Renal Hemodynamic Control by Endothelin and Nitric Oxide Under Angiotensin II Blockade in Man

Alberto Montanari, Nicoletta Carra, Patrizia Perinotto, Veronica Iori, Elena Fasoli, Almerina Biggi, Almerico Novarini

Abstract—To investigate whether endothelin-A receptors and nitric oxide modulate renal hemodynamics in man under angiotensin II receptor-1 blockade, 6 healthy volunteers, on a 240 mmol Na diet, underwent 4 separate renal hemodynamic measurements, in 3 of which endothelin-A blocker BQ-123 0.2 nmol · kg · min⁻¹ was infused for 90 minutes after pretreatment with either placebo, telmisartan 1 mg · kg · day⁻¹ for 3 days, or telmisartan as well, but with co-infusion of both BQ-123 and N⁵-nitro-L-arginine methylester 0.5 μg · kg · min⁻¹. A fourth infusion was made with N⁵-nitro-L-arginine methylester alone. No change followed infusion of either N⁵-nitro-L-arginine methylester alone or BQ-123 alone. With BQ-123 after telmisartan, renal blood flow rose from 916±56 mL · min⁻¹ · 1.73 m² to 1047±51.2 (P<0.001), and renal vascular resistances fell from 89±7 mm Hg · min · L⁻¹ to 74±4 (P<0.001). These changes were fully abolished by the co-infused N⁵-nitro-L-arginine methylester. Infusion of BQ-123, devoid of renal hemodynamic effects at baseline, produces significant renal vasodilation when angiotensin II receptors are blocked, indicating an increasing renal hemodynamic role of endothelin-A–receptor activity. Because such a vasodilation is prevented by nonvasoconstricting microdoses of N⁵-nitro-L-arginine methylester, nitric oxide–endothelin balance controls substantially renal hemodynamics under angiotensin II blockade. These findings are consistent with a rationale of the association of endothelin-A blockers with angiotensin II blockers or angiotensin-converting enzyme inhibitors in treating nitric oxide–deficient conditions such as arterial hypertension, heart failure, and chronic renal diseases. (Hypertension. 2002;39[part 2]:715-720.)

Key Words: angiotensin II ■ nitric oxide ■ endothelin ■ kidney ■ hemodynamics ■ L-NAME ■ receptors, endothelin

Hemodynamic control in both systemic circulation and kidney results physiologically from a balance of opposing vasodilator systems such as nitric oxide (NO) and prostaglandins, and vasoconstrictor systems.¹ These latter include, besides sympathetic nervous system, the 2 potent peptide vasoconstrictors angiotensin II (Ang II) and endothelin-1 (ET-1),¹ this latter being the predominant isoform of endothelin family expressed in human vasculature.² Vasoactive properties of ET-1 are mediated by 2 receptor subtypes, ETₐ and ETₐb, both leading to vasoconstriction in vascular smooth muscle cells, whereas activation of ETₐb in endothelial cells may cause vasodilation through the release of prostacyclin and NO.²,³ At the kidney level, however, studies in both dogs⁴,⁵ and humans⁶,⁷,⁸ have indicated the ETₐ receptor as the main mediator of the vasoconstrictor effect of ET-1.

A considerable body of evidence has shown that ET-1, although it is the most potent endogenous vasoconstrictor, assumes a major hemodynamic role and contributes to the end-organ damage, mainly under experimental and clinical pathophysiological conditions.²,³ Furthermore, a number of interactions have been recognized between Ang II and ET-1, such as an ET-1–mediated vasoconstriction from Ang II infusion,⁹ a marked stimulation by Ang II of ET-1 synthesis and release,¹⁰ and a synergistic effect of nonhypertensive amounts of ET-1 to induce a pressor action of otherwise nonpressor doses of infused Ang II.¹¹ ET-1 is also known to contribute substantially through its hypertrophic and mitogenic effect to the functional and structural abnormalities of both the cardiovascular system and the kidney in experimental, salt-sensitive, hypertension because of low-dose Ang II infusion.¹⁰,¹²,¹³ Thus, ET-1 is now considered as a powerful mediator of Ang II–dependent vasoconstriction and organ damage.

