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=NS 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.1 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.1–3 In human kidney, we and others4–5 have previously shown that NOSI with low-rate systemic infusion of Nω-monomethyl-L-arginine (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.1

In addition to NO (and prostacyclin), the 21–amino acid peptide endothelin (ET) is of special importance in endothelium-mediated hemodynamic regulation.6 As an autocrine-paracrine substance, ET is known to exert a potent, vasoconstrictive action in both systemic and renal vasculature.7 Interactions between ET and NO are well known, including potentiation of ET-induced vasoconstriction and elevation in MAP8,9 and enhancement of synthesis and release of ET after acute NOSI.9 Vasoactive properties of ET are mediated by 2 receptor subtypes, ETα and ETβ.6 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.6 However, local ETβ blockade in the human forearm predominantly produces vasoconstriction, which suggests a prevalent, net vasodilatory NO-mediated effect of ETβ receptors.10 In both normotensive10 and hypertensive humans,11 forearm ETα blockade results in vasodilation that is almost completely inhibited by concomitant NOSI.10 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 vasodilation12 or to abrogate renal vasoconstriction due to exogenous ET-1 infusion.13

Until now, no studies have been conducted in humans on the relationship between tonic, NO-dependent regulation of
TABLE 1. Basic Clinical and Laboratory Characteristics of the 8 Healthy Subjects Participating in the Study

<table>
<thead>
<tr>
<th>Males/females, n</th>
<th>3/5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>34.0±2.6</td>
</tr>
<tr>
<td>Height, cm</td>
<td>170±3</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>69±6</td>
</tr>
<tr>
<td>Body surface area, m²</td>
<td>1.77±0.05</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>77.5±2.0</td>
</tr>
<tr>
<td>Fractional hematocrit</td>
<td>0.430±0.015</td>
</tr>
<tr>
<td>Plasma sodium, mmol/L</td>
<td>140±0.5</td>
</tr>
<tr>
<td>Plasma potassium, mmol/L</td>
<td>4.3±0.1</td>
</tr>
<tr>
<td>Plasma creatinine, μmol/L</td>
<td>80.9±3.0</td>
</tr>
<tr>
<td>Plasma uric acid, μmol/L</td>
<td>228.0±26.5</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

After 60 minutes of equilibration (~45 minutes time), subjects emptied their bladders, then a 45-minute baseline clearance period was initiated. After 45 minutes, subjects again voided their bladders, then a pump infusion of either 0.125 mmol·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 NO₃ plus NO₂ (NOₓ).

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 infused, 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 Clinalfa.

Analytical Methods
Na was measured by flame photometry and Li by atomic absorption spectrophotometry. Plasma and infusate PAH and inulin and urinary NOₓ were measured as described previously.²,¹⁴

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%.
(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 infusion, was not significantly different between L-NAME and 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, CILi, 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 NO injury, including a marked rise in both afferent and efferent arteriolar tone with consequent elevation in FF. Conversely, local intrarenal NO injury leads to smaller, although still substantial, increases in afferent tone and, to a lesser extent, in efferent tone. In addition, antinatriuresis after intrarenal NO injury may be reversed, with consequent natriuresis, when MAP rises markedly, mainly because of a “pressure natriuresis” mechanism. Finally, these latter rat studies also showed that ET receptor blockade was highly effective in preventing just the RPP-dependent portion of renal vasoconstriction during acute systemic NO injury. 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 al 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. Because forearm vasodilation secondary to ET_{A} blockade was almost completely abolished by simultaneous NO injury with L-NMMA, 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. 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 dog and in humans. Finally, infusion of the selective ET_{B} receptor agonist ET3 did not affect kidney function of healthy humans. 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 failure or hepatorenal patients. These pathophysiological conditions, however, are potentially characterized by abnormally elevated activity of the ET system. 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. However, it was able to vasodilate forearm when infused locally, and, more
TABLE 3. Effects of Infusion of 0.125 μmol · kg · min⁻¹ BQ-123, 3.0 μg · kg · min⁻¹ L-NAME, and L-NAME+BQ-123 on Renal Handling of Na and Li and Urinary Excretion of NO₂ Plus NO₃ (NOₓ) in 8 Na-Repleted Healthy Subjects

