Human renal and systemic hemodynamic, natriuretic, and neurohumoral responses to different doses of L-NAME

A. BROERE, A. H. VAN DEN MEIRACKER, F. BOOMSMA, F. H. M. DERKX, A. J. MAN IN'T VELD, AND M. A. D. H. SCHALEKAMP
Department of Internal Medicine I, University Hospital Dijkzigt, 3015 GD Rotterdam, The Netherlands

Broere, A., A. H. van den Meiracker, F. Boomsma, F. H. M. Derkx, A. J. Man in't Veld, and M. A. D. H. Schalekamp. Human renal and systemic hemodynamic, natriuretic, and neurohumoral responses to different doses of L-NAME. *Am. J. Physiol.* 275 (*Renal Physiol.* 44): F870–F877, 1998.—Experimental evidence indicates that the renal circulation is more sensitive to the effects of nitric oxide (NO) synthesis inhibition than other vascular beds. To explore whether in men the NO-mediated vasodilator tone is greater in the renal than in the systemic circulation, the effects of three different intravenous infusions of N^G-nitro-L-arginine methyl ester (L-NAME; 1, 5, and 25 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 30 min) or placebo on mean arterial pressure (MAP), systemic vascular resistance (SVR), renal blood flow (RBF), renal vascular resistance (RVR), glomerular filtration rate (GFR), and fractional sodium and lithium excretion (FE_{Na} and FE_{Li}) were studied in 12 healthy subjects, each receiving randomly two of the four treatments on two different occasions. MAP was measured continuously by means of the Finapres device, and stroke volume was calculated by a model flow method. GFR and RBF were estimated from the clearances of radiolabeled thalamate and hippuran. Systemic and renal hemodynamics were followed for 2 h after start of infusions. During placebo, renal and systemic hemodynamics and FE_{Na} and FE_{Li} remained stable. With the low and intermediate L-NAME doses, maximal increments in SVR and RVR were similar: 20.4 ± 19.6 and 23.5 ± 16.0%, respectively, with the low dose and 31.4 ± 26.7 and 31.2 ± 14.4%, respectively, with the intermediate dose (means ± SD). With the high L-NAME dose, the increment in RVR was greater than the increment in SVR. Despite a decrease in RBF, FE_{Na} and FE_{Li} did not change with the low L-NAME dose, but they decreased by 31.2 ± 11.0 and 20.2 ± 6.3%, respectively, with the intermediate dose and by 70.8 ± 8.1 and 31.5 ± 15.9% with the high L-NAME dose, respectively. It is concluded that in men the renal circulation is not more sensitive to the effects of NO synthesis inhibition than the systemic circulation and that the threshold for NO synthesis inhibition to produce antinatriuresis is higher than the threshold level to cause renal vasoconstriction.

nitric oxide synthesis inhibition; renal vascular resistance; systemic vascular resistance; fractional sodium excretion; fractional lithium excretion

EXPERIMENTAL STUDIES with L-arginine analogs that inhibit nitric oxide (NO) synthesis have provided unequivocal evidence that NO is an important regulator of renal blood flow (RBF), diuresis, and natriuresis. Consequently, it has been suggested that disorders in the NO-forming pathway may be involved in the pathogenesis of certain forms of hypertension (9, 19, 28). Dose-response studies with short-lasting systemic infusions of the L-arginine analog N^G-nitro-L-arginine methyl ester (L-NAME) in rats have shown that a low degree of NO synthesis inhibition can evoke a decrease in diuresis and natriuresis without affecting RBF (20). At a somewhat higher degree of NO synthesis inhibition, RBF decreases as well, whereas a still higher degree of NO synthesis inhibition is required to induce a rise in systemic blood pressure. On the basis of these observations, it has been hypothesized that the kidney is particularly sensitive to the effects of NO synthesis inhibition, suggesting a greater NO vasodilator tone in the renal than in the systemic circulation (20). In this respect, some caution is required, however, because studies were performed in anesthetized animals. As has been shown, anesthesia itself can influence the hemodynamic responses to NO synthesis inhibition in the various regional vascular beds (29).

So far, few studies have investigated the effects of acute systemic NO synthesis inhibition on renal function and systemic hemodynamics in humans (3, 10, 22, 38). These studies do not allow for a conclusion regarding whether in humans, like in animals, the renal circulation is particularly sensitive to the effects of NO synthesis inhibition, since studies comparing the effects of different degrees of NO synthesis inhibition on systemic and renal hemodynamics and renal sodium handling have not been performed. In an attempt to further clarify this issue, we investigated in healthy human volunteers the effects of different degrees of acute systemic NO synthesis inhibition on the above-mentioned parameters as well as on vasoactive hormones.

