstrated that a mild impairment of EDNO synthesis produces a sustained decrease in sodium excretion, which renders blood pressure susceptible to increase during high sodium intake.

The results obtained with the intravenous infusion of L-NAME alone are similar to those found in a previous study and suggest that renal function is much more sensitive than arterial pressure to the effects of prolonged inhibition of EDNO synthesis. The administration of a low dose of L-NAME, while having no systemic pressor effects, caused a significant reduction in natriuresis and diuresis and a transitory decrease in GFR. The mechanism responsible for the transitory escape in the sodium-retaining effect of L-NAME infusion during the fourth consecutive day of EDNO synthesis inhibition is not known. Our results could be interpreted as indicating that the low dose of L-NAME used in our study (0.1 μg/kg per minute) produces only a partial inhibition of EDNO synthesis, because other groups of investigators have found that long-term administration of larger doses of L-NAME induces an increase in...
arterial pressure. However, the purpose of this study was to determine whether the renal ability to eliminate a prolonged increase in sodium intake is altered during the inhibition of EDNO synthesis with an L-NAME dose that does not increase arterial pressure but induces a sustained decrease in natriuresis and diuresis. Our results indicate that the resultant expansion of extracellular fluid volume elevates arterial pressure to overcome this excess volume. In anesthetized dogs, Perrella et al. demonstrated that total peripheral resistance increased after EDNO synthesis inhibition even when a subpressor dose of a nitric oxide synthesis inhibitor is infused. The possible increase in total peripheral resistance induced by the administration of L-NAME in our study was not high enough to increase arterial pressure when sodium intake was not modified. However, this change in total peripheral resistance could contribute to the rise in arterial pressure that occurs simultaneously with a rapid increase in sodium retention.

Changes in cumulative sodium balance and arterial pressure were similar during the first 3 days that sodium increased in the L-NAME-treated dogs. As was previously shown in anesthetized dogs, our results demonstrated that the infusion of L-NAME during several consecutive days shifts the normal renal pressure-natriuresis relation toward higher perfusion pressures necessary to achieve similar rates of UNaV. The significant increase in cumulative sodium balance (33.8 mmol sodium) the last day that sodium intake was enhanced suggests that, at least for a few days, the L-NAME-treated animals failed to undergo “escape” from the sodium-retaining effects of nitric oxide blockade. It is possible that arterial pressure would increase more if the elevation in sodium intake and administration of L-NAME were maintained for a longer time.

The renal effects induced by the L-NAME infusion during increases in sodium intake can be attributed to the inhibition of EDNO synthesis, because the increment in arterial pressure and the larger elevation in cumulative sodium balance were prevented by the simultaneous administration of the nitric oxide precursor L-Arg. This hypothesis is supported by in vitro studies showing that the mechanism of action of L-NAME is by competitive inhibition, which is specifically reversed by L-Arg. Other in vivo studies performed by our group also have found that the renal effects of L-NAME infusion are completely reversed by L-Arg infusion. It was also demonstrated that the administration of L-Arg alone during 3 consecutive days at the same dose used in the present study (5 μg/kg per minute) did not induce significant changes in arterial pressure and renal function. Similar results were obtained in preliminary experiments when L-Arg was infused alone during 5 consecutive days at a dose of 5 μg/kg per minute.

Our study suggests that a mild deficiency in EDNO synthesis induces a volume-dependent situation comparable to that observed in salt-sensitive hypertensive patients and other salt-sensitive hypertensive models. In support of this hypothesis, it has recently been reported that long-term supplementation with the EDNO precursor L-Arg prevents the development of hypertension in genetically salt-sensitive rats fed a high-salt diet. On the other hand, it is hypothesized that the sodium sensitivity of blood pressure in the elderly population could be secondary, at least partly, to a decreased release of EDNO. This hypothesis is supported by the fact that aging is associated with a reduced production, release, or both of relaxing factors and that sodium sensitivity of arterial pressure increases significantly with increasing age. This age-related arterial pressure elevation is not observed in cultures with low dietary salt consumption.

Our results suggest that regulation of renal excretory function during prolonged increases in sodium intake appears to be EDNO dependent. Mild inhibition of EDNO synthesis seems to sufficiently alter renal function in such a way that the renal ability to elevate UNaV in response to a sodium load is reduced. Under these circumstances, the resultant expansion of extracellular fluid volume elevates arterial pressure to overcome this excretory deficit and thereby to return body fluid volumes to a normal state. Thus, the EDNO synthesis is vitally important in the control of arterial pressure by the regulation of UNaV during prolonged increments in sodium intake.

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