Endothelin-A Blockade Attenuates Systemic and Renal Hemodynamic Effects of L-NAME in Humans

Alberto Montanari, Almerina Biggi, Nicoletta Carra, Elena Fasoli, Massimo Calzolari, Francesca Corsini, Patrizia Perinotto, Almerico Novarini

**Abstract**—Eight Na-repleted volunteers underwent 3 separate 90-minute infusions of either Nω-nitro-L-arginine methyl ester (L-NAME) 3.0 mg · kg⁻¹ · min⁻¹ or endothelin-A receptor (ET-A) blocker BQ-123 (BQ) 0.125 nmol · kg⁻¹ · min⁻¹ or both. Mean arterial pressure (MAP), glomerular filtration rate (GFR), renal blood flow (RBF), renal vascular resistances (RVR), and sodium excretion rate (UNaV) were measured at baseline (b) and from 0 to 45 minutes (period 1) and 45 to 90 minutes (period 2) of infusion. BQ alone had no effect. GFR declined by 4.9% (P<0.001 versus b) in period 1, to 9.9% (P<0.001) in period 2 with L-NAME, and by 3.3% (P<0.01) to 6.6% (P<0.001) with L-NAME plus BQ (P=NS between L-NAME and L-NAME plus BQ). UNaV fell equally with L-NAME or L-NAME plus BQ. MAP rose significantly in period 2 with L-NAME (6.9%; P<0.001) but not with coinfused BQ (2.1%; P=NA versus b, P=0.005 versus L-NAME alone). RBF declined by 12.2% (P<0.001) to 18.3% (P<0.001) with L-NAME and by 4.6% (P<0.005) to 8.2% (P<0.001) with L-NAME plus BQ. These changes were smaller with L-NAME plus BQ (P<0.05 in period 1 and P<0.02 in period 2). Blunted changes were also seen for RVR (P<0.005 in period 1 and P<0.001 in period 2 between L-NAME alone and L-NAME plus BQ). These findings show that systemic and renal vasoconstriction due to L-NAME are attenuated by BQ, which suggests that an interaction between endogenous nitric oxide production and ET-A activity participates in the maintenance of baseline systemic and renal vascular tone in humans. (Hypertension. 2000;35[part 2]:518-523.)

**Key Words:** nitric oxide ■ endothelin ■ kidney ■ hemodynamics ■ L-NAME ■ man ■ BQ-123

It is now well established that endothelium-derived nitric oxide (NO) plays a major role in the physiological maintenance of baseline systemic and renal circulation. When tonic NO production is acutely abrogated by NO synthase inhibition (NOSI) in animals and humans, vasoconstriction takes place, with a dose-dependent increase in mean arterial pressure (MAP) and vascular resistance in many organs, including renal circulation. In human kidney, we and others have previously shown that NOSI with low-rate systemic infusion of Nω-nitro-L-arginine methyl ester (L-NMMA) or Nω-nitro-L-arginine methyl ester (L-NAME) may reduce effective renal plasma flow (ERPF), renal blood flow (RBF), glomerular filtration rate (GFR), and Na excretion independently of any apparent change in MAP. Both withdrawal of tonic NO production and amplification of underlying endogenous vasoconstrictor systems, such as angiotensin II (Ang II) and sympathetic nervous system, may participate, at least under certain experimental conditions, in renal vasoconstriction secondary to NOSI.

In addition to NO (and prostacyclin), the 21-amino acid peptide endothelin (ET) is of special importance in endothelium-mediated hemodynamic regulation. As an autocrine-paracrine substance, ET is known to exert a potent, vasoconstrictive action in both systemic and renal vasculature.

Interactions between ET and NO are well known, including potentiation of ET-induced vasoconstriction and elevation in MAP and enhancement of synthesis and release of ET after acute NOSI. Vasoactive properties of ET are mediated by 2 receptor subtypes, ETα and ETβ. Both ETα and ETβ in vascular smooth muscle cells seem to mediate vasoconstriction, whereas activation of ETβ receptors in endothelial cells may cause vasodilation through the release of prostacyclin and NO. However, local ETβ blockade in the human forearm predominantly produces vasoconstriction, which suggests a prevalent, net vasodilatory NO-mediated effect of ETα receptors. In both normotensive and hypertensive humans, forearm ETα blockade results in vasodilation that is almost completely inhibited by concomitant NOSI. At the kidney level, studies in dogs and humans indicate the ETα receptor as the main mediator of the vasoconstrictor effect of ET, on the basis of the demonstrated capacity of selective ETα blockade to either produce renal vasodilation or to abrogate renal vasoconstriction due to exogenous ET-1 infusion.