MATERIALS AND METHODS

Subjects

Twelve nonsmoking apparently healthy men, who used no drugs, age 24 ± 6 yr (means ± SD) participated in the study. Their dietary salt intake was unrestricted. On the evening before the study day each subject took a single dose of 400 mg of lithium carbonate. The study protocol was approved by the Medical Ethics Committee of the University Hospital Dijkzigt, and written informed consent was given by all subjects.

Study Protocol

On the study days, the participants arrived at the cardiovascular research unit at 7:30 AM after an overnight fast. Indwelling catheters were placed in veins of both forearms for

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infusions and blood sampling. All subjects received an initial load of tap water (12 ml/kg body wt) and, to maintain diuresis, water input matched urinary output (UV) throughout the study. The subjects remained supine except when voiding. Renal clearance studies started at 8:00 AM with an intravenous loading dose of [^{125}I]iothalamate and [^{131}I]orthoiodohippuran after which continuous infusion of both tracers was started (41). After a 60-min equilibration period, the subjects passed urine to empty the bladder. This was followed by six 30-min clearance periods. The study drug was administered during the third clearance period. Throughout the study, finger blood pressure was recorded continuously.

Urine for determination of urine flow rate, tracers, lithium, sodium, cGMP, and nitrite + nitrate was collected at the end of each clearance period. Blood samples were drawn at the end of the clearance periods, and they were analyzed for tracers, hematocrit, lithium, sodium, L-citrulline, cGMP, and vasoactive hormones.

Four different conditions were studied. These consisted of a 30-min intravenous infusion of either L-NAME (Clinalpha), at infusion rates of 1 ($n = 6$), 5 ($n = 6$), or 25 $\mu g \cdot kg^{-1} \cdot min^{-1}$ ($n = 6$), or saline ($n = 6$).

Each subject was studied two times on different occasions with an interval of at least 10 days between study days. Subjects were randomly assigned to the various treatments with the restriction that they received different infusions on the two study days.

Methods

Renal function. Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were assessed by calculating clearances of [^{125}I]iothalamate and [^{131}I]orthoiodohippuran, respectively. After a loading dose, a continuous constant infusion technique with timed urine sampling as previously described was used (41). Effective renal blood flow (ERBF) was calculated as ERPF/(1 − hematocrit). Renal vascular resistance (RVR), expressed in resistance units (RU), was the ratio between mean arterial pressure (MAP) and ERBF. Filtration fraction (FF) was calculated as GFR divided by ERPF. Fractional excretions of sodium (FE_{Na}) and lithium (FE_{Li}) were the percentages of filtered sodium and lithium that were excreted in the urine, whereas filtered sodium and lithium were calculated as GFR times their plasma concentrations.

Systemic hemodynamics. Finger blood pressure and heart rate (HR) were recorded continuously with a model 2300 Finapres (Ohmeda, Louisville, CO) and data were stored into a computer at a sampling frequency of 1,000 Hz. The stored data were analyzed by the BMI model flow program (TNO, The Netherlands) to compute beat-to-beat values of MAP, HR, and stroke volume (SV) (37). Cardiac output (CO) was calculated as SV times HR. Systemic vascular resistance (SVR), expressed in resistance units, was calculated as MAP divided by CO. Averages of the hemodynamic parameters of the last 10 min of each clearance period were used for analysis.

Analytical methods. Blood samples for determination of atrial natriuretic peptide (ANP), endothelin-1, and cGMP were collected in chilled tubes containing EDTA and aprotinin. Samples for determination of renin, norepinephrine, L-citrulline, and nitrite + nitrate were collected in chilled heparinized tubes containing glutathione. All samples were immediately centrifuged at 4°C, and plasma was stored at −80°C. Norepinephrine was measured with fluorometric detection after HPLC separation (16). Commercially available kits were used to measure the plasma concentration of ANP (Nichols Institute, Wijchen, The Netherlands), endothelin-1 (Nichols Institute Diagnostics, San Juan Capistrano, CA), and cGMP (Amersham, Little Chalfont, Buckinghamshire, UK). Active plasma renin concentration was measured by an immunoradiometric assay using a commercially available kit (Nichols Institute Diagnostics). L-Citrulline in plasma was measured by HPLC with fluorometric detection after derivatization with *O*-pthaldialdehyde and 3-mercaptopropionic acid as described (34a). Nitrite + nitrate in urine samples were measured by capillary electrophoresis with conductivity detection. The intra- and interassay variation coefficient of this measurement is 8%. Lithium concentrations in serum and urine were measured by flame photometry. Sodium in serum and urine was measured by a routine method at the Department of Clinical Chemistry of our hospital.

