Endothelin-A Receptors Mediate Renal Hemodynamic Effects of Exogenous Angiotensin II in Humans

Alberto Montanari, Almerina Biggi, Nicoletta Carra, Maurizio Ziliotti, Elena Fasoli, Luisa Musiari, Patrizia Perinotto, Almerico Novarini

Abstract—To investigate whether endothelin-A receptors mediate hemodynamic changes caused by exogenous Angiotensin II in humans, 7 healthy volunteers on a 250-mmol sodium diet underwent 3 separate p-aminohippurate and inulin-based renal hemodynamic studies. In 2 studies, Angiotensin II (increasing rates of 0.625, 1.25, and 2.5 ng/kg per minute, each for 30 minutes) was infused either alone or combined with endothelin-A blocker, BQ123, 0.4 nmol/kg per minute. A third infusion of BQ123 alone was not followed by any change. Angiotensin II infusion alone produced a progressive decrease in renal blood flow (1080 ± 94 mL/min × 1.73 m² to 801 ± 52, P < 0.001, versus baseline) and glomerular filtration rate (115 ± 7 mL/min × 1.73 m² to 97 ± 7, P < 0.001) with increase in filtration fraction (0.188 ± 0.017 to 0.220 ± 0.030, P < 0.01). Mean arterial pressure and renal vascular resistance increased markedly (86.8 ± 3.1 to 97.5 ± 4.4 mm Hg, P < 0.001 and 83 ± 7 to 133 ± 20 mm Hg/min per liter, P < 0.001, respectively). With Angiotensin II + BQ 123, mean arterial pressure still rose (86.2 ± 3.1 to 91.1 ± 4.3, P < 0.05 versus both baseline and BQ123 alone) but significantly less than with Angiotensin II alone (P < 0.05). Renal blood flow (1077 ± 76 to 993 ± 79, P < 0.001) and glomerular filtration rate (115 ± 7 to 105 ± 7, P < 0.05) also changed to a significantly lesser extent than with Angiotensin II alone (P < 0.05 for both), whereas filtration fraction remained unchanged (0.185 ± 0.015 to 0.186 ± 0.016). Renal vascular resistance rose only by 17% (82 ± 5 to 95 ± 9, P < 0.001 versus baseline as well as versus BQ123 or Angiotensin II alone). The results show that endothelin through Endothelin-A receptors contributes substantially to the systemic and renal vasoconstriction of low-dose exogenous Angiotensin II in healthy humans. (Hypertension. 2003;42[part 2]:825-830.)

Key Words: receptors, endothelin • angiotensin II • kidney • hemodynamics • human

Angiotensin II (Ang II), a potent endogenous vasoconstricting and sodium-retaining peptide, plays a key role in the regulation of renal function and arterial pressure, mostly by activating Ang II-type 1 (AT1) receptors, which leads to elevation in blood pressure, renal vasoconstriction, and sodium retention. Several of such actions are similar to those of endothelin-1 (ET-1), which is the predominant isoform of the endothelin family in human vasculature. Vasoactive properties of ET-1 are mediated by two receptor subtypes, ETA and ETB, both leading to vasoconstriction in vascular smooth cells, whereas activation of ETB in endothelial cells causes vasodilation as the result of release of prostacyclin and nitric oxide (NO). In humans, however, the ETα receptor is the main mediator of ET-1 renal vasoconstriction, although it is the most potent endogenous vasoconstrictor, assumes a major hemodynamic role and contributes to the end-organ damage, mainly under experimental pathophysiological conditions. In most of these, interactions between Ang II and ET-1 may contribute largely to the observed changes. For instance, salt-sensitive hypertension, renal vasoconstriction, and cardiovascular and renal fibrotic damage, produced by chronic administration of exogenous Ang II, can be prevented by inhibition of endogenous ET-1, indicating that ET-1 mediates much of the vasoconstriction caused by chronic Ang II. Furthermore, ET-1 participates in the acute pressor effects of exogenous Ang II and partly mediates the vasoconstriction from exogenous Ang II in different vascular beds, including kidney. Interestingly, studies in rats have shown that such Ang II–ET-1 interaction may be sodium-dependent, because under elevated sodium intake, ET A blockade inhibited Ang II hypertension much more than under sodium restriction. Ang II also stimulates both ET-1 synthesis and release, whereas Ang II enhances the pressor action of Ang II. Finally, ET-1 and Ang II share the same intracellular signaling pathways. Thus, ET-1 is generally considered as a powerful mediator of Ang II–dependent vasoconstriction and organ damage under experimental conditions.

