L-Arginine Does Not Prevent the Renal Effects of Endothelin in Humans

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ABSTRACT

The infusion of endothelin to obtain plasma levels as present in sodium-retaining conditions such as heart failure and hepatorenal syndrome has been shown to cause sodium retention and renal vasoconstriction. Whether these renal effects of endothelin could be modulated by the stimulation of nitric oxide production by the infusion of L-arginine was examined. Therefore, the renal and endocrine effects of the systemic administration of endothelin (2.5 mg/kg per minute for 90 min), L-arginine (5 mg/kg per minute for 90 min), or the combination of endothelin and L-arginine were studied in healthy subjects under clearance conditions. During endothelin infusion, plasma endothelin levels rose from 3.0 ± 0.2 to 14.1 ± 2.4 pmol/L (P < 0.01). Mean arterial pressure increased by 7 mm Hg (P < 0.01). The effects on renal function were disproportionately large: renal vascular resistance increased from 77.5 ± 3.2 to 124.1 ± 6.7 mm Hg/min per liter (P < 0.01), and sodium excretion fell from 178 ± 30 to 83 ± 11 μmol/min (P < 0.01). Endothelin had no effect on urinary nitrite excretion. L-Arginine caused a fall in blood pressure of 5 mm Hg (P < 0.01) and decreased renal vascular resistance by 12% (P < 0.05). Sodium excretion increased twofold. This was associated with an increase in urinary nitrite excretion from 112 ± 36 to 465 ± 190 nmol/min (P < 0.01), suggesting stimulation of renal nitric oxide production. During the combination of endothelin and L-arginine, urinary nitrite excretion increased similarly. L-Arginine prevented the effects of endothelin on blood pressure, and in fact, blood pressure decreased by 4 mm Hg during the combination of endothelin and L-arginine (P < 0.01). However, L-arginine could not prevent the renal vasoconstrictive and sodium-retaining effects of endothelin. These findings suggest that the role of nitric oxide as a physiologic antagonist of renal vasoconstriction is limited.

Key Words: Human, endothelin, L-arginine, nitric oxide, renal hemodynamics, sodium handling

We recently reported sodium retention and profound renal vasoconstriction during pathophysiologic increments in plasma endothelin after the exogenous infusion of endothelin-1 (1). Compared with time control data, endothelin infusion was associated with a major increase in renal vascular resistance and a decrease in sodium excretion. These effects disappeared within the first hour after the cessation of endothelin infusion (1). These findings suggested an important role of endothelin in human renal pathophysiology. It is unknown which counterregulatory mechanisms modulate these effects of endothelin. One such mechanism may be stimulation of the endothelium-dependent relaxing factor nitric oxide (2). Interaction between endothelin and nitric oxide occurs in the endothelium, where nitric oxide can inhibit endothelin production (3) and endothelin can stimulate nitric oxide production (4). Functional interactions at the level of the vascular smooth muscle have been demonstrated as well. In rings of conduit and resistance arteries (5-8), the stimulation of nitric oxide release by acetylcholine or the administration of exogenous nitric oxide completely abolished endothelin-induced contractions. However, in the human forearm circulation, the effects of endothelin could not be modified by nitric oxide (9). This suggests that susceptibility to the antagonist potency of nitric oxide may depend on the vascular bed studied or on the experimental method used. Although the importance of both nitric oxide and endothelin for renal function and pathophysiology is supported by many experiments, only little is known about the interactions of these endothelium-derived factors in the kidney. In this study, we therefore tested the hypothesis of whether increased availability of the stereospecific nitric oxide precursor L-arginine could prevent or attenuate the renal vasoconstrictive and sodium-retaining effects of pathophysiologic dosages of endothelin.

METHODS

Studies were carried out in six healthy male volunteers. Age ranged from 21 to 26 yr. The protocol was approved by the University Hospital Ethical Committee for study in humans.

All subjects underwent three clearance studies (see below) during which endothelin, L-arginine, or the combination of endothelin and L-arginine was infused. The studies were performed with intervals of at least 5 days. The order of the
studies was randomized. Our previous study (1) showed that the renal effects of endothelin were very large compared with a time-control study and that these effects had returned to baseline after a 1-h recovery. To reduce the experimental burden for the participating volunteers in this study, we did not include time-control experiments and limited the recovery observation period to 1 h.