Until now, no studies have been conducted in humans on the relationship between tonic, NO-dependent regulation of
renal hemodynamics and endogenous ET. Therefore, in view of the unequivocal importance of ET$_A$ receptors in both systemic$^{10,11}$ and kidney$^3$ circulation of humans, we designed the present study to investigate whether activation of ET$_A$ receptors by endogenous ET contributes to the renal effects of acute NOSI. For this purpose, healthy, Na-repleted humans underwent infusion of L-NAME alone or in combination with the ET$_A$ receptor blocker BQ-123.

**Materials and Methods**

**Subjects**

Eight healthy subjects (3 men and 5 women), chosen from among the medical staff of Patologia Medica and Semeiotica Medica Institutes at the University of Parma and who provided written informed consent, participated in the study, which was conducted according to the ethical protocols of our institution. All the subjects were aged <40 years; had no history or evidence of disease of heart, liver, kidneys, or endocrine organs; had not abused alcohol or drugs; and were not currently undergoing medical treatment. Before the study, all subjects had a clinical examination, blood pressure measurement, ECG, and laboratory screening. Clinical data and laboratory results are given in Table 1.

**Experimental Procedure**

In a randomized order, each subject underwent 3 infusion studies, the first with BQ-123 alone, the second with L-NAME alone, and the third with both drugs. In women, experiments were performed around the midpoint of the menstrual cycle. Before each experiment, subjects were maintained for 5 days on a controlled diet that provided 250 mmol/L Na, 80 mmol/L K, and 1800 to 2400 kcal per day.

At 10 PM of the day before the study, each subject received a priming dose of inulin (Inutest 25% solution; 3000 mg/1.73 m$^2$ body surface area) and PAH (20% solution) (600 mg/1.73 m$^2$ body surface area) was injected. Then, an infusion of PAH and inulin in saline was initiated. After 45 minutes, subjects again voided their bladders, emptied their bladders, then a 45-minute baseline clearance period was started. After 45 minutes, subjects again voided their bladders, then a pump infusion of either 0.125 nmol·kg$^{-1}$·min$^{-1}$ BQ-123 or 3.0 μg·kg$^{-1}$·min$^{-1}$ of L-NAME in saline solution or both drugs, respectively, was initiated and maintained until the end of the study. Two additional 45-minute clearance periods were performed (0 to 45 minutes [P1] and 45 to 90 minutes [P2]), then the experiment was stopped. A 300-mL tap-water load was administered hourly throughout the study to ensure an appropriate urine flow. Blood pressure was measured every 5 minutes with an automatic oscillometric monitoring device (TM 2421, A and D Co Ltd). Samples from urine volume excreted during each clearance period were taken for Na, Li, and NO$_2$ plus NO$_3$ (NO$_x$).

Samples for plasma PAH and inulin were drawn every 15 minutes during the entire study. Samples for plasma Na and Li were taken at -45, 0, +45, and +90 minutes.

**Calculations**

A satisfactory steady state of plasma concentration of PAH and inulin was obtained with our infusion technique. The variability in plasma PAH and inulin measured throughout infusion was of the same order of magnitude as the coefficient of variability found in duplicate analysis of single plasma samples (2.4% for PAH and 3.6% for inulin). Thus, we calculated ERPF and GFR without measuring urinary PAH and inulin. For this purpose, PAH and inulin were measured in the infusate, and we calculated infusion rates by multiplying their concentrations for the volume of infused solution per minute. By dividing the infusion rate for each measured plasma concentration, we obtained 4 values in the baseline period and 3 in each drug period for both ERPF and GFR. The mean values were used in the expression of data for each clearance period. We calculated filtration fraction (FF) by dividing GFR for ERPF, RBF by dividing ERPF for (1-hematocrit), and renal vascular resistances (RVR) from MAP and RBF. Clearances of Li (CLI) and Na (CNa) were calculated with the mean plasma value for each period. Baseline plasma Li ranged from 0.10 to 0.18 mmol/L. We obtained values for the fractional excretion of Li and Na (FEli and FENA) by dividing CNa and CLI for GFR.

**Study Drugs**

PAH (20% solution) was purchased from J. Monico and Inutest from Laevosan Gesellschaft. Pharmaceutical grade L-NAME and BQ-123 were obtained from Clnalfa.

**Analytical Methods**

Na was measured by flame photometry and Li by atomic absorption spectrophotometry. Plasma and infusate PAH and inulin and urinary NO$_x$ were measured as described previously.$^3,14$

**Statistical Methods**

Data are expressed as the mean±SEM. ANOVA and paired Student t test were used to compare the results obtained in different study periods in the same experiment and those of the same study period among the 3 experiments.