Conversely, little is known on the role of ET-1 and of its potential interactions with Ang II on systemic and renal hemodynamics in humans. In normal humans, systemic ET A blockade markedly blunts systemic and renal vasoconstriction as the result of either infusion of exogenous ET-1 or to exogenous ET-1 left unopposed by NO synthesis inhibition.
However, the same doses of ET\textsubscript{A} antagonists produce only minor effects on systemic circulation and no changes in baseline renal hemodynamics.\textsuperscript{8–10} Under clinical conditions, in patients with disease characterized by marked activation of both the renin-angiotensin system (RAS) and the ET system, such as liver cirrhosis\textsuperscript{19} and chronic heart failure (CHF),\textsuperscript{21} systemic ET\textsubscript{A} blockade has been shown to produce vasodilation. Evidence for favorable effects of ET-1 antagonistic drugs in humans is only preliminary at the present for essential hypertension\textsuperscript{22} and renal disease,\textsuperscript{23} whereas trials in patients with CHF have shown substantial improvement in hemodynamic status and prognosis.\textsuperscript{24,25} Because such positive findings have been mostly obtained in patients already treated with ACE inhibitors (ACEI),\textsuperscript{24,25} the effects of ET-1 blockade might reflect, at least in part, an interaction between ET-1 and Ang II under simultaneous stimulation of both systems, as it is known in CHF.\textsuperscript{24} In a recent human study aimed to directly investigate interactions between Ang II and ET-1, Wenzel et al.\textsuperscript{26} have shown that selective ET\textsubscript{A} antagonism inhibits Ang II vasoconstriction in the skin microcirculation of healthy subjects.

The aim of the present study was to investigate whether and to what extent ET\textsubscript{A} activity participates in renal hemodynamic changes produced in normal humans by systemic infusion of low-dose Ang II.

The study was based on the hypothesis of a physiological mechanism by which an activated RAS, to the extent that it can be reproduced by low-dose exogenous Ang II, exerts, at least in part, its renal hemodynamic actions through ET\textsubscript{A} receptors. Apart from being relevant in normal physiology, such a result may be of importance in view of the proposed beneficial effects of ET-1 receptor blockade in human cardiovascular and renal disease,\textsuperscript{21–25} in which ACEI or AT1 receptor blockers (AIIRA) are now among the most generally accepted therapies.\textsuperscript{2}

For the purpose of our study, healthy humans underwent either acute ETA blockade by infusion of the specific peptide ET\textsubscript{A} antagonist BQ123 (BQ) or short-term infusion of low-dose Ang II or simultaneous coinfusion of both BQ and Ang II. Because both renal response to exogenous Ang II in humans\textsuperscript{27} and inhibitory action of ET\textsubscript{A} blockade on Ang II hypertension in animals\textsuperscript{16} are much more pronounced at an elevated sodium intake, our experiments were made with subjects kept on a 250-mmol sodium diet.

**Methods**

**Participants**

Seven healthy volunteers (4 men and 3 women; age, 33±1 years; height, 171±5 cm; body weight, 69.7±2.0 kg), after giving written informed consent, participated in the study, according to the ethical protocols of our institution. None had evidence or history of heart, liver, kidney, or endocrine diseases, alcohol or drug abuse, and was under medical treatment. Before the study, all participants had a clinical examination, repeated blood pressure measurements, an ECG, and a routine laboratory screening.