The subjects received a diet containing 200 mmol of sodium and 100 mmol of potassium. Adherence to the diet was controlled by 24-h urine collections. Lithium carbonate (400 mg) was taken at 10 p.m. on the eve of the clearance studies. The studies were performed after an overnight fast in the supine position. Maximal water diuresis was induced by an oral water load of 25 mL/kg body wt and maintained by the subjects drinking amounts of water matching urinary output. At 9 a.m., a priming dose of a solution containing 2.5% inulin, to measure glomerular filtration rate (GFR), and 2.5% p-aminobenzoic acid, to measure estimated renal plasma flow (ERPF), was administered, followed by continuous infusion of this solution throughout the remainder of the study. After at least 1 h of equilibration, and only when urine osmolality was 70 mOsmol/kg or less, two 30-min urine baseline collections were obtained by spontaneous voiding. Hereafter, infusions of endothelin, l-arginine, or the combination of endothelin and l-arginine were started (see below) via a separate antecubital vein for 90 min. The infusion period was followed by a recovery period of 60 min. Urine and blood sampling was continued at 30-min intervals throughout the whole study. Blood specimens were drawn at the midpoint of each collection period from the contralateral forearm. Samples for the determination of plasma endothelin were obtained before infusion, at 45 and 75 min (during the infusion of endothelin, l-arginine, or the combination of endothelin and l-arginine), and at 135 min (during recovery). Plasma renin activity (PRA) and atrial natriuretic peptide (ANP) were measured in blood samples drawn before, at 75 min (during infusion), and at 135 min (during recovery).

Endothelin-1 (Peptide Institute Inc., Scientific Marketing Associates, Herst, United Kingdom) was dissolved in Haemaccel (Behring Pharma, Hoechst Holland NV) and administered for 15 min in a dose of 0.5 ng/kg per minute. This low starting dose was applied to exclude the possibility that adverse effects would occur. From then on, endothelin was infused for another 75 min in a dosage of 2.5 ng/kg per minute. l-Arginine-HCl was given as a 10% solution at a rate of 5 mg/kg per minute for 90 min. During the combined infusion of endothelin and l-arginine, the same dosages were given.

Blood pressure and heart rate were recorded at 4-min intervals during the clearance studies with an automatic oscillometer device (Omega 2000; Invivo Research Laboratory Inc., Tulsa, OK). All blood and urine samples were analyzed for sodium (flame photometry), chloride, and uric acid (Hitachi 717 auto analyzer), lithium (Perkin-Elmer 3030 Atomic Absorption Spectrophotometer; Perkin-Elmer, Norwalk, CT), and inulin and p-aminobenzoic acid (by photometry [10,11]). Bicarbonate, ammonia, and titrable acid concentration were assessed by potentiometric titration (Radiometer PHM 60 pH-meter). Urine samples for the determination of nitrite were stored at −70°C until the assay, and before determination, it was extracted with Sep-Pak octadecyl solid phase extraction cartridges (Waters, Milford, MA). After the cartridges were conditioned with methanol, deionized water, and 4% acetic acid, duplicate extractions were performed of 1.0 mL of plasma acidified with 3 mL of 4% acetic acid. After washing with 3 mL of deionized water and 3 mL of 25% ethanol, the cartridges were eluted with 2 mL of 86% ethanol:glacial acetic acid (96:4 vol/vol). The eluates were dried under nitrogen at room temperature, and the residues were dissolved in 200 µL of assay buffer and analyzed by radioimmunoassay (Nichols Institute, Wychen, The Netherlands). The recovery of endothelin-1 throughout the extraction was 85%. Reported concentrations (in picomoles per liter) are corrected for procedural losses. Cross-reactivities with endothelin-2, endothelin-3, and proendothelin-1 are 52, 96, and 7%, respectively. The detection limit of the assay is 0.4 pmol/L.

