Plasma Renin Activity and the Renal Response to Nitric Oxide Synthesis Inhibition¹,²

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ABSTRACT
Inhibition of systemic endothelium-derived relaxing factor (EDRF) synthesis with L-N²-nitroarginine (L-NAME) results in decreased RBF, which can be reversed by acute blockade of angiotensin II (All). Because All is particularly elevated in the renal circulation, it was hypothesized that the degree of renal vasoconstriction produced by L-NAME in anesthetized rats is related to PRA. To test this, PRA was chronically increased or suppressed by the manipulation of dietary sodium (eating 0.03% sodium chow or deoxycorticosterone acetate plus drinking 1% NaCl, respectively). After 10 days, rats were anesthetized for determination of blood pressure (BP) and RBF before and after L-NAME (10 mg/kg body wt). In rats with high PRA (61.6 ± 10.4 ng of angiotensin I (AI) /mL/h; N = 8), L-NAME increased BP by 29 ± 2 mm Hg (from 110 ± 4 to 139 ± 5 mm Hg; P < 0.001), decreased RBF by 27% (from 7.9 ± 0.3 to 5.8 ± 0.3 mL/min/g kidney wt; P < 0.001), and increased renal vascular resistance (RVR) by 67% (from 14.5 ± 0.9 to 24.2 ± 1.1 resistance units (RU); P < 0.001). When rats with high PRA (N = 8) were treated with 10 mg/kg body wt of DuP 753, an All receptor antagonist, L-NAME similarly increased BP by 30 ± 5 mm Hg (from 81 ± 3 to 111 ± 5; P < 0.001) but RBF did not change and RVR increased by only 31% (from 10.9 ± 0.8 to 13.3 ± 0.7 RU; P < 0.005). In rats with low PRA (0.8 ± 0.1 ng of AI/h/mL; N = 8), L-NAME increased BP by 23 ± 2 mm Hg (from 104 ± 4 to 127 ± 3 mm Hg; P < 0.001); there was, however, no decrease in RBF, whereas RVR increased by only 30% (from 16.2 ± 0.9 to 21.1 ± 1.5 RU; P < 0.005). Therefore, neither chronically suppressing All nor acutely inhibiting it with DuP 753 reduced the systemic pressor response, whereas either manipulation greatly attenuated the decrease in RBF produced by L-NAME. These results suggest that, in the anesthetized rat, the renal but not the systemic response to EDRF synthesis inhibition is predominantly mediated by All, suggesting that a particular balance between EDRF and All maintains the renal circulation.

Key Words: Endothelium-derived relaxing factor, nitric oxide, l-nitroarginine methyl ester, PRA, RBF

The vascular endothelium plays an important role in the modulation of vascular tone because of the production of a number of endothelium-derived factors, in particular, nitric oxide, the endothelium-derived relaxing factor (EDRF) (1, 2). The importance of basal EDRF release in maintaining normal renal function has been demonstrated by a number of investigators. Inhibition of EDRF synthesis with either L-N²-nitroarginine (L-NAME) or N⁵-monomethyl-L-arginine results in increased blood pressure (BP), decreased RBF, and increased renal vascular resistance (RVR) (3–6). In addition to these renal hemodynamic changes, Baylis et al. (3) also demonstrated that after EDRF synthesis inhibition there is a disproportionately smaller decrease in GFR, resulting in an increased filtration fraction. Lahena et al. (7) have suggested that the renal circulation is particularly sensitive to EDRF in that they found that low doses of L-NAME producing only partial inhibition of EDRF synthesis increased RVR without a significant change in systemic BP. All of these findings suggest that, under basal conditions, tonic release of EDRF from the renal endothelium serves as an important regulator of RBF and renal function.

