Interaction Between Nitric Oxide and Endogenous Vasoconstrictors in Control of Renal Blood Flow

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Abstract—The level of renal blood flow (RBF) is controlled by opposing vasoconstrictor and vasodilator influences. In a recent investigation in normotensive dogs, we found that combined blockade of endothelin type A (ET\textsubscript{A}) receptors and angiotensin II formation induces marked increases in RBF that were much larger than the effects of blocking either system alone. The aim of the present study was to determine the contribution of nitric oxide (NO) to this vasodilator response. Experiments were made in 6 conscious, chronically instrumented dogs subjected to 5 different experimental treatments on separate days. Blockade of ET\textsubscript{A} receptors alone by the selective antagonist LU 135252 had only minor effects on RBF compared with time-control experiments. Additional blockade of angiotensin II formation by angiotensin-converting enzyme inhibition with trandolaprilat caused a substantial increase of RBF by \(\approx\)50%. This vasodilatation was entirely suppressed when NO formation was prevented by inhibition of NO synthase with \(N\textsuperscript{G}\)-nitro-L-arginine methyl ester HCl. However, when during NO synthase inhibition renal vascular NO concentrations were clamped at control levels by infusing the NO donor \(S\)-nitroso-\(N\)-acetyl-d,L-penicillamine, the vasodilator response to combined blockade of ET\textsubscript{A} receptors and angiotensin II formation was completely restored (\(\Delta\text{RBF} \approx\)60%). These results indicate that the vasodilatation after combined ET\textsubscript{A} receptor blockade and angiotensin-converting enzyme inhibition is not mediated by an increase in NO release but results from the unmasking of the tonic influence that is normally exerted by constitutively released NO. Accordingly, the tonic activity of endothelial NO synthase appears to be of major importance in the physiological regulation of renal vascular resistance by determining the vasomotor responses to endothelin and angiotensin II. (Hypertension. 1999;34:1254-1258.)

Key Words: endothelin \textsubscript{A} receptors, endothelin \textsubscript{A} nitric oxide \textsubscript{B} renal circulation \textsubscript{A} renin-angiotensin system

Under physiological conditions, renal blood flow (RBF) is maintained at its normal level by a balance of opposing vasoconstrictor and vasodilator influences.\textsuperscript{1} Besides the renal nerves, angiotensin II (Ang II) and endothelin-1 (ET-1) are the most potent renal vasoconstrictors. The renal circulation is particularly sensitive to small changes in Ang II plasma concentrations even within the physiological range,\textsuperscript{2,3} and chronic infusions of low doses of Ang II cause sustained reductions of RBF and increases in systemic blood pressure in dogs.\textsuperscript{4,5} The physiological function of ET-1 in the regulation of renal hemodynamics is less clear; under pathophysiological conditions, it apparently can assume a major vasoconstrictor role and contribute to end-organ damage.\textsuperscript{6-10}

In a recent investigation in normotensive dogs, we have observed that combining angiotensin-converting enzyme (ACE) inhibition and endothelin type A (ET\textsubscript{A}) receptor blockade caused a pronounced renal vasodilation, whereas blockade of either system alone had only minor effects on RBF.\textsuperscript{11} These findings suggest that Ang II and ET-1 closely interact in the normal control of RBF. The mechanism underlying the increase in RBF after blockade of both systems, however, is still unclear. In the renal circulation, the vasoconstrictor effects elicited by Ang II and ET-1 are tonically antagonized by nitric oxide (NO) produced by endothelial cells.\textsuperscript{1} Accordingly, after withdrawal of the vasoconstrictor tone elicited by Ang II and ET-1, the vasodilator influence of constitutively released NO may predominate, thus inducing a pronounced increase in RBF. Alternatively, during ET\textsubscript{A} receptor blockade, the local release of NO may be stimulated by an enhanced binding of ET-1 to endothelin type B (ET\textsubscript{B}) receptors.\textsuperscript{12-14} This latter mechanism has recently been identified in the human forearm circulation, where the vasodilator response to ET\textsubscript{A} receptor blockade was nearly completely abolished by either ET\textsubscript{B} receptor blockade or by preventing changes in NO release.\textsuperscript{15} Because the renal circulation is particularly sensitive to the vasoconstrictor effects of Ang II and because acute ET\textsubscript{A} receptor blockade induces a substantial stimulation of the renin-angiotensin system,\textsuperscript{16} the effects of an increased formation of NO on RBF may become apparent only after additional ACE inhibition.\textsuperscript{11}

To determine the relative contribution of the constitutive and the stimulated mode of NO release to vasodilation after blockade of the ET and renin-angiotensin system, we investigated the effects of combined blockade of ET\textsubscript{A} receptors and...
Ang II formation in the presence of NO synthase activity, after inhibition of NO formation, and after the clamping of renal vascular NO concentrations at control levels.