Statistics

Values of ERBF, GFR, RVR, CO, and SVR are expressed per 1.73 m^2 of body surface area. Data are given as mean values ± SD in text and in Tables 1–4 and as mean values ± SE in Figs. 1–3. Baseline values are averages of the data of the first two clearance periods. A one-way ANOVA was used to compare the baseline values in the four treatment groups, whereas a repeated measures ANOVA, followed by Student-Newman-Keuls multiple comparison test, was used to compare the treatment-induced changes (all time points) in the four groups. If the Student-Newman-Keuls multiple comparison test revealed a significant difference, then Student's paired *t*-test with Bonferroni correction for multiple comparisons was used to compare the L-NAME-induced changes vs. baseline values within one treatment group. $P < 0.05$ was considered to indicate a significant difference.

RESULTS

Baseline values of systemic and renal hemodynamics between the four groups did not differ. Values are given in Table 1. L-NAME infusions were well tolerated and side effects were not reported.

Systemic Hemodynamics

MAP, HR, CO, and SVR in the placebo group did not change throughout the observation period (Fig. 1). Compared with the time course in the placebo group, MAP did not rise with the low and the intermediate

Table 1. *Baseline values of systemic and renal hemodynamics in the four groups*

		L-NAME ($\mu g \cdot kg^{-1} \cdot min^{-1}$)			
	Placebo	1	5	25	*P*
n	6	6	6	6	
HR, beats/min	59 ± 12	56 ± 10	57 ± 9	59 ± 10	0.95
MAP, mmHg	89 ± 5	93 ± 8	92 ± 9	90 ± 9	0.86
CO, l/min	6.2 ± 1.3	5.9 ± 2.2	6.6 ± 1.1	5.4 ± 0.8	0.44
SVR, RU	15 ± 3	18 ± 6	14 ± 3	17 ± 2	0.39
GFR, ml/min	105 ± 13	115 ± 8	114 ± 11	112 ± 9	0.39
ERBF, ml/min	772 ± 77	944 ± 157	899 ± 153	846 ± 148	0.19
RVR, RU	117 ± 17	101 ± 19	105 ± 19	110 ± 25	0.57
RBF/CO, l/l	0.13 ± 0.04	0.17 ± 0.03	0.14 ± 0.04	0.16 ± 0.04	0.13

Values are means ± SD; *n* = no. of experiments. HR, heart rate; MAP, mean arterial pressure; CO, cardiac output; SVR, systemic vascular resistance; GFR, glomerular filtration rate; ERBF, effective renal blood flow; RVR, renal vascular resistance; RBF/CO, renal blood flow vs. cardiac output; L-NAME, N^G-nitro-L-arginine methyl ester; RU, resistance units.

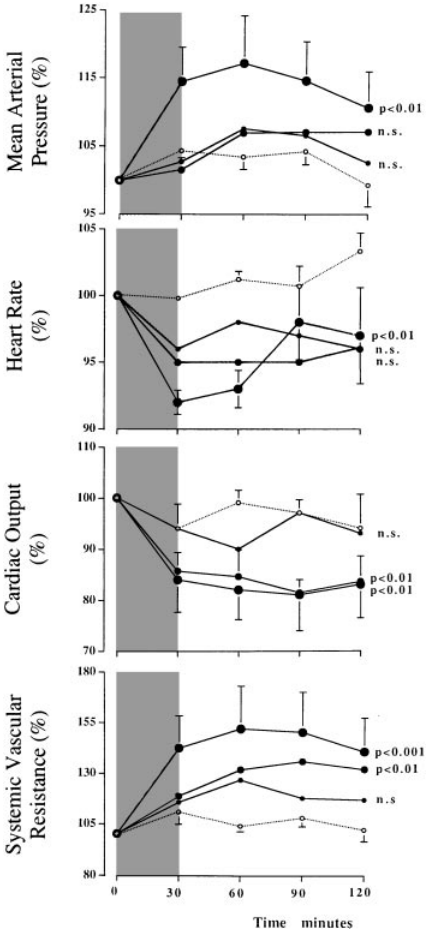

Fig. 1. Time course of changes in mean arterial pressure, heart rate, cardiac output, and systemic vascular resistance in the 4 treatment groups. Percentage changes compared with baseline are given. Placebo (○) or different doses of N^G-nitro-L-arginine methyl ester (L-NAME, ●) were administered by intravenous infusion from 0 to 30 min (shaded area). For L-NAME infusion, small solid circles indicate L-NAME, 1 $\mu g \cdot kg^{-1} \cdot min^{-1}$; intermediate solid circles indicate L-NAME, 5 $\mu g \cdot kg^{-1} \cdot min^{-1}$; and large solid circles indicate L-NAME, 25 $\mu g \cdot kg^{-1} \cdot min^{-1}$; n.s., not significant.

