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

Heike Berthold, Armin Just, Hartmut R. Kirchheim, Heimo Ehmke

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_{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_{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 pronounced renal vasodilation, whereas blockade caused a pronounced renal vasodilation, whereas blockade of either system alone had only minor effects on RBF. These findings suggest that Ang II and ET-1 closely interact in the normal control of RBF. Alternatively, the vasodilation after combined ET_{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 receptors, endothelin nitric oxide renal circulation 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.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,2,3 and chronic infusions of low doses of Ang II cause sustained reductions of RBF and increases in systemic blood pressure in dogs.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.6–10

In a recent investigation in normotensive dogs, we have observed that combining angiotensin-converting enzyme (ACE) inhibition and endothelin type A (ET_{A}) receptor blockade caused a pronounced renal vasodilation, whereas blockade of either system alone had only minor effects on RBF.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.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_{A} receptor blockade, the local release of NO may be stimulated by an enhanced binding of ET-1 to endothelin type B (ET_{B}) receptors.12–14 This latter mechanism has recently been identified in the human forearm circulation, where the vasodilator response to ET_{A} receptor blockade was nearly completely abolished by either ET_{A} receptor blockade or by preventing changes in NO release.15 Because the renal circulation is particularly sensitive to the vasoconstrictor effects of Ang II and because acute ET_{A} receptor blockade induces a substantial stimulation of the renin-angiotensin system,16 the effects of an increased formation of NO on RBF may become apparent only after additional ACE inhibition.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_{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 (SSniff 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 ET_A receptor antagonist with a plasma half-life in dogs of ~12 hours. The selectivity for ET_A receptors, expressed as the ratio of the affinities for ET_A over ET_B receptors, is 131.17 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 mmol/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% 2 hours after the administration of trandolaprilat.18 NO synthase inhibition was induced by intravenous bolus infusion of 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 at a constant rate of 2 to 4 mg/kg per minute in 12 mL/h normal saline. The rate of infusion was adjusted to restore RBF to the control level measured before L-NAME. In preliminary time-control experiments, which were performed in 8 dogs during an earlier study from our laboratory,19 we found that RBF remains stable with this procedure for at least 120 minutes (data not shown). After the final dose adjustment of SNAP infusion, combined blockade of ET_A receptors and Ang II formation was induced by administration of LU 135252 (10 mg/kg IV) and trandolaprilat (2 mg/kg IV).

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

ET_A Receptor Blockade

The effects of selective ET_A receptor blockade on RBF compared with time-control experiments are shown in Figure 1. The ET_A receptor antagonist was administered intravenously at time 0. RBF started to increase 20 minutes after induction of the ET_A 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,
the changes were quantitatively very similar to those observed in a previous study from our laboratory,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 ETA Receptors and Ang II Formation**

Additional blockade of Ang II formation by ACE inhibition induced a substantial renal vasodilation (Figure 1). In contrast to ETA receptor blockade alone, RBF started to rise immediately after the experimental intervention (time 0) and reached a plateau after 60 minutes. Similar to ETA 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 ETA 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 ETA receptors and Ang II formation after L-NAME had almost no further effect on RBF, which remained at ~200 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 ETA receptors are coupled to NO synthase by a tyrosine kinase,13 it is conceivable that during blockade of ETA receptors, ET-1 preferentially binds to ETA receptors to stimulate NO formation,12,14,15 which may contribute substantially to the renal vasodilation after ETA 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 ETA 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 ETA 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 ETA 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 ETA 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 systemic levels of NO were elevated above normal values. tentently fell after clamping of NO levels, indicating that blocker and ACE inhibitor. In contrast to RBF, MAP 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, α2, and β 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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