**Experimental Procedure**

Participants underwent in a randomized order three 90-minute infusion studies, each preceded by 5 days of a controlled diet that provided 250 mmol/L sodium, 80 mmol/L potassium, and 1800 Kcal. The washout period between infusions was ~2 weeks for men and 4 weeks for women, who were studied during follicular phase of menstrual cycle. In one study, ET\textsubscript{A} blocker BQ (0.4 mmol/kg per minute) was infused alone, whereas in another study, human Ang II was infused at stepwise increasing rates of 0.625, 1.25, and 2.5 ng/kg per minute, each for 30 minutes. In the third study, both BQ and Ang II were simultaneously coinfused.

Doses of both drugs close to the ranges used in our experiments have been reported to produce in humans effective renal vasoconstriction with small systemic pressor changes for Ang II\textsuperscript{27–29} and inhibition of systemic and renal effects of endogenous ET-1 for BQ,\textsuperscript{19} respectively.

After an overnight fast, experiments were initiated at 8 AM, with the participant in a sitting position. A plastic indwelling catheter was placed into a cubital vein, a priming dose of 3000 mg/l.73 m\textsuperscript{2} body surface area of inulin and 600 mg/L of para-aminohippuric acid (PAH) was injected, and an infusion of PAH and inulin was initiated and continued throughout the entire study, with a 50-mL syringe precision pump (Perfusion Secura, Braun Melsungen) used to obtain plasma levels of ~15 mg/L for PAH and 200 mg/L for inulin. A second indwelling catheter for blood sampling was placed immediately at the contralateral arm. After 60 minutes of equilibration, participants emptied their bladder, then a 30-minute baseline (b) clearance period was performed. Then, after voiding, a pump infusion of either BQ, Ang II, or BQ plus Ang II was initiated. Three additional 30-minute clearance periods were performed (0 to 30 minutes, 30 to 60, and 60 to 90 minutes, respectively). Tap water (300 mL) was administered hourly to ensure an appropriate urine flow. Blood pressure was measured every 3 minutes with the use of an automatic oscillometric monitoring device (TM 2421, A and D Co). Samples from urine of each clearance period were taken for excretion rate of sodium (UNaV). Samples were drawn for plasma PAH and inulin every 10 minutes during the entire study and for plasma ET-1 concentration at the end of each 30-minute period.

**Calculations**

A satisfactory steady state for PAH and inulin in plasma was obtained with our infusion technique.\textsuperscript{19} Variability for plasma PAH and inulin was 2.4% and 3.6%, respectively. Effective renal plasma flow (ERPF) and glomerular filtration rate (GFR) were estimated without measuring urinary PAH and inulin through the use of a constant-infusion technique.\textsuperscript{19,28–30} PAH and inulin measured in the infused solution were 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 four clearance values at baseline and three in each infusion period.\textsuperscript{19} The mean values were used in the expression of data for each period. Filtration fraction (FF) was calculated as the ratio between GFR and ERPF, renal blood flow (RBF) 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. Pharmaceutical grade BQ and human Ang II were obtained by Clinalfa.

**Analytical Methods**

Sodium was measured by flame photometry. Plasma and infused PAH and inulin were measured as previously described.\textsuperscript{38} Plasma ET-1 was measured in extracted EDTA plasma samples with a commercially available immunoenzymatic method (R and D Systems).

**Statistical Methods**

Data are expressed as mean±SEM. Time-dependent effects of each infusion were analyzed by 1-way ANOVA. Differences between various infusions were analyzed by 2-way ANOVA followed by post hoc multiple comparisons with the Student-Newman-Keuls test. Differences at the 5% level or less were considered to be statistically significant.
**Results**

The results of MAP, GFR, RBF, FF, RVR, UNaV, and plasma ET-1 are summarized in the Table. RVR also is represented in the Figure.

### Acute ET₄ Blockade Does Not Affect Baseline Renal Hemodynamics

Acute ET₄ blockade alone, as performed with BQ infusion alone, did not produce changes in any measured values.