L-NAME doses. With the high L-NAME dose, MAP rose, with a maximal increment occurring 60 min after start of infusion. At that time, MAP had increased by 17.4 ± 17.2% ($P = 0.048$) compared with baseline.

The L-NAME-induced rise in MAP was caused by a rise in SVR. With the low, intermediate, and high dose of L-NAME, the maximal rise in SVR was, respectively, 20.4 ± 19.6% ($P = 0.042$), 31.4 ± 26.7% ($P = 0.002$), and 52.9 ± 50.1% ($P = 0.027$). With the intermediate and high doses of L-NAME, SVR at the end of the observation period was still increased in all subjects (Fig. 1). The already low values of HR tended to decrease further with the different L-NAME infusions, but the decrease was significant only with the high dose of L-NAME (7.8 ± 1.8%; $P < 0.001$).

CO tended to decrease with the low dose of L-NAME. With the intermediate and high L-NAME dose, CO decreased significantly by, respectively, 15.9 ± 13.0% ($P = 0.025$) and 20.6 ± 11.6% ($P = 0.015$). Considering the relatively small decrease in HR, the decrease in CO was predominantly due to a decrease in SV.

Renal Hemodynamics

Values of ERBF, GFR, RVR, and FF in the placebo group did not change throughout the observation period (Fig. 2). In response to the three L-NAME doses, ERBF decreased with maximal decrements observed 60–90 min after start of infusions. Compared with baseline, ERBF decreased maximally by 13.3 ± 8.0% ($P = 0.011$) with the low dose, by 18.1 ± 5.6% ($P = 0.0006$), with the intermediate does, and by 39.5 ± 3.9% ($P = 0.0002$) with the high L-NAME dose. Compared with placebo, RVR increased with the intermediate and high L-NAME dose (Fig. 2). Compared with baseline, maximal increments in RVR were 23.5 ± 16% ($P = 0.046$) with the low dose, 31.2 ± 14.4% ($P = 0.005$) with the intermediate dose, and 80.2 ± 25.2% ($P < 0.001$) with the high L-NAME dose. The ratio of ERBF to CO did not change with the low and intermediate L-NAME dose, but it decreased maximally by 22.5 ± 11.6% ($P = 0.012$) with the high L-NAME dose.

The marked decrements in ERBF were not associated with parallel decrements in GFR. GFR did not change with the low and intermediate L-NAME dose

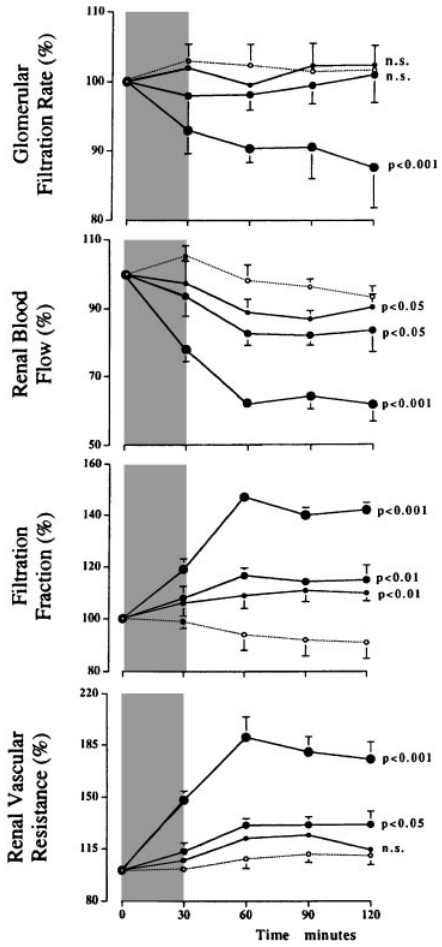

Fig. 2. Time course of changes in glomerular filtration rate, renal blood flow, filtration fraction, and renal vascular resistance in the 4 treatment groups. Percentage changes compared with baseline are given. Placebo (○) or different doses of L-NAME (●, as described in legend to Fig. 1) were administered by intravenous infusion from 0 to 30 min (shaded area).

and decreased maximally by only 11.7 ± 7.2% (P = 0.012) with the high L-NAME dose. FF increased with all three doses. The maximal increment was 15.6 ± 10.6% (P = 0.016) with the low dose, 21.0 ± 14% (P = 0.0018) with the intermediate dose, and 47.0 ± 7.6% (P = 0.024) with the high L-NAME dose.