Calculations and Statistics

Mean arterial pressure (MAP) was calculated as the sum of one-third of the systolic pressure and two-thirds of the diastolic pressure. RBF was calculated by dividing ERPF by (1 − packed cell volume), and renal vascular resistance was calculated by dividing MAP by RBF. Net acid excretion was calculated by adding the amount of H+ excreted as titratable acid and NH4+ and subtracting urinary HCO3− loss.

Values are presented as means ± SE. PRA was analyzed after logarithmic transformation. Statistical analysis was performed by the two-way analysis of variance of a randomized block design with the infusion of endothelin, l-arginine, and the combination of endothelin and l-arginine as independent variables. If the variance ratios reached statistical significance, the differences between the means were analyzed with the least significant difference test for P < 0.05 and P < 0.01.

RESULTS

Twenty-four-hour urine sodium excretions were 187 ± 40, 198 ± 23, and 206 ± 17 mmol on the days before endothelin infusion, l-arginine infusion, and the combined infusion of endothelin and l-arginine, respectively.

Hemodynamic Responses

Endothelin Infusion. Endothelin infusion was well tolerated in the dosage used, and no side effects were observed. The cumulative dosage over 90 min was 16.5 ± 0.5 µg, varying between 15.4 and 19.3 µg. The MAP increased during sustained endothelin infusion from 95.4 ± 2.0 to 102.4 ± 2.3 mm Hg (P < 0.01) (Figure 1). During recovery, MAP decreased to 98.4 ± 2.3 mm Hg, a value still significantly elevated compared with baseline (P < 0.05). The heart rate declined from a baseline value of 54.5 ± 1.1 to 50.5 ± 1.8 beats/min (P < 0.05; Figure 1). The ERPF was reduced by ∼32% at the end of the endothelin infusion (Table 1) and remained decreased during recovery. The GFR was reduced by ∼10% during endothelin infusion. As a result, the filtration fraction increased. Calculated renal vascular resistance increased from 78 ± 3 to 124 ± 7 mm Hg/min per liter (P < 0.01; Figure 1).

l-Arginine Infusion. l-Arginine infusion was well...
L-Arginine and Endothelin

Figure 1. Effects of the infusion of endothelin (open circles), L-arginine (closed circles), or the combination of endothelin + L-arginine (triangles) on heart rate, MAP, and renal vascular resistance (RVR). Compared with baseline values, significant changes (P < 0.05) were as follows. Endothelin decreased heart rate at 90 min and increased MAP and RVR at all times from 30 to 150 min. L-Arginine increased heart rate at 60 min, decreased MAP at 30, 60, and 90 min, and decreased RVR at 60 and 90 min. The combined infusion had no significant effect on heart rate and decreased MAP at 30, 60, and 90 min, but increased RVR at 60, 90, 120, and 150 min.

This represented an acid load of 177.8 ± 5.9 mmol, varying between 166.3 and 208.3 mmol. MAP decreased during L-arginine infusion from 96.8 ± 1.4 to a minimum of 91.9 ± 1.0 mm Hg (P < 0.01; Figure 1). Heart rate slightly but significantly increased from 54.8 ± 1.4 to a maximum of 57.8 ± 1.0 beats/min (P < 0.05). ERPF increased by ~12% during L-arginine infusion (Table 1). GFR did not change in response to L-arginine infusion, and as a result, filtration fraction decreased. Calculated renal vascular resistance decreased during L-arginine infusion from 80 ± 3 to 71 ± 4 mmHg/min per liter (P < 0.05; Figure 1).

Combined Endothelin and L-Arginine Infusion. L-Arginine completely prevented the increase in blood pressure observed during endothelin infusion. In fact, MAP decreased significantly from 97.8 ± 1.5 mm Hg to a minimum of 94.0 ± 2.1 mm Hg (P < 0.01; Figure 1), which was not different from the change in MAP during L-arginine infusion alone. Heart rate during the combined infusion did not change significantly. ERPF was reduced by ~27%. This response was not different from the response during endothelin infusion alone. The GFR decreased and filtration fraction increased (Table 1), again not different from the response observed during endothelin infusion alone. Renal vascular resistance increased during the combined infusion from 82 ± 3 to 114 ± 5 mm Hg/min per liter (P < 0.01). This increase was less than that observed during endothelin infusion alone (P < 0.05).