Methods

Animals

Experiments were performed in 6 conscious foxhounds of either gender weighing 29 to 38 kg. The dogs received a commercially available dog diet (SNNiff Spezialdiäten GmbH) containing ~100 mEq sodium per day and had free access to water. At least 10 days before the first experiment was performed, each animal was instrumented with a chronic catheter in the abdominal aorta and a renal flow probe. Details of the implantation surgery and postoperative care have been described previously. All interventions and experiments were performed in accordance with the national law for the care and use of research animals.

Circulatory Measurements and Blood Sampling

Blood pressure was measured via the catheter in the abdominal aorta by use of Statham pressure transducers (P23Db) and Gould pressure processors. Heart rate (HR) was recorded instantaneously with a rate meter (Gould pressure processor). RBF was measured with the implanted ultrasound transit-time flow probe (6-mm diameter; Transonic Systems) connected to a flowmeter (Transonic T 106 or T 108). The flow probe signals were passed through a 10-Hz filter (Transonic). An analog recorder (Gould 2600) was used to display directly the measured variables. All data were sampled at 20 Hz and stored as 1-second mean values on-line (IBM PC 386) after analog-to-digital conversion.

Drugs

LU 135252 is a nonpeptide, selective ETA receptor antagonist with a plasma half-life in dogs of ~12 hours. The selectivity for ETA receptors, expressed as the ratio of the affinities for ETA over ETB receptors, is 131. LU 135252 was dissolved in 10 mL saline and given slowly as a bolus (10 mg/kg IV for 5 minutes). In anesthetized dogs, this dose completely inhibits the vasoconstrictor response to an intravenous injection of 0.75 nmol/kg ET-1, which increases plasma ET-1 concentrations into the nanomolar range, ie, 100- to 1000-fold higher than normal.18

The ACE inhibitor trandolaprilat (2 mg/kg IV) was used to inhibit the formation of Ang II. This dose was found to significantly suppress the pressor response to exogenous angiotensin I by 74% (6 SEM, 74% SEM, P<0.05) 2 hours after the administration of trandolaprilat.18

NO synthase inhibition was induced by intravenous bolus infusion of N'-nitro-L-arginine methyl ester HCl (L-NAME, Sigma Chemical Co) at a dose of 50 mg/kg dissolved in 10 mL normal saline. The NO donor SNAP was infused at a rate that restored mean RBF to the level observed during the initial period before the start of the experiment before L-NAME was given.

Experimental Protocols

All experiments were performed in 6 trained, conscious dogs lying quietly on their right sides on a bench. The experiments started between 8 and 9 AM, 16 to 20 hours after the last feeding. The dogs were connected to the recording instruments via extension cables, and mean arterial pressure (MAP), HR, and RBF were measured for 20 minutes before starting the experimental intervention. After the experimental intervention was completed, MAP, HR, and RBF were measured for another 120 minutes. At least a 2-day interval was left between each experiment in individual dogs. Experiments were performed in random order. Each dog was subjected to 5 different treatments, as described below.

Control

The dogs received an intravenous bolus infusion of 10 mL normal saline.

Results

ETA Receptor Blockade

The effects of selective ETA receptor blockade on RBF compared with time-control experiments are shown in Figure 1. The ETA receptor antagonist was administered intravenously at time 0. RBF started to increase 20 minutes after induction of the ETA receptor blockade and was 13% higher (309±37 versus 249±34 mL/min) than after normal saline administration by the end of the observation period. Even though this difference failed to reach statistical significance,

Figure 1. Effects of acute ETA receptor blockade on RBF in conscious dogs. Depicted are mean values of RBF under control conditions (○), during blockade of ETA receptors by LU 135252 (●), and during combined blockade of ETA receptors and Ang II formation (○, LU 135252 plus trandolaprilat). *P<0.05 compared with control. Values represent mean±SEM (n=6 for each protocol).
the changes were quantitatively very similar to those observed in a previous study from our laboratory\textsuperscript{11} in a different group of dogs. Neither MAP nor HR was significantly altered by the end of the observation period after blockade compared with time-control experiments (Table).