Renal Water and Sodium Handling

Baseline values of UV, urinary sodium excretion ($U_{Na}V$), FE_{Na}, and FE_{Li} between the four treatment groups did not differ (Table 2). In the placebo group UV, $U_{Na}V$, FE_{Na}, and FE_{Li} remained stable throughout the observation period (Fig. 3). In response to the different doses of L-NAME, UV, $U_{Na}V$, FE_{Na}, and FE_{Li} decreased dose dependently. The relative decrease in FE_{Li} was less pronounced than the decrease in FE_{Na}.

Vasoactive Hormones

Baseline values of concentrations of norepinephrine, active renin, and endothelin-1 in plasma between the four treatment groups did not differ, but baseline values of ANP were significantly higher in the group of subjects receiving the intermediate L-NAME dose compared with the other three treatment groups (Table 3). In the placebo group, the values of the above-mentioned parameters remained stable throughout the study. In response to the low and intermediate doses of L-NAME, the plasma concentrations of norepinephrine and renin did not change, whereas these two parameters decreased in response to the high L-NAME dose (Table 3). Plasma concentrations of endothelin-1 and ANP did not change in response to the three different doses of L-NAME.

cGMP, L-Citrulline, and Nitrite + Nitrate

Baseline values of plasma cGMP and L-citrulline between the four treatment groups were similar (Table 3). Values did not change in response to the different doses of L-NAME.

Baseline values of urinary excretion of cGMP and nitrite + nitrate between the four treatment groups did not differ (Table 4). L-NAME infusion was not associated with a decrease in the urinary excretion of cGMP. The urinary excretion of nitrite + nitrate moderately decreased ($P < 0.05$ vs. change in placebo group) in response to the high L-NAME dose.

Table 2. *Baseline values of urinary output, sodium excretion, fractional sodium excretion, and fractional lithium excretion in the four groups*

	L-NAME ($\mu g \cdot kg^{-1} \cdot min^{-1}$)				
	Placebo	1	5	25	P
n	6	6	6	6	
UV, ml/min	13.7 ± 3.2	15.3 ± 1.7	14.7 ± 1.8	15.0 ± 1.8	0.74
$U_{Na}V$, μmol/min	139 ± 70	253 ± 183	205 ± 108	181 ± 74	0.74
FE_{Na}, %	0.91 ± 0.46	1.32 ± 0.87	1.21 ± 0.64	1.06 ± 0.42	0.91
FE_{Li}, %	25 ± 8	23 ± 4	20 ± 3	20 ± 4	0.09

Values are means ± SD; n = no. of experiments. UV, urinary output; $U_{Na}V$, urinary sodium excretion; FE_x, fractional excretion of ion x.

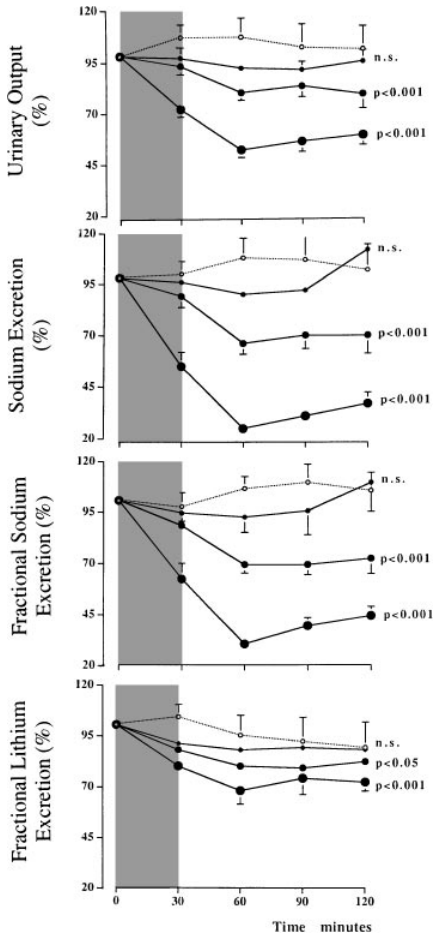

Fig. 3. Time course of changes in urinary output, sodium excretion, fractional sodium, and fractional lithium excretion. Percentage changes compared with baseline are given. Placebo (○) or different doses of L-NAME (●, as described in legend to Fig. 1) were administered by intravenous infusion from 0 to 30 min (shaded area).

DISCUSSION

This is the first study in men comparing the effects of different degrees of NO synthesis inhibition by L-NAME on renal and systemic hemodynamics, sodium handling, and neurohormones in healthy volunteers. The different doses of L-NAME induced clearly distinguishable effects, both in magnitude and duration, on systemic and renal hemodynamics, diuresis, and natriuresis. With the exception of the highest infusion rate of L-NAME, increments in RVR and SVR were of comparable magnitude. These observations therefore do not support the hypothesis based on animal experiments that the renal vascular bed is more sensitive to the effects of NO synthesis inhibition than other vascular beds, but do suggest a comparable basal NO-mediated vasodilator tone in the renal and systemic circulation in healthy men.