Effects on Electrolyte Excretion

Endothelin Infusion. Sodium excretion and chloride excretion decreased progressively during endothelin infusion. After the cessation of the endothelin infusion, sodium and chloride excretion returned to baseline levels within 60 min. Net acid excretion did not change (Figure 2). Fractional sodium excretion decreased as well, and this was accompanied by a decrease in the fractional excretions of lithium and uric acid and in maximal urine flow. In addition, the minimal urinary sodium concentration decreased (Table 2).

L-Arginine Infusion. Sodium excretion did not change during the first 60 min of L-arginine infusion, but increased thereafter until the end of the study, with its maximum value in the last recovery period (Figure 2). Chloride excretion on the other hand increased immediately after the start of L-arginine infusion, reaching its peak in the first recovery period. Net acid excretion also showed a steep and immediate increase. During the last recovery period, net acid excretion still was significantly elevated compared with baseline (P < 0.01). Maximal urine flow and fractional excretions of lithium and uric acid as well as urinary sodium concentration increased (Table 2).

Combined Endothelin and L-Arginine Infusion. Sodium excretion decreased during the first 60 min of the combined infusion. This decrease in sodium excretion was identical to that observed during endothelin...
whereas the fractional excretion of uric acid in-
and L-arginine excretion started to rise, reaching a maximal value in response during L-arginine and both differed from the values are 
urine alone, the decrease in absolute and fractional sodium decrease in minimal urinary sodium concentration bin alone (Figure 2) and was again accompanied by a decrease in minimal urinary sodium concentration (Table 2). However, in contrast to endothelin infusion alone, the decrease in absolute and fractional sodium excretion was not accompanied by a change in the fractional excretion of lithium or maximal urine flow, whereas the fractional excretion of uric acid increased. In the last infusion period, however, sodium excretion started to rise, reaching a maximal value in the recovery period that was not significantly different from the maximal sodium excretion during L-arginine alone. The excretion of chloride increased immediately after the start of the infusion. However, chloride excretion was less than during L-arginine infusion alone (P < 0.01). Net acid excretion showed a pattern similar to that seen during the infusion of L-arginine alone.

Effects on Hormones and Nitrite Excretion

Endothelin Infusion. The infusion of endothelin resulted in a four- to fivefold increase of plasma endothelin, reaching maximal values of 14.1 ± 2.4 pmol/L (P < 0.01 compared with baseline; Figure 3). During the infusion of endothelin, the urinary excretion of nitrite was unchanged (Figure 4). PRA decreased during endothelin infusion and remained decreased during recovery (Table 3). ANP levels did not change.

L-Arginine Infusion. Plasma endothelin levels did not change during L-arginine infusion (Figure 3). The urinary excretion of nitrite increased more than fourfold (P < 0.01; Figure 4). PRA decreased during L-arginine infusion and remained decreased during recovery. ANP did not change during the infusion of L-arginine (Table 3).

Combined Endothelin and L-Arginine Infusion. Plasma endothelin levels rose similarly as during endothelin infusion alone, reaching maximal values of 12.5 ± 2.6 pmol/L (P < 0.01 compared with baseline; Figure 3). This increase in plasma endothelin levels was not different from the increase observed during endothelin infusion alone. The urinary excretion of nitrite increased fourfold (P < 0.01; Figure 4), not different from the increase found during the infusion of L-arginine alone. PRA decreased during the combined infusion and remained decreased during recovery (Table 3). The decrease in PRA was not different from that found during endothelin or L-arginine infusion alone. ANP again did not change.