### Ang II Infusion Leads to Renal Vasoconstriction, Antinatriuresis, and Late Rise in MAP

The lowest rate of Ang II infusion (0.625 ng/kg per minute) produced significant decrease in RBF (−6.3%, *P*<0.05) and rise in RVR (+9.6%, *P*<0.05), with no change in MAP and GFR. FF increased significantly (+5.9%, *P*<0.05) and UNaV fell substantially (*P*<0.001). In the subsequent 2 steps of Ang II infusion, RBF decreased further (*P*<0.01 versus BQ at 60 minutes, *P*<0.001 at 90 minutes), and MAP rose

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### Table: Effects of 90-Minute Infusion of BQ, of AngII at a Rate Increasing Stepwise Every 30 Minutes, or of Both Drugs on MAP, Renal Hemodynamics, UNaV, and Plasma ET-1, in 7 Healthy Humans

<table>
<thead>
<tr>
<th>Variables</th>
<th>BQ, 0.4 nmol · kg⁻¹ · min⁻¹</th>
<th>ANG II</th>
<th>ANG II+BQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBF, mL · min⁻¹ · 1.73 m²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>1050±77</td>
<td>1080±94</td>
<td>1077±76</td>
</tr>
<tr>
<td>30'</td>
<td>1061±84</td>
<td>1012±89‡</td>
<td>1062±82</td>
</tr>
<tr>
<td>60'</td>
<td>1040±70</td>
<td>875±116†</td>
<td>1031±74*</td>
</tr>
<tr>
<td>90'</td>
<td>1066±78</td>
<td>801±52†</td>
<td>993±79‡</td>
</tr>
<tr>
<td>GFR, mL · min⁻¹ · 1.73 m²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>116±8</td>
<td>115±7</td>
<td>115±7</td>
</tr>
<tr>
<td>30'</td>
<td>115±7</td>
<td>114±8</td>
<td>113±7</td>
</tr>
<tr>
<td>60'</td>
<td>115±9</td>
<td>105±8†</td>
<td>113±8</td>
</tr>
<tr>
<td>90'</td>
<td>116±7</td>
<td>97±7†</td>
<td>105±7‡</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>86.0±3.5</td>
<td>86.8±3.1</td>
<td>86.2±3.6</td>
</tr>
<tr>
<td>30'</td>
<td>87.1±3.9</td>
<td>89.4±4.7</td>
<td>86.9±3.9</td>
</tr>
<tr>
<td>60'</td>
<td>85.9±4.1</td>
<td>92.2±4.1†</td>
<td>88.3±4.3</td>
</tr>
<tr>
<td>90'</td>
<td>86.4±3.6</td>
<td>97.5±4.4†</td>
<td>91.1±4.3‡</td>
</tr>
<tr>
<td>RVR, mm Hg · min⁻¹ · L⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>82±8</td>
<td>83±7</td>
<td>81±7</td>
</tr>
<tr>
<td>30'</td>
<td>82±7</td>
<td>91±8†</td>
<td>84±7</td>
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<tr>
<td>60'</td>
<td>83±9</td>
<td>113±15†</td>
<td>86±7</td>
</tr>
<tr>
<td>90'</td>
<td>82±8</td>
<td>133±20†</td>
<td>95±9‡</td>
</tr>
<tr>
<td>FF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>0.196±0.020</td>
<td>0.188±0.017</td>
<td>0.185±0.015</td>
</tr>
<tr>
<td>30'</td>
<td>0.192±0.019</td>
<td>0.199±0.018†</td>
<td>0.187±0.015</td>
</tr>
<tr>
<td>60'</td>
<td>0.196±0.023</td>
<td>0.218±0.025†</td>
<td>0.192±0.016</td>
</tr>
<tr>
<td>90'</td>
<td>0.194±0.026</td>
<td>0.220±0.030†</td>
<td>0.186±0.016</td>
</tr>
<tr>
<td>UNaV, µmol/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>212±9</td>
<td>202±10</td>
<td>203±11</td>
</tr>
<tr>
<td>30'</td>
<td>219±7</td>
<td>151±9*</td>
<td>159±13‡</td>
</tr>
<tr>
<td>60'</td>
<td>200±11</td>
<td>97±13*</td>
<td>116±9‡</td>
</tr>
<tr>
<td>90'</td>
<td>205±10</td>
<td>64±10*</td>
<td>75±13‡</td>
</tr>
<tr>
<td>Plasma endothelin-1, pg/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>0.82±0.03</td>
<td>0.86±0.04</td>
<td>0.81±0.02</td>
</tr>
<tr>
<td>30'</td>
<td>0.85±0.05</td>
<td>0.80±0.06</td>
<td>0.80±0.03</td>
</tr>
<tr>
<td>60'</td>
<td>0.80±0.06</td>
<td>0.79±0.07</td>
<td>0.85±0.04</td>
</tr>
<tr>
<td>90'</td>
<td>0.79±0.05</td>
<td>0.83±0.07</td>
<td>0.89±0.06</td>
</tr>
</tbody>
</table>