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>P1</th>
<th>P2</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNaV, μmol/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BQ-123</td>
<td>234±22</td>
<td>223±19</td>
<td>230±24</td>
</tr>
<tr>
<td>L-NAME</td>
<td>219±27</td>
<td>149±20†</td>
<td>112±14*††</td>
</tr>
<tr>
<td>L-NAME+BQ-123</td>
<td>249±26</td>
<td>176±23††</td>
<td>130±22*††</td>
</tr>
<tr>
<td>FENa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BQ-123</td>
<td>0.015±0.003</td>
<td>0.015±0.002</td>
<td>0.014±0.003</td>
</tr>
<tr>
<td>L-NAME</td>
<td>0.014±0.003</td>
<td>0.010±0.002*†</td>
<td>0.008±0.002*††</td>
</tr>
<tr>
<td>L-NAME+BQ-123</td>
<td>0.015±0.002</td>
<td>0.011±0.001*†</td>
<td>0.009±0.001*††</td>
</tr>
<tr>
<td>FELi</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BQ-123</td>
<td>0.289±0.025</td>
<td>0.285±0.020</td>
<td>0.295±0.018</td>
</tr>
<tr>
<td>L-NAME</td>
<td>0.299±0.029</td>
<td>0.246±0.022*†</td>
<td>0.210±0.019*††</td>
</tr>
<tr>
<td>L-NAME+BQ-123</td>
<td>0.223±0.027</td>
<td>0.214±0.023*†</td>
<td>0.192±0.019*††</td>
</tr>
<tr>
<td>UNOxV, μmol/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BQ-123</td>
<td>1.18±0.20</td>
<td>1.28±0.24</td>
<td>1.10±0.29</td>
</tr>
<tr>
<td>L-NAME</td>
<td>1.30±0.24</td>
<td>0.79±0.19†</td>
<td>0.52±0.23*††</td>
</tr>
<tr>
<td>L-NAME+BQ-123</td>
<td>1.41±0.17</td>
<td>0.88±0.25*†</td>
<td>0.59±0.23*††</td>
</tr>
</tbody>
</table>

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

Importantly, to prevent renal vasoconstriction when confused systemically with ET-1.13

In agreement with previous reports in healthy humans,13 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.3,14 During coinfusion of L-NAME and BQ-123, UNOxV fell to the same extent as during L-NAME alone, which indicates that the different magnitude of renal changes between the 2 infusions was not due to variations in the degree of NOSI, at least up to the limits of UNOxV as an approximate marker of whole body and, presumably, renal metabolism of NO.3,14

As the main finding from the present study, changes in ERPF, RBF, and RVR due to L-NAME were markedly attenuated when BQ-123 was simultaneously confused. The drop in GFR also showed a trend to be prevented by BQ-123, although without statistical significance. Finally, the increases in MAP and FF, both observed in the later period of L-NAME infusion, were abrogated.

These results point to a physiological interaction between ET₄ receptor activity and NO in the control of baseline vascular tone, not only in systemic circulation2,10,11 but also in renal circulation. The inhibitory action of BQ-123 on increased MAP is in keeping with previous studies21 showing that the marginal effect of ET receptor blockers on baseline MAP is markedly enhanced by NOSI. Our data therefore confirm a potential role of such drugs in treating hypertension in NO-deficient states.22

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 ET₄ seemed 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.8,15,16 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 rat15 and healthy humans3 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.23,24 Recent studies in dogs indicated that ET₄ blockade may lead to an increase in renin production.25 This effect took place, however, only when RPP was lowered, which suggests an enhanced sensitivity of the renin-angiotensin system to the changes in RPP secondary to ET₄ blockade.25 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.9,26 The hypothesis may therefore be advanced that the interaction between NO and ET₄ 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 from 40% to 70% in rat experiments,25,28 in which MAP rose by ≥20% to 30%. Such changes in circulating ET-1 are much smaller than those that occur after anesthesia in rats,29 and they are in any case still below the vasoconstrictor threshold both in vitro30 and in vivo, as indicated for systemic and renal circulation in studies in humans undergoing ET-1 infusion.8,31 We did not measure plasma ET-1 in this study, first because the above-reported evidence suggests that under our experimental conditions, significant variations in plasma ET-1 are very unlikely. In addition, plasma ET-1 may not reflect an increase in its tissue concentration due to an accentuated release of peptide on the basolateral side of endothelial cells.6,27 Thus, although the participation of relatively enhanced ET production in the kidney in response to NOSI cannot be excluded, such an effect should be minimized because of low doses of infused L-NAME and only modest, slow changes in MAP.

Coinfusion of BQ-123 did not affect Na and Li retention due to L-NAME, which indicates that at least under our
experimental conditions, ET<sub>A</sub> 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 ET<sub>A</sub> receptor activation.17,18 Because low doses of ET-1 may be natriuretic in animals via activation of ET<sub>B</sub> receptors,6,7 it is conceivable that ET<sub>B</sub> receptor blockade unMASKS an ET<sub>B</sub>-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 ET<sub>B</sub>-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 ET<sub>A</sub> 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 ET<sub>A</sub> 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
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 and Almerico Novarini

Hypertension. 2000;35:518-523
doi: 10.1161/01.HYP.35.1.518
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2000 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/35/1/518

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/