DISCUSSION

Effects on Hemodynamics

Endothelin infusion at a rate of 2.5 ng/kg per minute for 90 minutes resulted in plasma levels that can also be observed during pathologic conditions such as heart failure (14), renal failure (15, 16), and hepatorenal syndrome (17). During this infusion estimated renal plasma flow fell by ~32%. This decrease in RPF was proportionally greater than the decrease in GFR, resulting in a substantial increase in filtration fraction. The mean blood pressure increased by ~7 mm Hg, and as a result, calculated renal vascular resistance increased by ~60%. In a previous study in healthy volunteers, we infused endothelin in increasing dosages of 0.5, 1.0, and 2.5 ng/kg per minute, 40 min for each dosage (1). Compared with this study, the final infusion rate of the previous study was similar, but the hemodynamic response was milder: blood pressure did not increase, and the renal vascular resistance increased by only ~37% and recovered completely within the first hour after the discontinuation of endothelin infusion. Possibly, this observation of a somewhat stronger hemodynamic effect has to do with individual variation in sensitivity. More likely,
level of ~14 pmol/L, whereas the previously applied regimen resulted in a threefold increase to an average plasma endothelin level of ~9 pmol/L (1).

The main purpose of this study was to examine whether L-arginine, known to stimulate nitric oxide production (18,19), can prevent the renal effects of endothelin. It has been demonstrated that the renal arteries (20) and microvessels (21) produce nitric oxide and that this nitric oxide production is an important determinant of both basal renovascular tone (20,21) and the response to vasoconstrictors such as angiotensin II and noradrenaline (22). Renal nitric oxide production may also act as a physiologic antagonist of endothelin. The inhibition of nitric oxide synthase could potentiate the effects of endothelin on systemic and renal vascular resistance in dogs (23), on rabbit afferent arterioles (7), and on rat renal artery (24). Converting enzyme inhibition, but not angiotensin II blockade, has been shown to block most of the renal actions of endothelin, presumably by the inhibition of kinin degradation and the stimulation of nitric oxide production (25).

The infusion of L-arginine increased ERPF. Because GFR was unaltered, filtration fraction decreased. As a result of the increase in ERPF and the decrease in MAP of ~5 mm Hg, renal vascular resistance decreased by ~12%. These data are in agreement with previous reports on the systemic and renal effects of L-arginine in humans (26,27). The systemic and renal vasodilatory effects of L-arginine are probably caused through the activation of the nitric oxide pathway (28–32), although the specificity of this mode of action has been doubted in one study (33). To assess the effects of L-arginine on local nitric oxide production, we measured urinary nitrite excretion. Urinary nitrite excretion is likely to reflect local nitric oxide production (25).

Figure 2. Effects of the infusion of endothelin (open circles), L-arginine (closed circles), and the combination of endothelin + L-arginine (triangles) on urinary electrolyte excretion. Compared with baseline values, significant changes (P < 0.05) were as follows. Endothelin decreased sodium and chloride excretion at 60, 90, and 120 min and did not change acid excretion. L-Arginine increased the excretion of sodium (90 to 150 min), chloride (30 to 150 min), and acid (30 to 150 min). During the combined infusion, the excretion of sodium was initially decreased (at 60 min) and later increased (at 120 and 150 min). Both chloride excretion (60 to 150 min) and net acid excretion (30 to 150 min) increased.

However, the difference is related to the different infusion regimen. In this study, the 2.5 ng/kg per minute infusion rate was continued for 90 min, resulting in a fivefold increase in plasma endothelin to an average
TABLE 2. Water and electrolyte excretion during the infusion of endothelin, L-arginine, or the combination of endothelin and L-arginine