**Results**

No adverse effect due to BQ-123 or L-NAME administration was observed.

Table 2 summarizes the results of our experiments for MAP and renal hemodynamics. Whereas BQ-123 alone did not exert any effect, L-NAME alone was followed by a slight but significant rise in MAP after only 45 minutes of infusion (+7% from baseline, P<0.001 versus both baseline and BQ-123 alone). Renal hemodynamics were widely altered by L-NAME even at 0 to 45 minutes (P1), when MAP was unchanged. In that period, GFR diminished by ≈5%.
TABLE 2. Effects of Infusion of 0.125 \( \mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) BQ-123, 3.0 \( \mu \text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) L-NAME, and L-NAME+BQ-123 on MAP and Renal Hemodynamics in 8 Na-Repleted Healthy Subjects

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>P1</th>
<th>P2</th>
</tr>
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<tbody>
<tr>
<td>MAP, mm Hg</td>
<td>77.0±2.1</td>
<td>76.6±2.0</td>
<td>76.8±2.4</td>
</tr>
<tr>
<td>BQ-123</td>
<td>76.7±2.3</td>
<td>77.8±2.3</td>
<td>82.0±2.2‡‡</td>
</tr>
<tr>
<td>L-NAME</td>
<td>76.7±2.2</td>
<td>77.2±2.2</td>
<td>78.3±2.3§</td>
</tr>
<tr>
<td>ERPF, mL \cdot min(^{-1}) \cdot 1.73 m(^{-2})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BQ-123</td>
<td>122±7</td>
<td>123±6</td>
<td>122±8</td>
</tr>
<tr>
<td>L-NAME</td>
<td>121±9</td>
<td>115±9†</td>
<td>109±8†‡</td>
</tr>
<tr>
<td>L-NAME+BQ-123</td>
<td>121±8</td>
<td>117±8†</td>
<td>113±8†</td>
</tr>
<tr>
<td>RBF, mL \cdot min(^{-1}) \cdot 1.73 m(^{-2})</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BQ-123</td>
<td>561±44</td>
<td>576±46*</td>
<td>564±41</td>
</tr>
<tr>
<td>L-NAME</td>
<td>552±41</td>
<td>485±33†</td>
<td>443±33†‡</td>
</tr>
<tr>
<td>L-NAME+BQ-123</td>
<td>576±54</td>
<td>547±52§</td>
<td>553±51§</td>
</tr>
<tr>
<td>RVR, mm Hg \cdot min(^{-1}) \cdot mL(^{-1})×10(^3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BQ-123</td>
<td>85±9</td>
<td>84±8</td>
<td>85±9</td>
</tr>
<tr>
<td>L-NAME</td>
<td>85±7</td>
<td>97±7†</td>
<td>112±8†</td>
</tr>
<tr>
<td>L-NAME+BQ-123</td>
<td>83±8</td>
<td>88±7§</td>
<td>92±8§</td>
</tr>
</tbody>
</table>

Significant differences (\(P<0.05\) to \(P<0.001\)): * vs baseline; † vs BQ123; ‡ vs P1; § vs L-NAME (for individual \(P\) values, see Results section).

\((P<0.001\) versus both baseline and BQ-123 alone), ERPF declined by 12.2\% \((P<0.005\), and RVR increased by 14\% \((P<0.001\). All these changes were more pronounced in P2, when ERPF and RBF were 18.3\% lower and RVR was 31.4\% higher than that calculated at baseline. With L-NAME plus BQ-123, changes in MAP in P2 were markedly blunted (+2\%; not significant versus baseline, \(P<0.005\) versus L-NAME), whereas those in ERPF, RBF, and RVR were all significantly smaller than those with L-NAME in both P1 and P2. In particular, in P1, ERPF and RBF decreased by only 4.6\% \((P<0.005\) versus baseline, \(P<0.05\) versus L-NAME) and RVR increased by 5.6\% \((P<0.01\) versus baseline, \(P<0.005\) versus L-NAME). In P2, ERPF and RBF were diminished with respect to both baseline and BQ-123 \((P<0.001\), but they were less reduced than with L-NAME \((P<0.02\), whereas RVR was only 10\% higher than at baseline \((P<0.001\) versus L-NAME). Conversely, GFR, although it showed a trend toward a smaller decrease than with L-NAME, was not significantly different between L-NAME and L-NAME plus BQ-123. FF, which showed a modest but significant increase in P2 with L-NAME (11\%), did not change during infusion of L-NAME plus BQ-123 \((P<0.005\) versus L-NAME). In the Figure, the results obtained for MAP, GFR, RBF, and RVR are summarized in a graph form.