**Combined Blockade of ET\textsubscript{A} Receptors and Ang II Formation**

Additional blockade of Ang II formation by ACE inhibition induced a substantial renal vasodilation (Figure 1). In contrast to ET\textsubscript{A} receptor blockade alone, RBF started to rise immediately after the experimental intervention (time 0) and reached a plateau after \(\sim60\) minutes. Similar to ET\textsubscript{A}, receptor blockade alone, MAP tended to decrease, whereas HR significantly increased (Table).

**Effect of NO Synthase Inhibition**

To investigate whether NO contributes to this renal vasodilation, NO synthase was inhibited by L-NAME before the ET\textsubscript{A} receptor antagonist and the ACE inhibitor were administered. L-NAME rapidly reduced RBF (from 283±35 to 194±22 mL/min, \(P<0.05\); Figure 2) and HR (from 78±5 to 45±3 bpm, \(P<0.05\)) and moderately increased MAP (from 84±2 to 97±3 mm Hg, \(P<0.05\)). Additional blockade of ET\textsubscript{A} receptors and Ang II formation after L-NAME had almost no further effect on RBF, which remained at \(\sim200\) mL/min until the end of the observation period. Similarly, MAP and HR remained at the levels attained after L-NAME (Table).

**Effect of Clamping NO Levels**

Because ET\textsubscript{A} receptors are coupled to NO synthase by a tyrosine kinase,\textsuperscript{13} it is conceivable that during blockade of ET\textsubscript{A} receptors, ET-1 preferentially binds to ET\textsubscript{B} receptors to stimulate NO formation,\textsuperscript{12,14,15} which may contribute substantially to the renal vasodilation after ET\textsubscript{A} receptor blockade and ACE inhibition. To test this hypothesis, the NO clamp technique was used: after inhibition of endogenous formation of NO by L-NAME, the NO donor SNAP was infused at a constant rate that restored RBF to the baseline level measured before administration of L-NAME. Implementation of the NO clamp slightly decreased MAP and moderately elevated HR (Table). Contrary to the hypothesis, combined blockade of ET\textsubscript{A} receptors and Ang II formation during clamped renal vascular NO levels induced a marked increase in RBF, which was quantitatively not different from the response in the presence of intact NO synthase (Figures 2 and 3). MAP fell further (to 63±5 mm Hg) in response to ET\textsubscript{A} receptor blockade and ACE inhibition (\(P<0.05\)), whereas HR increased to 112±10 bpm (\(P<0.05\)), suggesting that ET-1 and Ang II significantly contribute to total peripheral resistance during NO clamp conditions (Table).

**Discussion**

The results of the present study indicate that tonically released NO is essential for the renal vasodilator response after acute blockade of the influences of ET-1 and Ang II. Furthermore, because clamping of renal vascular NO levels by blockade of NO synthase and infusion of the NO donor SNAP completely restored the vasodilator response, a contribution of a stimulated formation of NO to the increase in RBF seems very unlikely. Thus, the renal vasodilation after combined ET\textsubscript{A} receptor blockade and ACE inhibition appears to result from an unmasking of the influence of constitutively released NO.

The most unexpected finding of the present study was that combined ET\textsubscript{A} receptor blockade and ACE inhibition induced a renal vasodilator response only if the renal vessels were already dilated by NO. Previous studies have shown that the
Because the rate of SNAP infusion was adjusted to return the mean level of RBF to baseline values measured before NO inhibition of NO synthase. In preliminary time-control experiments of ET-1 and Ang II was completely restored when NO present observations at the cellular level.