*Rate of AngII infusion increasing from 0.625, 1.25 to 2.5 ng · kg⁻¹ · min⁻¹. b indicates baseline; 30', 60', 90' indicate 30-minute clearance periods.

Significant differences (*P*<0.05 to <0.001): *vs b; †vs AngII+BQ; ‡vs BQ alone. (For individual *P* values, see Results section.)
Renal hemodynamic changes after the lowest rate of Ang II (0.625 ng/kg per minute) were fully prevented by blockade of ETA receptors with coinfused BQ, whereas fall in UNaV was unaffected.

At the rate of 1.25 ng/kg per minute Ang II+BQ, RBF declined significantly (P<0.05 versus b) but significantly less than with Ang II alone (P<0.01). Changes in GFR, MAP, RVR, and FF were fully prevented.

At the highest rate of 2.5 ng/kg per minute, Ang II+BQ, significant changes in RBF (P<0.001 versus b), GFR (P<0.05), MAP (P<0.05), and RVR (P<0.001) were detectable. Such variations, however, although still significant versus BQ alone (P<0.05 for all), also were significantly smaller than with Ang II alone (P<0.001 for RBF, MAP, and RVR; P<0.01 for GFR). FF did not change with Ang II+BQ. UNaV fell to the same extent than with Ang II alone. Neither BQ nor Ang II nor their combination were followed by changes in plasma ET-1.

**Discussion**

In the present study, healthy humans were studied on an elevated sodium intake to inhibit as much as possible endogenous Ang II production. It is also known that renal vascular responses to infused Ang II is altered by changes in sodium intake, with much less vasoconstriction when subjects are placed on a low sodium diet.\(^{27}\) Previous human studies performed under similar experimental conditions have shown that plasma Ang II may increase from 15 to 57 pmol/L after a 2-hour infusion of 1.5 ng/kg per minute\(^ {29}\) or from 20 to 25 to 50 to 70 pmol/L with stepwise increasing doses of Ang II from 0.3 to 3.0 ng/kg per minute each for 30 minutes.\(^ {28}\) Such levels of plasma Ang II are roughly the same observed in human pathophysiological conditions such as CHF\(^ {31}\) and renovascular hypertension.\(^ {32}\) Thus, although we could not measure plasma Ang II, our infusion protocol was designed in the assumption that a stepwise increasing rate of 0.625 to 2.5 ng/kg per minute Ang II would have produced a progressive elevation in plasma Ang II from low baseline levels toward physiological and, later, pathophysiological concentrations. Accordingly, at the lowest rate of Ang II infusion, renal vasocostriction but no change in GFR took place. The consequent increased FF indicates a selective effenter vasoconstriction by low doses of Ang II.\(^ {1,2,27–29}\) In addition, MAP remained unchanged, not only confirming the well-known sensitivity of the kidney vasculature to Ang II\(^ {1,2}\) but also ruling out any unspecific autoregulatory renal vasoconstriction in response to an increased renal perfusion pressure. Conversely, such an effect cannot be excluded in the two subsequent steps of Ang II infusion, in which MAP increased substantially, RBF declined further and GFR fell. FF, however, continued to increase progressively, still indicating the effenter arteriole as the main site of Ang II vasoconstriction. UNaV declined substantially even at the lowest Ang II infusion rate, then fell by \(\approx70\%\) at the highest rate. ET-1 concentration was not affected by Ang II infusion. This is in keeping with previous human studies\(^ {33}\) indicating a lack of regulation of plasma ET-1 by Ang II, although in vitro investigations with human cultured cells or tissues have clearly shown that Ang II promotes both synthesis and release of ET-1 by endothelial cells.\(^ {3,17,33}\)

The main finding in the present study was that blockade of ETA receptors markedly attenuated pressor and renal vasoconstrictor actions of Ang II, without significant effects on Ang II-induced reduction in UNaV.