Previously, we have shown that inhibition of angiotensin II (All) with either converting enzyme inhibition or an All receptor antagonist dissociates the renal vascular response from the systemic effect of EDRF synthesis inhibition (8). Both of these pharmacologic manipulations blocked the decrease in RBF and attenuated the increase in RVR observed with L-NAME under control conditions but did not impair the systemic response to EDRF synthesis inhibition. These findings suggested that within the

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renal vasculature there is a unique interaction between the vasodilator influence of EDRF and the vasoconstictor influence of AI. In our model, barbiturate anesthesia induces a twofold to threefold increase in PRA (2 to 4 ng of angiotensin I [AI]/mL/h in conscious rats versus 9 to 10 ng of AI/mL/h in anesthetized rats); thus, changes in RBF in response to EDRF synthesis inhibition may be due to the enhanced participation of AI in the mediation of renal hemodynamics in this model. Acute pharmacologic inhibition of the renin-angiotensin system lowers BP by around 20 mm Hg. This reduction of renal perfusion pressure could suppress shear-mediated EDRF production independently of AI blockade. Our study was designed to chronically alter the level of PRA so as to eliminate the decline in BP seen after acute pharmacologic blockade of the renin-angiotensin system. For this reason, the activity of the renin-angiotensin system was modified to test whether chronically increasing or decreasing PRA would alter the interaction between angiotensin and EDRF in controlling renal hemodynamics. PRA was chronically elevated or suppressed by the placement of rats on either a low-(9, 10) or high-(9, 11) salt diet for 10 days, respectively.

MATERIALS AND METHODS

Male Sprague-Dawley rats (Charles River Laboratory, Wilmington, MA) were fasted overnight but were allowed free access to water. On the day of the experiment, they were anesthetized by an ip injection of 125 mg/kg body wt thiobutabarbital (Inactin; Andrew Lockwood Co., Milwaukee, WI) and placed on a heating pad to maintain constant body temperature. The rats were surgically prepared with a tracheostomy with PE 260 tubing (Fisher Scientific, Chicago, IL) for spontaneous breathing of room air. Then, the femoral artery was catheterized with PE 50 tubing to monitor femoral BP with a Statham pressure transducer and flow meter connected to a flow meter (Carolina Medical Electronics, King, NC). The flow probe was calibrated in vivo by treating the rat with heparin, cannulating the renal artery distal to the flow probe, and collecting timed blood samples. Absolute zero flow through the renal artery was determined by occluding the artery distal to the probe with shielded hemostats. The pressure transducer and flow meter were connected to a Gould chart recorder (Gould Inc., Valley View, OH) for simultaneous recording of RBF and BP.

PRA was measured by RIA of angiotensin I generation by a modification of the technique of Haber et al. (12) as described previously (13). Results are expressed as nanograms of angiotensin I/mL/h.

A bolus dose of 10 mg/kg body wt of L-NAME (Sigma Chemical Co., St. Louis, MO) was used to inhibit systemic EDRF synthesis. We have previously shown that this dose induces sustained inhibition of EDRF in the systemic and renal circulation (4).

The experiment was divided into four protocols, as described below.

Effect of EDRF Synthesis Inhibition by L-NAME on BP and RBF in Rats With Unmanipulated PRA

Eight anesthetized rats were prepared as described above. After surgery, the rats were allowed a 60-min recovery period during which BP and RBF were monitored. After this period, the rats received a bolus of 10 mg/kg body wt of L-NAME and changes in BP and RBF were monitored over 30 min, by which time these parameters had again stabilized.

Effect of EDRF Synthesis Inhibition by L-NAME in Rats With Chronically Elevated PRA

In eight rats, PRA was chronically elevated by the placement of the rats on a low-sodium diet (0.03% NaCl; Ralston Purina, Richmond, IN) for 10 days (9, 10). On the day of the experiment, the rats were prepared as described above. After surgery, the rats were allowed a 60-min recovery period during which BP and RBF were monitored. After this period, the rats received a bolus of 10 mg/kg body wt of L-NAME and changes in BP and RBF were monitored over 30 min, by which time these parameters had again stabilized.