Inhibition of ET A receptors and Ang II formation. When vessels are preconstricted. Therefore, the influence of endogenously generated ET-1 and Ang II should be more pronounced after inhibition of NO synthase; consequently, their blockade should have had larger effects than with an intact NO production. By contrast, inhibition of NO release by L-NAME completely eliminated the vasodilator response to combined blockade of ET A receptors and Ang II formation. The mechanisms underlying this paradoxical response are not clear. Because NO has been shown to attenuate the myogenic response in resistance vessels, the latter may overshoot during NO synthase inhibition, accounting nearly completely for the vasoconstriction induced by L-NAME. Alternatively, NO may modulate a common signal transduction step downstream from the effects of Ang II and ET-1, which renders the vascular smooth muscle cell unresponsive to changes in Ang II and ET-1 within the physiological range if NO levels are very low. This latter explanation, however, is entirely speculative, and further experiments are necessary to explain the present observations at the cellular level.

Figure 3. Comparison of the effects of the different experimental interventions on RBF. Depicted are mean values of RBF obtained during the last 20 minutes of the recording periods, ie, 100 to 120 minutes after the experimental treatment. Whereas blockade of ET A receptors alone (hatched bar) had only minor effects on RBF compared with the time control (open bar), additional blockade of Ang II formation (light gray bar) caused a marked increase in RBF. This vasodilation was entirely suppressed during inhibition of NO formation by L-NAME (dark gray bar). Clamping of renal vascular NO concentrations at control levels by supplementing NO exogenously via infusion of the NO donor SNAP during NO synthase inhibition (filled bar) completely restored the vasodilator response to combined blockade of ET A receptors and Ang II formation. *P<0.05 compared with control. Values represent mean±SEM.

The vasodilator response to acute blockade of the influences of ET-1 and Ang II was completely restored when NO was substituted by a constant infusion of SNAP during inhibition of NO synthase. In preliminary time-control experiments, we found that infusions of SNAP alone did not cause a progressive renal vasodilatation in dogs treated with L-NAME. This demonstrates that the increase in RBF is a specific response to the administration of the ET A receptor blocker and ACE inhibitor. In contrast to RBF, MAP consistently fell after clamping of NO levels, indicating that systemic levels of NO were elevated above normal values. Because the rate of SNAP infusion was adjusted to return the mean level of RBF to baseline values measured before NO synthase inhibition, the different systemic and renal hemodynamic responses suggest that local NO concentrations are particularly high in the renal vascular bed, which is in line with previous reports. After additional combined ET A receptor blockade and ACE inhibition, MAP was reduced by approximately 20 mm Hg from control values. This hypotensive response was associated with an elevated HR, most likely because of an inhibition of the baroreceptor reflex. Baroreflex-mediated increments in sympathetic nerve activity can shift the lower limit of RBF autoregulation by 20 to 25 mm Hg to higher renal perfusion pressures. Therefore, the increase in RBF after combined antagonism and clamped NO levels may have been underestimated. It should be noted, however, that autoregulation of RBF is completely preserved down to 60 to 65 mm Hg in dogs even after combined ET A receptor blockade and ACE inhibition.

In contrast to the present observations in the renal circulation, the increase in forearm blood flow induced by ET A receptor antagonism was substantially attenuated during clamped NO levels in humans. In the same study, a similar attenuation was observed when, in addition to ET A receptors, ET B receptors were blocked. This latter finding indicates that in the human forearm circulation an additional stimulation of NO release by ET B receptor activation is required for the vasodilator response induced by ET A receptor antagonism. Even though the experimental protocols were not identical and species differences cannot be excluded, these discrepant responses may indicate important differences in the role played by NO in the kidney and in the muscle circulation. In support of this interpretation, the renal circulation has been consistently found to be much more sensitive to acute inhibition of NO synthase than other circulatory beds.

Taken together, the present results suggest that tonically released NO, in addition to its well-established function in setting the mean level of RBF, is of major importance in the physiological regulation of renal vascular resistance by determining the renal vasomotor responses to vasoconstrictor influences. Accordingly, one might expect that the gene for endothelial NO synthase cosegregates with renal hemodynamic abnormalities or elevated blood pressure, but all previous studies have failed to reveal such an association in experimental models of hypertension or in the human population. It should be noted, however, that in the Dahl salt-sensitive rat, the gene loci for the α1, and β1 subunits of the soluble guanylyl cyclase were found to be closely linked to chromosomal regions that have been previously shown to cosegregate with blood pressure. Thus, further studies may identify genetic alterations in the signaling cascade that lie downstream from NO synthase.

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References


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