ETA blockade was obtained by infusing systemically the peptide antagonist BQ at a rate devoid of any systemic and renal hemodynamic action at baseline, as previously found by us\(^ {19}\) and others.\(^ {7,8}\) Comparable amounts of BQ produced systemic vasodilation in both CHF\(^ {31}\) and patients with liver cirrhosis\(^ {20}\) with presumably activated ET, and RAS systems. In addition, BQ prevented in humans renal vasoconstriction caused by either infused ET-1\(^ {8,9}\) or NO synthase inhibition.\(^ {19}\) Thus, doses of BQ as used in the present study should ensure a substantial blockade of ETA receptor.

When ETA blockade was combined with Ang II infusion, not only renal vasoconstriction was largely prevented, but also changes in MAP were blunted, indicating therefore that Ang II–ET-1 interaction is involved in the Ang II systemic vasoconstriction as well. On the other hand, during Ang II plus BQ infusion, a relatively lower renal perfusion pressure may have contribute unspecifically to the lesser degree of renal vasoconstriction. However, the rise in both RVR and FF was prevented in the absence of any difference in MAP as observed at 0.625 ng/kg per minute Ang II. In addition, FF did not rise with Ang II+BQ, even when significant renal vasoconstriction still took place, as it was at 2.5 ng/kg per minute Ang II. Our findings indicate therefore that ETA receptors mediate substantially Ang II vasoconstriction in the
kidney, through an interaction located just at the efferent arteriolar side of glomerulus.

Studies of ET-1 infusion in humans have shown that GFR fell to a significantly lesser extent than ERPF, whereas the resulting rise in FF was fully prevented by BQ7 or the oral nonpeptide ET$_A$ antagonist ABT-627. Furthermore, BQ largely prevented both renal vasoconstriction and rise in FF mediated in humans by endogenous ET-1 left unopposed by inhibition of NO synthesis. These studies indicate collectively that ET$_A$ receptors at the level of efferent arteriole mediate both ET-1 and Ang II vasoconstriction. Taking into account the range of plasma Ang II concentrations generally reached with infusion protocols such as adopted in our study, one can hypothesize that of ET$_A$ receptors also participate in hemodynamic regulation under physiological or pathophysiological conditions of endogenously activated RAS. On the other hand, because of the high sodium diet adopted in our study, the possibility cannot be ruled out that ET-1–Ang II interaction is no longer detectable at a lower sodium intake, despite an activated endogenous RAS, as it can be suggested by the blunted action of ET$_A$ blockade on changes in MAP in Ang II–infused, sodium-restricted rats.

At variance of previous human studies in which antinatriuresis after ET-1 infusion was effectively blunted by ET$_A$ blockade, BQ did not affect the decline in UNaV secondary to Ang II. It is generally believed that sodium retention after ET-1 infusion in humans results merely from renal vasoconstriction, which should obscure the specific natriuretic action of ET-1 caused by stimulation of NO production through ET$_B$ receptors. Thus, ET$_A$ blockade during ET-1 infusion might result in a blunted antinatriuresis as the result of simultaneous reduction in vasoconstriction, unmasking of ET$_B$-mediated natriuresis, and increased availability of ET-1 displaced from ET$_A$ receptors for ET$_B$ activation. On the contrary, Ang II is known to stimulate directly proximal tubular sodium reabsorption through an AT$_1$ receptor–mediated, highly sensitive mechanism independent of hemodynamics. If we assume that direct tubular Ang II action is predominant in reducing UNaV, it is not surprising that UNaV with Ang II+BQ fell to the same extent as that with Ang II alone.