Effect of EDRF Synthesis Inhibition by L-NAME in DuP 753-Pretreated Rats With Chronically Elevated PRA

In eight rats, PRA was chronically elevated by the placement of the rats on a low-sodium diet (0.03% NaCl; Ralston Purina) for 10 days (9, 10). On the day of the experiment, the rats were prepared as described above. After surgery, the rats were allowed a 30-min recovery period during which BP and RBF were recorded. After this period, the rats received 10 mg/kg of DuP 753 (DuPont Corp., Wilmington, DE), an AI receptor antagonist without agonistic properties (14), and the BP and RBF were monitored over 30 min. After this period, the rats received a bolus of 10 mg/kg body wt of L-NAME and BP and RBF were again recorded over 30 min, by which time these parameters had again stabilized.
Effect of EDRF Synthesis Inhibition by L-NAME in Rats With Chronically Suppressed PRA

In eight rats, PRA was chronically suppressed by the placement of the rats on a high-salt diet (1.0% NaCl in the drinking water) in the presence of deoxycorticosterone acetate (Sigma Chemical Co.) with RTV Silastic (Dow Corning Co., Midland, MI) implants (100 mg/kg body wt) for 10 days as described previously (9, 11). On the day of the experiment, the rats were prepared as described above. After surgery, the rats were allowed a 60-min recovery period during which BP and RBF were monitored. After this period, the rats received a bolus of 10 mg/kg body wt of L-NAME and changes in BP and RBF were monitored over 30 min, by which time these parameters had again stabilized.

Analysis

Blood flow to the left kidney was determined directly from the flow meter and normalized to flow per gram of kidney weight. BP and RBF were used to calculate RVR. Units for RVR are mm Hg per milliliter per minute per gram of kidney weight and will be designated hereafter as resistance units (RU). Analysis of comparisons between groups was performed by one-way analysis of variance and then was analyzed further by Dunnett’s t-test. We considered a P value (or an adjusted P value) of less than 0.05 to be statistically significant.

RESULTS

Effect of EDRF Synthesis Inhibition by L-NAME on BP and RBF in Rats With Unmanipulated PRA

The mean body weight of the eight rats used in these studies was 296 ± 13 g, and the mean left kidney weight was 1.33 ± 0.06 g. The PRA for this group of rats was 9.0 ± 1.6 ng of Al/mL/h.

The changes in BP, RBF, and RVR in response to L-NAME are shown in Figure 1. Basal BP was 104 ± 3 mm Hg, RBF was 7.6 ± 0.4 mL/min/g kidney wt, and RVR was 13.5 ± 0.7 RU. L-NAME significantly increased BP by 22 ± 1 mm Hg to 125 ± 1 mm Hg (P < 0.001). RBF decreased by 28% to 5.6 ± 0.4 mL/min/g kidney wt (P < 0.001), and RVR was increased by 70% to 23.0 ± 1.6 RU (P < 0.001).

Effect of EDRF Synthesis Inhibition by L-NAME in Rats With Chronically Elevated PRA

The mean body weight of the eight rats used in these studies was 258 ± 8 g, and the mean left kidney weight was 1.32 ± 0.07 g. The PRA of this group of rats was 61.6 ± 10.4 ng of Al/mL/h.

The changes in BP, RBF, and RVR in response to L-NAME are shown in Figure 1. Basal BP was 110 ± 4 mm Hg, RBF was 7.9 ± 0.3 mL/min/g kidney wt, and RVR was 14.5 ± 0.9 RU. L-NAME significantly increased BP by 29 ± 2 mm Hg to 139 mm Hg (P < 0.001). RBF decreased by 27% to 5.8 mL/min/g kidney wt (P < 0.001), and RVR increased by 67% to 24.2 ± 1.1 RU (P < 0.001).

Effect of EDRF Synthesis Inhibition by L-NAME in DuP 753-Pretreated Rats With Chronically Elevated PRA

The mean body weight of the eight rats used in these studies was 306 ± 11 g, and the mean left
kidney weight was 1.13 ± 0.03 g. The PRA of this group of rats was 81.0 ± 8.0 ng of AI/mL/h.