So far, most NO synthesis inhibition studies performed in healthy volunteers have used N^G-monomethyl-L-arginine (L-NMMA) as a NO synthase (NOS) inhibitor (3, 6, 10, 15, 31, 38). In most of these studies, a maximal dose of 3 mg/kg has been administered as an extended bolus injection over a 10-min period. With

Table 3. *Baseline values and time courses of plasma concentration of norepinephrine, renin, endothelin-1, and ANP in the 4 groups*

	L-NAME ($\mu g \cdot kg^{-1} \cdot min^{-1}$)				
	Placebo	1	5	25	*P**
n	6	6	6	6	
Norepinephrine, pg/ml					
Baseline	174±103	166±67	158±90	185±64	0.95
30 min	192±139	156±62	154±83	162±59	
60 min	205±145	167±78	165±69	122±33†	
120 min	193±119	186±87	146±61	123±35†	
Renin, μU/ml					
Baseline	27.8±4.7	24.7±13.8	26.9±6.1	27.4±4.0	0.91
30 min	27.5±5.0	24.7±13.9	23.7±5.9	24.4±5.3	
60 min	27.1±6.8	24.1±17.1	23.3±7.2	18.6±3.7†	
120 min	27.4±5.6	22.8±14.2	24.0±6.5	18.7±3.5†	
Endothelin-1, $pg \cdot ml^{-1} \cdot ml^{-1}$					
Baseline	4.1±1.1	3.1±1.4	3.0±0.7	5.2±3.5	0.61
30 min	3.9±1.3	3.0±1.1	2.8±0.9	3.9±0.6	
60 min	3.7±1.0	3.4±1.2	3.3±0.7	4.7±3.6	
120 min	4.3±3.3	3.0±1.2	3.0±1.0	6.2±6.2	
ANP, pg/ml					
Baseline	62±20	109±39	92±49	44±16	0.015
30 min	62±13	103±32	95±51	42±16	
60 min	63±25	108±37	85±45	46±15	
120 min	60±17	95±25	79±43	41±10	

Values are means ± SD; *n* = no. of experiments. ANP, atrial natriuretic peptide. *For differences between baseline values. †$P <$ 0.05 vs. baseline.

this intravenous dose of L-NMMA, the reported average rise in MAP ranged from 5 to 10% and the reported average rise in SVR from 28 to 36%. Similar hemodynamic effects were observed with the intermediate dose of L-NAME (0.005 $mg \cdot kg^{-1} \cdot min^{-1}$ for 30 min, i.e., 0.15 mg/kg in total) that was used in the present study. From these findings, it can be estimated that, with respect to its hemodynamic effects, L-NAME is ~20 times more potent than L-NMMA. This fits well with observations in anesthetized rats, showing that L-NAME is about 10-fold more potent than L-NMMA in increasing blood pressure and causing bradycardia (25).

L-NAME induced a dose-dependent renal vasoconstrictor response, confirming previous experimental and human studies that NO exerts a basal relaxing effect on the renal circulation (3, 7, 10, 20, 22, 26). Especially, the highest dose of L-NAME produced pronounced vasoconstriction in the renal circulation, which exceeded the systemic vasoconstrictor response. If a predominant NO-mediated vasodilator tone existed in the renal circulation of our subjects, then we would a priori expect a more pronounced increase in resistance in the renal than in the systemic circulation with the two lowest doses of L-NAME and not just with the highest dose. Possibly, the divergence in effect of the highest dose of L-NAME on the renal and systemic circulation could be explained by autoregulatory adjustment of the renal circulation to the L-NAME-induced rise in systemic arterial pressure. However, studies in rats have shown that the renal hemodynamic and excretory responses to systemic L-NAME infusion were similar, either when renal perfusion pressure was allowed to increase (from 122 to 157 mmHg) or when it was servocontrolled at baseline level (23).

The marked decrements in effective RBF associated with the various doses of L-NAME were not accompanied by parallel decrements in GFR. A relative preservation of GFR after NO synthesis inhibition has been noticed before (3, 10). Glomerular micropuncture studies have shown a decrease in glomerular capillary ultrafiltration coefficient (K_f) in response to either local or systemic NO synthesis inhibition (8, 40). This decrease in K_f appears to be mediated by a rise in mesangial cell tone (7). The relative preservation of GFR is therefore likely caused by a rise in the hydrostatic pressure of the glomerular capillaries, indicating that systemic NO synthesis inhibition elicits more vasoconstriction in the efferent than in the afferent arteriole of the glomerulus. Although it is tempting to speculate that unopposed activity of angiotensin II underlies this preferential efferent arteriolar vasoconstriction, this is not supported by our preliminary observations in losartan-pretreated subjects and by a recent publication showing that the L-NAME-induced effects on renal hemodynamics in sodium-repleted healthy volunteers were not altered by administration of the angiotensin II receptor blocker losartan (22).