Disparate mechanisms are potentially involved in the interaction between Ang II and ET-1. Although Ang II stimulates ET-1 synthesis in endothelial cells, several hours are required for this effect, whereas ET$_A$ blockade blunts very rapidly the systemic or renal actions of Ang II in our healthy humans as well as in rats. However, release of ET-1 takes place within minutes from in vitro vessels or human vascular cultured cells exposed to Ang II, implying that preformed ET-1 is stored in cells and that its release may be Ang II–dependent. Such a mechanism cannot, therefore, be excluded in our human model despite no change in plasma ET-1 levels. In fact, plasma ET-1 may not reflect an increase in its tissue concentration due to the preferential release of peptide on basolateral side of endothelial cells. Alternatively, blockade of the single receptor subtype A could amplify the actions of the unblocked receptor subtype B, the activation of which by endogenous ET-1 displaced from ET$_A$ receptors may result in overproduction of NO able to partially offset vasoconstriction from Ang II. This mechanism, however, is unlikely in our study, because BQ has been shown to displace significantly ET-1 from ET$_A$ receptors. Finally, because both ET-1 and Ang II signal through multiple common intracellular pathways, endogenous ET-1 also could act as a “primer” of intracellular cascade activated by Ang II. Such an interaction may be of particular interest because it should be conceivably operating even without important stimulation of endogenous ET-1 system as in the present human study.

To summarize, our results demonstrate in healthy humans an important contribution of ET-1 system, through activation of ET$_A$ receptors, to the systemic, and, more importantly, renal hemodynamic response to exogenous Ang II. The precise mechanism responsible for the substantial mediation by ET$_A$ receptors of Ang II renal vasoconstriction in human kidney remains obscure. Because ET$_A$ receptors and Ang II appear to interact into (or very close to) a physiological to pathophysiological range of plasma Ang II concentrations, our findings support the hypothesis that such an interaction could play a role under human pathophysiological conditions of activation of both RAS and ET-1 system.

Since the pathophysiological role of ET-1 as a potent endogenous vasoactive substance has been established, numerous peptide and orally active, nonpeptide ET-1 receptor antagonists have been introduced for research and clinical purposes, pointing to a potential role of these drugs in a variety of renal and cardiovascular diseases. Beneficial effects of ET-1 antagonists in humans have been reported mainly in CHF, in which recent trials with selective ET$_A$ or mixed ET$_A$/ET$_B$ blockers also appear to indicate that ET-1 receptor antagonism may improve substantially prognosis, even in association with or superimposed to a standard ACEI treatment.

The results the present study may contribute to explain why a further hemodynamic improvement follows ET-1 blockade in the presence of a presumed effective RAS blockade. If we hypothesize that RAS may be inhibited only partially by standard ACEI or AIIRA therapy in CHF in the presence of an activated ET-1 system, amplification of Ang II effects by ET$_A$ receptors activated by an excess of ET-1 could not be prevented.

Our data in healthy humans support the concept of a potential usefulness of ET-1 antagonism in association with RAS blockade in the management of clinical conditions of activation of both systems such as CHF and hypertensive or renal diseases. Caution should be taken, however, in extending our results of acute studies of Ang II infusion combined with ET$_A$ blockade in young healthy individuals to chronic conditions, such as CHF, with marked activation of all vasoconstricting and sodium-retaining systems. Further studies are needed to investigate the contribution of the interaction between ET-1 and Ang II in patients treated with ACEI or AIIRA.

Perspectives

The ET-1 system, through ET$_A$ receptors, appears to mediate significantly some of the renal hemodynamic actions of infused Ang II in normal human kidney. We could assume
that such an interaction also operates under pathophysiological conditions in which endogenous RAS and ET1 systems are stimulated. More work is needed in the view to ascertain the impact of relations between RAS and ET-1 systems in human diseases.

The main perspective message coming from the present study has obvious clinical and therapeutic relevance and pertains the rationale of an association of ET-1–blocking agents and RAS-inhibiting drugs in humans with cardiovascular and renal diseases.

References

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