The changes in BP, RBF, and RVR in response to L-NAME are shown in Figure 2. The initial BP was 114 ± 4 mm Hg, RBF was 8.0 ± 0.4 mL/min/g kidney wt, and RVR was 14.8 ± 0.8 RU. DuP 753 lowered BP to 81 ± 3 mm Hg (P < 0.001). Although there was no significant change in RBF (from 8.0 ± 0.4 to 7.8 ± 0.6), RVR decreased to 10.9 ± 0.8 RU (P < 0.001). L-NAME significantly increased BP by 30 ± 3 mm Hg.

Effect of EDRF Synthesis Inhibition by L-NAME in Rats With Chronically Suppressed PRA

The mean body weight of the eight rats used in these studies was 304 ± 10 g, and the mean left kidney weight was 1.53 ± 0.06 g. The PRA of this group of rats was 0.8 ± 0.1 ng of AI/mL/h.

Changes in BP, RBF, and RVR in response to L-NAME are shown in Figure 3. Basal BP was 104 ± 4 mm Hg, RBF was 6.5 ± 0.3 mL/min/g kidney wt, and RVR was 16.2 ± 0.9 RU. L-NAME significantly increased BP by 23 ± 2 mm Hg to 127 ± 3 mm Hg (P < 0.001). There was no change in RBF in response to L-NAME (6.5 ± 0.3 versus 6.2 ± 0.4 mL/min/g kidney wt), while RVR increased by only 30% to 21.1 ± 1.5 RU (P < 0.001). This increase was significantly less than that observed after L-NAME in controls (P < 0.05).

DISCUSSION

Our results suggest that renal hemodynamics in barbiturate-anesthetized rats are largely mediated by the balance between EDRF and All. We found that the decrease in RBF we observed after EDRF synthesis inhibition could be completely abolished by acute pharmacologic blockade of All on chronic dietary suppression of the renin-angiotensin system. Thus, it would appear that, in our experimental model, EDRF balances the effect of endogenous All in the renal circulation. Ito et al. (15) have suggested that EDRF synthesis inhibition may result in increased sensitivity of the renal resistance vessels to All on the basis of their observations in isolated perfused renal afferent arterioles. Additionally, we observed that inhibition of the renal response to L-NAME occurred without diminution of the systemic pressor effect, suggesting that the influence of All is more important in the renal circulation than as a mediator of total peripheral resistance (TPR). On the other hand, chronic stimulation of the renin-angiotensin system resulted in a similar change in RBF and RVR compared with controls. This suggests that in control (anesthetized) rats, the intrarenal levels of All are high enough to cause maximum renal vasoconstriction after EDRF synthesis inhibition. This is in contrast to the peripheral circulation, where an increase in PRA is associated with a potentiation of the pressor response to EDRF synthesis inhibition. Thus, our results suggest that as circulating levels of All are increased, the role of EDRF in regulating BP and TPR...
Inhibition of EDRF synthesis in the renal or systemic circulation results in an increased perfusion pressure, decreased RBF, and increased RVR (2–4), while suppressor doses of L-NAME still increase RVR (7). In addition to decreased RBF, Baylis et al. (3) have demonstrated that EDRF synthesis inhibition resulted in a concurrent but smaller decrease in GFR, causing filtration fraction to increase. The administration of exogenous All causes similar changes in renal hemodynamics in a variety of species (19–21). This led us to speculate that, as the vasodilator influence of EDRF is deleted from the renal circulation, vasoconstriction results because of endogenous All, which circulates at particularly higher concentrations in the kidney. We have previously demonstrated that acute pharmacologic blockade of the renin-angiotensin system with either DuP 753 or enalaprilat partly abolishes the renal response but not the systemic pressor response to L-NAME (8). We suggested that the inhibition of EDRF synthesis unmasked an All-mediated renal vasoconstriction. However, acute inhibition of All in anesthetized rats results in a significant decrease in BP (8). Reduced renal perfusion should reduce shear stress, a stimulus for EDRF (2), and therefore might also account for the diminished renal response to L-NAME. Therefore, we used chronic suppression of the renin-angiotensin system in which BP is maintained to demonstrate that the renal response to L-NAME is a function of All rather than a function of the decreased renal perfusion pressure and diminished EDRF.