Conversely, the role of ET-1 by itself and of its numerous interactions with Ang II in the maintenance of systemic and renal hemodynamics in humans under physiological conditions is much less clarified.

Systemic pharmacological blockade of the primarily vasoconstricting ETₐ receptor may produce vasodilation in patients suffering from diseases with an activated ET-1 system and markedly elevated plasma ET-1, such as chronic heart
failure (CHF) and liver cirrhosis. In healthy humans, systemic ET₄ blockade prevents or markedly blunts systemic and renal vasoconstriction due to either infusion of exogenous ET-1 or NO synthesis inhibition by systemic infusion of N^ω-nitro-L-arginine methylester (L-NAME). However, when doses of ET₄ antagonists, such as those used under experimental or pathophysiological conditions, have been administered systemically in healthy humans, only minor effects on systemic circulation and no changes in baseline renal hemodynamics have been detected. Even when selective ET₄ blockade has been performed by infusing drugs locally in the forearm of normal subjects, conflicting results have been obtained, ranging from no changes to a 40% increase in baseline blood flow.

On the other hand, studies in both normal dogs and rats undergoing simultaneous systemic blockade of renin-angiotensin system (RAS) with either ACE inhibitors (ACEI) or AT₁ receptor antagonists (AIIRA) have shown that hypertension and, primarily, marked renal vasodilation resulted from ET₄ or mixed ET₁/ET₄ blockade. For instance, Berthold et al have reported that, in dogs receiving both ACEI trandolapril and ET₄ blocker LU135252, renal blood flow (RBF) increased to an extent 3 to 4 times larger than that observed with ET₄ blockade alone.

Thus, the hypothesis may be advanced that a substantial ET₄ receptor–mediated contribution of ET-1 in the maintenance of systemic and, principally, renal hemodynamics takes place when RAS activity is blunted by ACE inhibition or AT₁ blockade. In addition, because renal vasodilation under combined ACE inhibition and ET₄ blockade was prevented in dogs by NO synthesis inhibition, intrarenal NO production seems to be involved in renal vasodilation due to ET₄ blockade.

The aim of the present study was to investigate whether and to what extent ET₄ activity and NO regulate renal hemodynamics in normal humans undergoing RAS blockade.

Apart from being relevant in normal physiology, this issue also may be of importance in view of the proposed beneficial role of ET-1 and NO synthesis inhibition by systemic infusion of L-NAME. However, when doses of ET₄ antagonists, such as those used under experimental or pathophysiological conditions, have been administered systemically in healthy humans, only minor effects on systemic circulation and no changes in baseline renal hemodynamics have been detected. Even when selective ET₄ blockade has been performed by infusing drugs locally in the forearm of normal subjects, conflicting results have been obtained, ranging from no changes to a 40% increase in baseline blood flow.

Experimental Procedure

Participants underwent 4 infusion studies in a randomized order, each preceded by 5 days of a controlled diet that provided 250 mmol Na, 80 mmol K, and 1800 Kcal. The washout period between infusions was approximately 2 weeks for males and 4 weeks for females, who were studied during the follicular phase of the menstrual cycle. In 3 studies ET₄ blocker BQ 0.2 mmol·Kg·min⁻¹ was infused after pretreatment with either PL, 1 mg·Kg·day⁻¹, TEML, or TEML as well, but with co-infusion of both BQ and 0.5 mg·Kg·min⁻¹ L-NAME. The fourth infusion was made with 0.5 mg·Kg·min⁻¹ L-NAME alone preceded by PL. Such a dose of L-NAME, which is one-half the lowest amount infused up to now in humans, seems to be involved in renal vasodilation due to ET₄ blockade.