<table>
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<th>Parameter</th>
<th>Baseline</th>
<th>Infusion 30 min</th>
<th>Infusion 60 min</th>
<th>Infusion 90 min</th>
<th>Infusion Recovery 120 min</th>
<th>Infusion Recovery 150 min</th>
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<td>Endothelin</td>
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<td>0.87 ± 0.13</td>
<td>0.60 ± 0.09^b</td>
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<td>0.63 ± 0.08^b</td>
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<td>1.03 ± 0.18</td>
<td>1.00 ± 0.19</td>
<td>1.13 ± 0.25</td>
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<td>2.26 ± 0.40^b</td>
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<tr>
<td>Combination</td>
<td>0.99 ± 0.22</td>
<td>0.85 ± 0.20</td>
<td>0.58 ± 0.18^b</td>
<td>0.85 ± 0.24</td>
<td>1.60 ± 0.45^b</td>
<td>2.52 ± 0.85^b</td>
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<td>U_{Na} (mmol/L)</td>
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<tr>
<td>Endothelin</td>
<td>10.9 ± 1.8</td>
<td>10.2 ± 1.5</td>
<td>8.5 ± 1.2</td>
<td>7.8 ± 0.8^c</td>
<td>9.2 ± 1.0</td>
<td>11.8 ± 1.1</td>
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<td>L-Arginine</td>
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<td>9.2 ± 1.5</td>
<td>8.7 ± 1.4</td>
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<td>18.8 ± 2.0^b</td>
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<td>8.0 ± 1.7</td>
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<td>6.8 ± 1.7</td>
<td>13.0 ± 3.0^c</td>
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<td>15.4 ± 1.1</td>
<td>11.8 ± 1.1^b</td>
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<td>13.1 ± 0.5^b</td>
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<td>19.2 ± 0.7</td>
<td>22.0 ± 0.9^b</td>
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<tr>
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<td>7.1 ± 0.5</td>
<td>9.0 ± 0.8^b</td>
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<td>10.8 ± 0.6^b</td>
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</tr>
<tr>
<td>Combination</td>
<td>7.9 ± 0.5</td>
<td>8.7 ± 0.4</td>
<td>8.9 ± 0.9</td>
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<td>26.0 ± 1.5</td>
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<tr>
<td>Combination</td>
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</table>

Values are means ± SE. FE_{Na}, fractional excretion of sodium; U_{Na}, urinary sodium concentration; V_{max}, maximal urine flow; FE_{K}, fractional excretion of potassium; FE_{UA}, fractional excretion of uric acid; FE_{Li}, fractional excretion of lithium. See text for differences between tests.

aP < 0.01 compared with baseline.
bP < 0.05 compared with baseline.

cP < 0.01 compared with baseline.

Figure 3. Plasma endothelin levels during the infusion of endothelin (open circles), L-arginine (closed circles), or the combination of endothelin + L-arginine (triangles). During the endothelin and the combined infusion, similar elevations in plasma endothelin levels were found at 45 and 75 min (P < 0.01 compared with baseline).

Figure 4. Urinary excretion of nitrite during the infusion of endothelin (open circles), L-arginine (closed circles), or the combination of endothelin + L-arginine (triangles). Significant (P < 0.01) and comparable elevations in urinary nitrite were found in the second and third half hour of the infusions of L-arginine or the combination of endothelin + L-arginine.
Importantly, endothelin infusion did not increase urinary nitrite excretion in this study, indicating that nitric oxide, as an endogenous antagonist system in the kidney, was not stimulated by endothelin. In addition, these data indicate that the counterregulatory potency of nitric oxide stimulation on the effects of endothelin in the renal vasculature is only limited. This study therefore not only confirms the relative resistance of endothelin-induced renal vasoconstriction to a vasodilator in humans, it also illustrates the limitations of nitric oxide as a physiologic system antagonizing the renal effects of endothelin.

### Effects on Electrolyte Excretion

The infusion of endothelin caused a ~53% reduction in sodium excretion. The antinatriuresis was associated with a fall in fractional sodium excretion, indicating a tubular effect of endothelin. Although water and electrolyte excretion rates are ultimately determined in the distal nephron, the large fall in maximal urine flow and fractional excretions of lithium and uric acid, markers of sodium delivery out of the proximal nephron (37), indicates that at least some of the decrease in sodium excretion during endothelin infusion was due to the stimulation of reabsorption in the proximal nephron. At the same time, the minimal urinary sodium concentration decreased. This phenomenon, taking place while maximal urine flow fell, indicates increased sodium reabsorption in the diluting segment, that is, distal to the point of isotonicity in the medullary ascending limb of Henle's loop (38). Therefore, endothelin probably increased sodium reabsorption in both the proximal and the distal nephron.