In a dose-response study of L-NAME performed in anesthetized rats, it was observed that the two lowest

Table 4. *Baseline values and time courses of plasma concentration of cGMP and L-citrulline and urinary excretion of cGMP and nitrite + nitrate in 4 treatment groups*

	L-NAME ($\mu g \cdot kg^{-1} \cdot min^{-1}$)				
	Placebo	1	5	25	*P**
n	6	6	6	6	
Plasma cGMP, pmol/l					
Baseline	4.6±0.7	8.8±1.3	6.8±2.2	6.3±2.1	0.09
30 min	5.0±0.4	8.5±1.4	7.0±2.2	6.5±1.8	
60 min	5.2±0.9	8.1±1.2	7.4±2.2	6.5±1.8	
120 min	4.8±0.6	8.5±2.3	6.7±2.4	6.6±2.1	
Plasma L-citrulline, μU/ml					
Baseline	23.2±3.5	24.2±5.9	22.5±2.7	20.3±3.1	0.43
30 min	22.1±1.9	24.6±4.9	21.4±2.5	22.7±6.6	
60 min	22.5±1.8	25.0±4.6	24.6±4.0	23.5±7.9	
120 min	22.8±2.6	25.5±4.6	23.2±3.9	22.0±2.5	
Urinary cGMP, pmol/min					
Baseline	365±80	591±188	488±248	416±158	0.02
30 min	316±91	543±152	447±241	331±130	
60 min	280±81	504±127	414±230	273±69	
120 min	257±103	479±89	373±137	349±140	
Urinary nitrite+nitrate, μmol/min					
Baseline	1.8±0.5	1.1±0.5	1.6±0.4	1.7±0.1	0.06
30 min	1.8±0.4	1.1±0.7	1.6±0.5	1.6±0.2†	
60 min	2.0±0.4	1.0±0.5	1.5±0.3	1.5±0.4	
120 min	2.2±0.5	1.0±0.8	1.4±0.4	1.5±0.3	

Values are means ± SD. *For differences between baseline values. †Time course of changes significantly different from placebo time control study.

intravenous doses of L-NAME (0.1 and 1.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 3 h) caused an antidiuretic and antinatriuretic effect not accompanied by detectable effects on GFR or RBF (20). This could not be confirmed in the present study. All three doses of L-NAME applied caused a decrease in RBF and a rise in FF, but only the intermediate and high L-NAME doses were associated with antidiuresis and antinatriuresis. Apparently, in healthy men, the threshold level of NO synthesis inhibition to affect renal and sodium water handling is not lower but appears to be higher than the threshold level to affect renal hemodynamics.

As evidenced by the pronounced decrease in FE_{Na}, the antinatriuresis observed with the intermediate and high L-NAME doses was a consequence of increased tubular sodium reabsorption. The FE_{Li} was used as a marker of proximal tubular sodium reabsorption (4). Like the FE_{Na}, the FE_{Li} decreased as well, albeit to a lesser degree, suggesting that the L-NAME-induced increase in sodium reabsorption not only occurred in the proximal tubule of the nephron but also at more distal nephron sites. Additional evidence that sodium reabsorption took place beyond the proximal tubule, particularly in the diluting segment of the nephron, is provided by the finding that the percentage decrease in $U_{Na}V$ was far greater than the decrease in UV (Fig. 3).

In vivo studies in rats and dogs have shown that NO-induced natriuresis can occur independently of changes in renal hemodynamics (1, 20), indicating that it is a result of a direct effect of NO on tubular sodium reabsorption. This is supported by a number of in vitro studies showing an inhibitory effect of NO on sodium reabsorption, for example in the proximal tubule (14, 27), the thick ascending limb of Henle's loop (24), and the cortical (21, 32, 34) and inner medullary collecting ducts (40). It cannot be excluded that the antinatriuresis observed in the present study was in part caused by direct effect of L-NAME on tubular sodium reabsorption. However, the observation that the threshold of L-NAME to cause changes in renal hemodynamics is lower than the threshold to cause any decrease in FE_{Na} might be considered in favor of the view that the antinatriuretic effect currently observed was a consequence of the L-NAME-induced changes in renal hemodynamics.