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Materials and Methods

Participants

Six healthy volunteers, 3 men and 3 women, chosen from among the medical staff of Dipartimento di Scienze Cliniche at the University of Parma, after written informed consent, participated in the study, which was conducted according to the ethical protocols of our institution. Their ages were 30±1 years, heights 166±7 cm, body weights 66.7±2.0 kg, body surface areas 1.71±0.05 m². None had evidence or history of heart, liver, kidney, or endocrine diseases, had abused alcohol or drugs, or were currently under medical treatment. Before the study, all participants had a clinical examination, a blood pressure measurement, and an ECG. A laboratory screening showed normal values for urinalysis, blood hematoctrit, and plasma creatinine, Na, K, uric acid, total cholesterol, and triglycerides.

Calculations

A satisfactory steady-state of plasma concentration of PAH and inulin was obtained with our infusion technique. Because variability in plasma PAH and inulin measured throughout infusion was comparable to that found in a duplicate analysis of single plasma samples (2.4% for PAH and 3.6% for inulin), effective renal plasma flow (ERPF) and glomerular filtration rate (GFR) were estimated without measuring urinary PAH and inulin using a constant-infusion technique. Such a procedure was preferred rather than standard urinary clearance because an unethical bladder catheterization should be necessary to avoid errors in urine collection, potentially of the same order of magnitude as that of measured changes in renal...
hemodynamics.\textsuperscript{16,24} Constant infusion technique already has been shown to detect in humans rapid changes in renal hemodynamics as a result of administration of different substances such as nitrendipine,\textsuperscript{29} L-NAME\textsuperscript{16,24} and Ang II.\textsuperscript{30} PAH and inulin concentrations were measured in the infused, then multiplied for the volume of infused solution per minute. The resulting infusion rate of PAH or inulin was divided for each measured plasma concentration, thus obtaining 4 clearance values in the baseline period and 3 in each drug period for both ERPF and GFR.\textsuperscript{16,24} The mean values were used in the expression of data for each period. Filtration fraction (FF) was calculated from GFR and ERPF, RBF was calculated from ERPF and hematocrit, and renal vascular resistances (RVR) from MAP and RBF.

**Study Drugs**

PAH (20% solution) and inulin (10% solution) were purchased from J. Monico, Ltd, Venice, Italy. A commercially available TELM preparation of 40- and 80-mg tablets was used, while pharmaceutical grade L-NAME.HCL and BQ were obtained from Cilinalfa.

**Analytical Methods**

Na was measured by flame photometry. Plasma and infusate PAH was measured in the infusate, then multiplied for the volume of infused

| Effects of Four Different Infusions on Mean Arterial Pressure, Glomerular Filtration Rate, Renal Blood Flow, Filtration Fraction, and Na Excretion in Six Healthy Volunteers |
|-----------------------------|-----------------------------|-----------------------------|
|                             | Baseline                    | Infusion                    |
|                             | First Period                | Second Period               |
|                             | -45-0 min                   | 0–45 min                    | 45–90 min                   |
| MAP, mm Hg                  |                             |                             |                             |
| PL+BQ                       | 77.4±2.4                    | 77.7±2.3                    | 77.7±2.3                    |
| TELM+BQ                     | 80.5±3.4                    | 78.1±3.9                    | 76.5±2.9                    |
| PL+L-NAME                   | 77.4±2.7                    | 77.5±2.5                    | 77.4±2.6                    |
| TELM+BQ+L-NAME              | 75.5±2.3                    | 77.7±2.0                    | 77.0±2.2                    |
| GFR, mL·min\(^{-1}\)·1.73 m\(^2\) |                             |                             |                             |
| PL+BQ                       | 111±5                       | 110±5                       | 110±5                       |
| TELM+BQ                     | 110±6                       | 108±6                       | 111±5                       |
| PL+L-NAME                   | 110±4                       | 109±5                       | 110±4                       |
| TELM+BQ+L-NAME              | 110±5                       | 110±6                       | 108±5                       |
| RBF, mL·min\(^{-1}\)·1.73 m\(^2\) |                             |                             |                             |
| PL+BQ                       | 938±58                      | 951±55                      | 942±54                      |
| TELM+BQ                     | 916±62                      | 998±55\(^\bullet\)          | 1047±56\(^\bullet\)         |
| PL+L-NAME                   | 941±50                      | 948±50                      | 937±56                      |
| TELM+BQ+L-NAME              | 931±57                      | 940±60                      | 946±65                      |
| RVR, mm Hg·min·L\(^{-1}\)   |                             |                             |                             |
| PL+BQ                       | 84±2                        | 83±4                        | 84±3                        |
| TELM+BQ                     | 89±7                        | 88±5\(^\bullet\)            | 74±5\(^\bullet\)            |
| PL+L-NAME                   | 83±3                        | 83±3                        | 83±4                        |
| TELM+BQ+L-NAME              | 84±4                        | 84±4                        | 83±5                        |
| FF                          |                             |                             |                             |
| PL+BQ                       | 0.203±0.006                 | 0.199±0.006                 | 0.200±0.007                 |
| TELM+BQ                     | 0.207±0.007                 | 0.188±0.006\(^\bullet\)     | 0.176±0.005\(^\bullet\)     |
| PL+L-NAME                   | 0.202±0.003                 | 0.199±0.004                 | 0.206±0.003                 |
| TELM+BQ+L-NAME              | 0.204±0.005                 | 0.202±0.005                 | 0.196±0.004                 |
| UNaV, μmol·min\(^{-1}\)     |                             |                             |                             |
| PL+BQ                       | 203±18                      | 210±18                      | 205±16                      |
| TELM+BQ                     | 200±19                      | 206±21                      | 200±14                      |
| PL+L-NAME                   | 192±17                      | 155±14\(^\bullet\)          | 128±11\(^\bullet\)          |
| TELM+BQ+L-NAME              | 195±15                      | 167±12\(^\bullet\)          | 137±11\(^\bullet\)          |