In Table 3, we report the effects of infusion experiments on renal handling of Li and Na and excretion rate of NOx (UNOxV). BQ-123 did not produce any significant variation in baseline Li, Na, and NOx excretion. Infusion of L-NAME alone elicited a progressive decline in both absolute and fractional excretion of Li and Na, with values of UNaV, FENa, CLi, and FELi significantly lower in P2 than in P1. These changes in Na and Li handling were not modified by the combined infusion of L-NAME and BQ, which produced decrements in UNOxV comparable to those observed with L-NAME alone.

**Discussion**

The aim of the present study was to investigate whether interactions between tonic NO production and endogenous ET play a physiological role in the maintenance of baseline vascular tone in human kidney. To this purpose, renal hemodynamic changes after acute NOSI were measured with and without simultaneous blockade of ET at the level of ETA receptors.

In our experiments, NOSI was obtained with a low-rate, systemic L-NAME infusion technique. Previous animal studies have shown that a simultaneous rise in renal perfusion pressure (RPP) by itself may be responsible for a consider-
able portion of renal hemodynamic responses to acute systemic NOSI, including a marked rise in both afferent and efferent arteriolar tone with consequent elevation in FF.8,15,16 Conversely, local intrarenal NOSI leads to smaller, although still substantial, increases in afferent tone and, to a lesser extent, in efferent tone.8,15,16 In addition, antinatriuresis after intrarenal NOSI may be reversed, with consequent natriuresis, when MAP rises markedly, mainly because of a “pressure natriuresis” mechanism.2,8,15,16 Finally, these latter rat studies16 also showed that ET receptor blockade was highly effective in preventing just the RPP-dependent portion of renal vasoconstriction during acute systemic NOSI. However, with our experimental design, a slight but significant change in MAP took place only after 45 minutes of L-NAME infusion. Thus, any confounding, unspecific effect of a marked increase in RPP on the L-NAME–induced renal changes should be minimized.

Available evidence from dog and human observations indicates that the vasoconstrictor action of ET in both systemic and renal circulation is mediated predominantly by ET_A receptors. Studies by Verhaar et al10 showed that local infusion of an ET_A blocker in the forearm produced marked vasodilation, whereas ET_B blockade led to only modest vasoconstriction. In the same human model, simultaneous ET_A and ET_B blockade was followed by vasodilation of a magnitude only slightly diminished with respect to ET_A blockade alone.10 Because forearm vasodilation secondary to ET_A blockade was almost completely abolished by simultaneous NOSI with L-NMMA,10 the contribution of endogenous ET to the baseline systemic vascular tone in equilibrium with tonic NO production appears to be mediated principally by ET_A receptors. A similar vasodilatory action of ET_A blockade was found in dog kidney, with a substantial increase in RBF in the absence of significant changes in MAP.12 In addition, ET_A blocking agents, even at doses devoid of systemic and renal vasodilatory effects, largely prevented renal vasoconstriction due to systemic infusion of ET-1 in conscious dog17 and in humans.13 Finally, infusion of the selective ET_B receptor agonist ET3 did not affect kidney function of healthy humans.18 In the present experiments, a very low infusion rate (0.125 nmol kg\(^{-1}\) min\(^{-1}\)) of the specific peptide ET_A receptor blocker BQ-123 was used. Previous studies in humans indicated that such an inhibitor produces systemic and renal vasodilation and a fall in MAP when infused at a rate up to 3 nmol kg\(^{-1}\) min\(^{-1}\) in heart failure19 or hepatorenal20 patients. These pathophysiological conditions, however, are potentially characterized by abnormally elevated activity of the ET system.6 Conversely, BQ-123, when infused systemically in healthy subjects at rates ranging from 0.3 to 2.5 nmol kg\(^{-1}\) min\(^{-1}\), did not exert any systemic or renal hemodynamic action.13 However, it was able to vasodilate forearm when infused locally,10 and, more
creased MAP is in keeping with previous studies showing modest, slow changes in MAP.

MAP is markedly enhanced by NOSI. Our data therefore confirm a potential role of such drugs in treating hypertension in NO-deficient states.

Because BQ-123 not only attenuated the overall decrease in RBF and increase in RVR but also prevented the late rise in FF, the effect of ET_A seems to be focused on both arteriolar sides of the glomerulus. One could assume, however, that the modest increase in FF we observed in our human model was related to the slight but definite elevation in MAP, as suggested in rat kidney. Thus, a specific interaction between ET and NO in the kidney should take place at the level of the afferent arteriole.