In an attempt to obtain further information about the potential mechanisms that underlie the systemic and renal hemodynamic effects of L-NAME, the time course of the plasma concentrations of several vasoactive hormones was monitored. No changes in the plasma concentrations of norepinephrine, renin, endothelin-1, or ANP were observed with the low and intermediate doses of L-NAME. The high L-NAME dose was associated with moderate decrements in the plasma concentrations of norepinephrine and renin. Most likely, the decrease in plasma norepinephrine was a consequence of the L-NAME-induced rise in blood pressure and indicates that acute systemic NO synthesis inhibition causes a decrease in sympathetic tone. It is well known that, in addition to the activity of the sympathetic nervous system, the concentration of norepinephrine in plasma is determined by its clearance from the intravascular compartment. As a rule, if CO decreases, then so does the clearance of norepinephrine, and, providing that sympathetic tone does not change, the plasma norepinephrine concentration increases. The absence of a rise in plasma norepinephrine observed with the low and intermediate doses of L-NAME, despite the supposed diminishment of its clearance, therefore does not completely exclude that with these two doses sympathetic tone was lowered as well.

In vitro studies have provided evidence for either a stimulatory or an inhibitory effect of NO on renin secretion (18, 35). In intact animals, acute NO synthesis inhibition with L-NAME is associated with a fall in plasma renin activity. This fall appears to be mediated by the L-NAME-induced increase in renal perfusion pressure and decrease in sympathetic tone, since it is reversed to a rise when renal perfusion pressure is kept at a constant level and the stimulatory influence of the sympathetic nervous system on renin release is abolished by administration of propranolol (30). We therefore suggest that the decrease in plasma renin concentration observed with the highest dose of L-NAME has been mediated by the concomitant increase in arterial pressure and decrease in sympathetic tone.

It has been shown that, notwithstanding the decrease in CO, cardiac filling pressures remained unchanged during NO synthesis inhibition, suggesting that withdrawal of the NO vasodilator tone is associated with both arterial and venous vasoconstriction (6). The present finding that the plasma ANP concentration did not increase with the various doses of L-NAME is in accordance with this view.

Plasma endothelin-1 has been proposed to be the natural antagonist of NO. There is experimental evidence that blockade of NO synthesis increases tissue endothelin mRNA expression as well as the plasma endothelin concentration (5, 17, 26). In the present study, no change in plasma endothelin concentration was observed in response to the various L-NAME infusions. This does not completely exclude that a change in the production of endothelin has occurred, since endothelin acts locally and appears to be predominantly secreted at the abluminal site of the circulation (36).

Since NO is short-lived in vivo, its direct measurement is extremely difficult (13). Instead of measuring NO, the plasma concentrations of cGMP and L-citrulline, as well as the urinary excretions of cGMP and nitrite + nitrate, were used as parameters to monitor the biochemical effects of NO synthesis inhibition. Despite the marked hemodynamic effects, the plasma concentrations of cGMP and L-citrulline in response to the L-NAME infusions did not change. Based on these findings, we conclude that these parameters, as determined with the current assays, are not suitable to monitor the inhibition of constitutive NOS. Unexpectedly, considering the pronounced effects on renal hemodynamics, the urinary excretion of cGMP also did not decrease and the nitrite + nitrate excretion only moderately decreased in response to the high L-NAME dose.

Possibly, the large amounts of tap water the subjects had to drink to guarantee a stable urinary flow during the study may have masked any potential effect of L-NAME on the urinary excretion of nitrite + nitrate. The reason for the absence of an effect of L-NAME on urinary cGMP excretion could be that cGMP production also depends on other mediators of cellular function such as ANP. In addition, removal of cGMP from the renal tubular cells may be preferentially at the basolateral and not at the luminal site of the cell, which limits the use of urinary cGMP excretion as an index of NO formation or inhibition (33).

In conclusion, this study further expands our knowledge about the important role of NO in controlling the vascular tone in both the systemic and renal circulation in humans. There is no evidence, at least not in healthy men, that the renal circulation is more sensitive to the effects of NOS inhibition than the systemic circulation. Compared with L-NMMA, the most frequently used NOS inhibitor for performing studies in healthy men, L-NAME appears to be about 20 times more potent in inducing systemic and renal hemodynamic effects. We were intrigued not only about the magnitude but also about the rather long duration of action of the L-NAME-induced systemic and renal hemodynamic effects. In this context, the availability of a simple biochemical marker by which the degree and duration of NOS inhibition could easily be monitored would be a valuable new tool.

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Address for reprint requests: A. Broere, Univ. Hospital Dijkzigt, Dept. of Internal Medicine I, Rm. L242, Dr. Molewaterplein 40, 3015 GD Rotterdam, The Netherlands.

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