*PL indicates placebo; BQ, BQ-123 0.2 nmol·Kg·min\(^{-1}\); TELM, telmisartan 1 mg/KgBW for 3 days before infusion; L-NAME, N\(^\text{2}\)-nitro-L-arginine-methylster 0.5 μg·Kg·min\(^{-1}\).

\(^\bullet\)*Indicates significant changes versus baseline (\(P<0.001\)); \(^\bullet\)Indicates significant differences versus PL+BQ, PL+L-NAME and TELM+BQ+L-NAME (\(P<0.001\)); \(^\bullet\)Indicates significant differences versus PL+BQ and TELM+BQ (\(P<0.001\)).

L-NAME was co-infused with BQ after TELM, it prevented any change in RBF (Figure 1) and RVR. In addition, a 30% to 33% decrease in UNaV took place when L-NAME was infused, either alone or with TELM+BQ.

**Discussion**

In the present human study, ET\(_1\)-receptor blockade was obtained by infusing systemically the peptide antagonist BQ at a rate devoid of any renal hemodynamic action at baseline as previously found by us\textsuperscript{16} and others.\textsuperscript{8} Comparable rates of infusion of BQ, however, were shown to produce systemic
vasodilation in both CHF and liver cirrhosis patients with presumably activated ET\(_1\) system. In addition, BQ prevented renal vasoconstriction in healthy men due to either exogenously infused ET-1 or endogenous ET-1 left unopposed by NO synthase inhibition with low-dose, systemic L-NAME infusion. Thus, amounts of BQ such as those used in the present study should ensure a substantial blockade of ET\(_A\) receptor. Similar results also have been reported in humans for the oral ET\(_A\) antagonist ABT-627, which fully prevented vasoconstriction both in the kidney during systemic ET-1 infusion and in the forearm during local infusion of such a peptide, in spite of only minor changes in MAP. Our findings indicate, therefore, that there is only a little contribution, if any, of endogenous ET-1 to the baseline renal vascular tone in normal humans.

However, when ET\(_A\) blockade was performed after a short-term treatment with the AIIRA TELM, substantial renal vasodilation took place, with rise in RBF and decrease in RVR. GFR remained unchanged and FF fell, in keeping with vasodilation that took place, with rise in RBF and decrease in intrarenal NO production was statistically significant \(P < 0.001\) versus both baseline and all other infusion treatments.