Several observations in chronically instrumented conscious rats showed no significant effects of blockade of the renin-angiotensin system on renal changes secondary to acute NOSI. This indicates that at least under baseline, unstimulated conditions of minimal Ang II concentration in the kidney, there are no interactions between NO and Ang II in controlling baseline renal hemodynamics. These latter results eliminate the possibility that the relationship between ET and NO we show here was mediated indirectly via a synergistic interaction between ET and Ang II. Recent studies in dogs indicated that ET_A blockade may lead to an increase in renin production. Because MAP was kept constant or was increased modestly rather than decreased in our experiments, any interference by a possibly activated intrarenal renin-angiotensin system seems to be unlikely.

It is also known that NO has a distinct inhibitory effect on both synthesis and release of ET. The hypothesis may therefore be advanced that the interaction between NO and ET_A receptor activity observed in the present study is not the expression of a balance between baseline endogenous NO and ET but rather of an increased ET production due to NOSI and ET but rather of an increased ET production due to NOSI and ET but rather of an increased ET production due to NOSI.

In agreement with previous reports in healthy humans, baseline MAP, renal hemodynamics, and cation excretion were not affected by BQ-123 infusion alone. Infusion of L-NAME resulted, as expected, in significant changes in basal renal hemodynamics and tubular reabsorption, with vasoconstriction, reduction in GFR, and fall in both absolute and fractional excretion of Li and Na. Both MAP and FF rose slightly after only 45 minutes of L-NAME infusion. These findings are consistent with previous animal and human investigations, including our recent studies performed with the same L-NAME infusion technique.

During coinfusion of L-NAME and BQ-123, concentration in the kidney, there are no interactions between NO and Ang II in controlling baseline renal hemodynamics. These latter results eliminate the possibility that the relationship between ET and NO in the kidney should take place at the level of the afferent arteriole.

In rat 15 and healthy humans 3 showed no significant effects of coinfusion of BQ-123 did not affect Na and Li retention secondary to acute NOSI. This indicates that at least under baseline, unstimulated conditions of minimal Ang II concentration in the kidney, there are no interactions between NO and Ang II in controlling baseline renal hemodynamics. These latter results eliminate the possibility that the relationship between ET and NO we show here was mediated indirectly via a synergistic interaction between ET and Ang II. Recent studies in dogs indicated that ET_A blockade may lead to an increase in renin production. Because MAP was kept constant or was increased modestly rather than decreased in our experiments, any interference by a possibly activated intrarenal renin-angiotensin system seems to be unlikely.

These results point to a physiological interaction between ET_A receptor activity and NO in the control of baseline vascular tone, not only in systemic circulation but also in renal circulation. The inhibitory action of BQ-123 on increased MAP is in keeping with previous studies showing that the marginal effect of ET receptor blockers on baseline...
experimental conditions, ET₄ blockade is unable to reverse the increased tubular reabsorption at both the proximal and postproximal level secondary to NOSI. It is generally believed that Na retention produced by infusion of ET-1 in both animals and humans results merely from renal vasoconstriction secondary to ET₄ receptor activation.¹⁷,¹⁸ Because low doses of ET-1 may be natriuretic in animals via activation of ET₆ receptors, it is conceivable that ET₄ receptor blockade unmasks an ET₆-dependent natriuresis. However, BQ-123 did not affect either baseline Li or Na excretion or their increased reabsorption during L-NAME infusion. This latter finding was expected because ET₆-mediated effects of ET on tubular reabsorption of both Na (inhibition) and water (stimulation) are dependent on NO synthesis,⁶ which was in turn inhibited by L-NAME.

Because the physiological role of ET as a potent, endogenous vasoactive substance has been established, a number of peptide and nonpeptide ET receptor antagonists have been introduced for research and clinical purposes,²²,²⁷ pointing to a potential role of these drugs in both cardiovascular and renal disease.²² An impairment in NO-dependent vascular function is a common finding in many cardiovascular diseases, including hypertension and heart failure.¹¹,³² The present findings corroborate the view that ET₄ receptor blockade is a useful tool in preventing the endogenous ET-dependent component of systemic and renal vasoconstriction secondary to NO deficiency.

In summary, this study supports the concept that baseline ET₄ receptor activity exerts in humans a physiological interaction with endogenous NO, thus participating in the tonic control of systemic and renal hemodynamics. Such an interaction might also be important from a therapeutic point of view under clinical conditions of impaired NO availability in both systemic and renal vasculature.

References
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