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Anesthesia modifies various regulatory reflex mechanisms, suppressing the central nervous system as well as increasing basal PRA. We observed a twofold to threefold increase in PRA after anesthesia. Thus, anesthesia may amplify the renal influence of All. In our experiments with rats in which the activity of the renin-angiotensin system was chronically suppressed, we found that the decrease in RBF seen after L-NAME was eliminated. However, in every case, we observed significant (although diminished) increases in RVR, suggesting that other vasoconstrictors besides angiotensin are involved in the renal vascular response to L-NAME. We have previously found that norepinephrine- or phenylephrine-induced vasoconstriction is particularly strong in L-NAME-treated rats (22), and it may be that the central nervous system accounts for the residual renal vasoconstriction; if so, such constriction could be more pronounced in the absence of general anesthesia. Regardless, the dissociation between the renal and systemic responses we observed with either acute or chronic suppression of the renin-angiotensin system illustrates the enhanced sensitivity of the renal circulation to the influence of All. Our results not only confirm our previous observations (8) but further demonstrate that, as with acute inh-
bition of the renin-angiotensin system, chronically suppressing PRA through dietary manipulation also attenuates the renal vascular response to EDRF synthesis inhibition. However, unlike the response to pharmacologic intervention, there is no decrease in basal systemic pressure. Similarly, Tolins and Raij (23), using a peptide AII antagonist that increased blood pressure compared with controls, demonstrated a qualitatively similar attenuation of the RBF response to EDRF synthesis inhibition, as we have demonstrated. Thus, we conclude that the renal vasoconstriction seen after EDRF synthesis inhibition in anesthetized rats is due predominantly (though not exclusively) to AII.

We observed that the systemic pressor response to EDRF synthesis inhibition in rats with high PRA was about 32% greater than that in controls, whereas the decrease in RBF and increase in RVR were the same. The increased pressor response in rats with high PRA could reflect an AII-mediated stimulation of EDRF. Increasing endogenous AII could induce EDRF synthesis by one of two mechanisms: either directly by increasing endothelial cell intracellular calcium or indirectly by increasing vascular resistance, resulting in increased shear stress. Both of these pathways have been shown to stimulate EDRF (2). It has been suggested that AII or its degradation products can stimulate EDRF release (24). This is supported by the work of Pegoraro et al. (22), who observed potentiation of the pressor response to L-NAME in pithed rats in which pressure was returned to normal levels with AII.

We also observed that the high-AIII rats treated with DuP 753 responded to L-NAME with an exaggerated pressor response. However, unlike the untreated high-AIII rats, the basal blood pressure was reduced by DuP 753. L-NAME returned BP only to the preblockade level. The amplified change in BP with L-NAME is probably due to reflex pressor signals other than AII that come into play when AII is abruptly blocked. We have previously found (22) that in pithed rats made normotensive with norepinephrine or phenylephrine, the pressor response to L-NAME is greatly exaggerated.

In summary, we have observed that EDRF synthesis inhibition with L-NAME results in decreased RBF and increased renal perfusion pressure. Suppressing PRA chronically with diet or acutely by pharmacologic manipulation dissociated the systemic pressor effect from the renal vascular response to L-NAME. This suggests that the decrease in RBF and increase in RVR seen after EDRF synthesis inhibition in anesthetized rats are largely mediated by AII. The fact that the pressor response was potentiated in rats with high PRA but not in controls or rats with low PRA suggests that chronic elevation of AII may have a more pronounced effect on systemic pressure than normal AII levels, perhaps serving as a stimulus for further EDRF release. These results complement our previous findings with acute pharmacologic manipulation (8) and suggest that the renal circulation is uniquely influenced by the interaction between the vasoconstrictor AII and the vasodilator EDRF in the regulation of renal hemodynamics.

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