Effects of different infusions (for abbreviations see Table 1) on renal blood flow (RBF) in 6 healthy volunteers. The rise in RBF with TELM+BQ is statistically significant \(P < 0.001\) versus both baseline and all other infusion treatments.
modest increase in its plasma levels may result from the withdrawal of the NO-dependent inhibition due to L-NAME.2,3 However, the rise in plasma ET-1 in acute NO synthesis inhibition in healthy humans may not exceed 20% of baseline values with a 5% to 7% increase in MAP.36,37 Such levels are in any case still below the in vivo vasoconstrictor threshold as indicated for renal circulation in human studies of ET-1 infusion.6,7,38 Furthermore, plasma ET-1 may not reflect an increase in its tissue concentration due to a preferential release of peptide on the basolateral side of endothelial cells.2,3 Thus, although we did not measure plasma ET-1, a participation of a relatively enhanced production of ET-1 in the kidney is unlikely because of the very low dose of L-NAME and slight decrease, rather than increase, in MAP. Taken together, these findings indicate therefore that, because neither NO nor ET-1 were significantly stimulated in our human experimental model, NO and ET receptors interact at (or very close to) their baseline level of activity in controlling renal hemodynamics under AT1 blockade.

To summarize, the present data show that the contribution of the intrarenal balance between baseline NO production and ETα receptor activity to the maintenance of baseline renal hemodynamics is markedly accentuated when RAS is blocked. Berthold et al in their previous dog studies4,5 have shown that combined blockade of both ET-1 and RAS causes pronounced renal vasodilation, which is fully prevented by L-NAME, whereas blockade of either system alone exerts only minor or no effects on RBF. These findings demonstrate not only that blockade of each system potentiates vasodilatation following blockade of the other, but also that such a vasodilatation is mediated by the intrarenal NO activity left unopposed by the simultaneous blockade of both vasoconstrictor systems. Thus, these animal data and our own human findings seem to indicate collectively that tonic activity of intrarenal NO not only participates in setting the baseline level of RBF but also is of major importance in determining the renal vasoconstrictor response to both Ang II and ET-1.

A substantial renal hemodynamic role of ETα receptor activity in humans undergoing RAS blockade also is relevant for our understanding of interactions among RAS, ET-1, and NO and their potential impact on drug treatment of renal and cardiovascular human diseases. For instance, recent trials with ET-1 blockers in CHF, seem to indicate that ET-1 receptor antagonism may not only ameliorate symptoms and hemodynamics but also may substantially improve prognosis.3,39 In most of such clinical studies, ET-1 blockade has produced hemodynamic improvement when superimposed to a standard ACEI treatment.3,39 Such results could parallel, at least in part, our finding of an enhanced, NO-dependent, renal vasodilation with ETα blockade in healthy humans pretreated with an AIIRA. In this view, our data may offer further support to the concept of a potential usefulness of ETα antagonism in association with RAS blockade in the management of CHF32 and of other clinical conditions such as hypertension21 or renal disease.20,22

Caution should be taken, however, in extending our results of acute studies of ETα blockade and NO synthesis inhibition in young healthy individuals with both RAS and ET-1 presumably not activated to chronic conditions, such as CHF, with marked activation of all vasoconstricting and Na-retaining systems.

In conclusion, the present study in healthy humans demonstrates that renal vasoconstrictor activity of endogenous ET-1 via ETα receptor, which exerts only marginal effects in the control of renal function under baseline conditions of intact RAS, assumes a major vasoconstrictor role and contributes substantially to renal hemodynamics when intrarenal RAS is blocked at the level of AT1 receptors. Because ETα-dependent vasoconstriction takes place in equilibrium with the intrarenally produced NO, our findings indicate an increasing role of ET-1/NO balance in humans undergoing RAS blockade. The clinical relevance of these observations in healthy humans pertains to CHF and, perhaps, to other human cardiovascular and renal diseases in which a disrupted ET-1/NO balance in the kidney could contribute to renal dysfunction even in the presence of an effective blockade of intrarenal RAS. These issues, which may further support the rationale of an association of ETα antagonistic drugs with standard treatment with ACEI or AIIRA, deserve further investigations.

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