In contrast to these observations, most animal studies demonstrated a natriuretic effect of the low-dose systemic administration of endothelin, despite a fall in GFR and RBF (39,40). This natriuresis may be due to stimulation of ANP (41) or pressure natriuresis secondary to a higher blood pressure during endothelin infusion (39). In addition, endothelin may also be an autocrine inhibitor of sodium and water in the inner medullary collecting duct (42). On the other hand, systemic infusions with high doses of endothelin in dogs decreased sodium excretion because of a reduction in filtered load and/or renin-angiotensin stimulation (43,44). In view of these observations, the net effect of endothelin on renal sodium excretion probably depends on the balance between its natriuretic and antinatriuretic effects. The decrease in sodium excretion was of a similar magnitude as in our previous study (1), despite higher plasma endothelin levels and more profound renal vasoconstriction in this study. A stronger antinatriuresis therefore might have been expected. Perhaps the concomitant increase in blood pressure, observed in this study but not in our previous one, attenuated the antinatriuretic effects of endothelin to some extent. Importantly, endothelin infusion had no effect on ANP. PRA decreased, but this was probably time related rather than a specific endothelin effect, because it remained in the recovery phase and a decrease was also found during L-arginine infusion.

L-Arginine infusion alone caused a twofold increase in sodium excretion. Sodium excretion increased even further after the cessation of the infusion, when urinary nitrite excretion returned to baseline, and therefore was not likely the result of the stimulation of nitric oxide production in the kidney. A major problem of L-arginine administration regarding the analysis of renal electrolyte handling is the obligatory concomitant acid load, approximately 180 mEq in this study. In normal volunteers, the administration of chloride-bound acid leads to enhanced excretion of electrolytes, primarily chloride and net acid, and, after some delay, also sodium and potassium (45). This is confirmed by our data, and it is hard to establish whether L-arginine had additional effects on sodium handling, independent of the effect of the acid load. For the same reason, it is difficult to say whether the L-arginine-associated increments in maximal urine flow and fractional excretions of lithium and uric acid were due to the acid load or were related to the observed renal vasodilatation.

Importantly, L-arginine did not prevent the antinatriuretic effect of concomitantly infused endothelin, although in the final half hour, some escape occurred. The latter was probably related to the increase in acid excretion, being unimpaired and maximal at this stage. Remarkably, the coinfusion of L-arginine did not prevent the fall in minimal urinary sodium concentration, but fully prevented the suppression of maximal urine flow and the lithium and uric acid excretion caused by endothelin infusion. Irrespective of the question of how this was established, this observation stresses that the influence of endothelin on distal rather than proximal tubular reabsorption is decisive for the effect on sodium excretion. How and where in the kidney endothelin accomplishes this effect remain to be determined.

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**TABLE 3. PRA and ANP during the infusion of endothelin, L-arginine, or the combination of endothelin and L-arginine**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Infusion, 75 min</th>
<th>Recovery, 135 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRA (pmol/L per second)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Endothelin</td>
<td>260 ± 55</td>
<td>182 ± 70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>167 ± 73&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>437 ± 142</td>
<td>290 ± 58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>222 ± 43&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Combination</td>
<td>386 ± 156</td>
<td>287 ± 98</td>
<td>251 ± 109&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>ANP (pmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endothelin</td>
<td>15.5 ± 0.9</td>
<td>14.5 ± 1.0</td>
<td>15.8 ± 1.0</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>12.5 ± 1.0</td>
<td>15.3 ± 1.1</td>
<td>14.7 ± 0.7</td>
</tr>
<tr>
<td>Combination</td>
<td>13.8 ± 1.0</td>
<td>18.3 ± 2.4</td>
<td>15.6 ± 1.6</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values are means ± SE. Values for PRA are the geometric mean. The responses in PRA during the three experiments were not different from each other.

<sup>b</sup> P < 0.05 compared with baseline.

<sup>c</sup> P < 0.01 compared with baseline.
In summary, the infusion of endothelin in pathophysiologic dosages caused a slight increase in blood pressure. Yet, this dosage had major effects in the kidney, consisting of vasoconstriction and sodium retention in association with enhanced reabsorption in proximal and distal segments. The coinfusion of L-arginine, in a dosage that was sufficient to stimulate renal nitric oxide production, prevented the effects of endothelin on blood pressure, but did not prevent endothelin’s renal vasoconstrictive and antinatriuretic effects.

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REFERENCES


