Role of Nitric Oxide in Modulating the Long-term Renal and Hypertensive Actions of Norepinephrine

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Abstract We have previously reported that nitric oxide (NO) plays an important role in protecting the renal vasculature from acute norepinephrine-induced vasoconstriction. The purpose of this study was to determine the importance of this interaction between NO and norepinephrine in long-term control of renal hemodynamics and arterial pressure. To achieve this goal, we examined the effects of an intrarenal infusion of norepinephrine (NE) (0.1 μg kg⁻¹ min⁻¹) for 7 days in conscious, chronically instrumented control dogs and in dogs pretreated with a synthesis inhibitor, L-NAME (3 μg kg⁻¹ min⁻¹ intrarenally). Both groups of dogs also received captopril (15 μg kg⁻¹ min⁻¹) plus angiotensin II intravenously to clamp the renin-angiotensin system throughout the protocol. In control dogs (n=6), intrarenal infusion of NE decreased renal plasma flow by 9% (134±10 to 122±14 mL/min) and glomerular filtration rate by 16% (49±4 to 41±5 mL/min) while having no effect on mean arterial pressure (100±3 to 98±4 mm Hg). In marked contrast, in dogs pretreated with intrarenal L-NAME (n=9), NE decreased renal plasma flow by 37% (129±8 to 81±16 mL/min) and glomerular filtration rate by 52% (47±5 to 22±7 mL/min) while increasing mean arterial pressure from 104±5 to 113±6 mm Hg. The results of this study demonstrate that NO plays an important role in modulating the long-term actions of NE on renal function and arterial pressure.
After recovery from surgery, dogs were housed in individual metabolic cages and fitted with harnesses that contained pressure transducers mounted at heart level. Arterial catheters were connected to the pressure transducer, and 24-hour MAP, pulsatile pressure, and heart rate were monitored via an analog-to-digital data collection system. The analog signal was sampled 60 times/h and recorded on a personal computer. For data analysis, all pressure and heart rate data were averaged over an 18-hour period excluding the maintenance period in the morning. The venous catheter was connected to a peristaltic pump (Wiz, ISCO Inc) for continuous infusion of solutions throughout the study. Isotonic saline was continuously infused intravenously at 450 mL/d to maintain the total sodium intake at 70 mEq/d. All solutions were prepared daily, and transducer lines were protected by a flexible vacuum hose to prevent air bubbles and possible contaminants from entering the venous system. The filters were changed frequently throughout the study as needed. Finally, the renal artery catheter was connected to a 60-mL syringe filled with isotonic saline, which was infused at 1.875 mL/h. The filters were changed frequently throughout the study as needed. Finally, the renal artery catheter was connected to a 60-mL syringe filled with isotonic saline, which was infused at 1.875 mL/h. Again, a disposable filter was used to prevent air bubbles and possible contaminants from entering the venous system. The filters were changed frequently throughout the study as needed. Finally, the renal artery catheter was connected to a 60-mL syringe filled with isotonic saline, which was infused at 1.875 mL/h. Again, a disposable filter was used to prevent air bubbles and contaminants from entering the kidney. The infusion tubing and transducer lines were protected by a flexible vacuum hose that was attached to the harness. The dogs were allowed to move freely in the cage but were unable to turn completely around.

**Daily Maintenance and Data Collection**

Daily maintenance of the dogs was performed in the morning between 8 and 10 AM. Dogs were fed with a sodium-deficient diet (H/D, Hill’s Pet Products) that provided 2 to 3 mEq of sodium and 65 mEq of potassium per day and allowed free access to water. Dicloxicillin (2×250 mg) and Sulfatrim (2×400 mg) were given orally for prophylaxis. Urine volume and electrolyte concentrations, water and saline intake, and body temperature were measured. Fresh solutions for intravenous and intrarenal infusions were prepared, catheters were flushed with heparinized saline, and cages were cleaned. Arterial pressure and heart rate data from the previous day were stored and analyzed.

**Experimental Protocol**

All dogs were divided into two groups, and arterial pressure, heart rate, and renal function were determined before, during, and after intrarenal norepinephrine infusion. Group I (vehicle) consisted of six dogs with intrarenal saline vehicle treatment. Group II (L-NAME) consisted of nine dogs with intrarenal NO synthesis blocked, established by infusion of L-NAME via the renal artery at 3 μg kg⁻¹ min⁻¹. We have previously shown that this dose of L-NAME infused via the renal artery blocks endothelium-dependent vasodilatation in the dog. In both groups, the renin-angiotensin system was clamped by infusing captopril (15 μg kg⁻¹ min⁻¹) plus angiotensin II (0.5 ng kg⁻¹ min⁻¹) intravenously throughout the study to assess the actions of norepinephrine infusion in the absence of changes in endogenous ANG II formation. The dogs were allowed to become acclimated to their daily routine, and a 7-day pretreatment period occurred before the control data were obtained. After a 7-day control period, norepinephrine (Levyphed, Winthrop Pharmaceuticals) was continuously infused intrarenally for 7 days at a rate of 0.1 μg kg⁻¹ min⁻¹. A 7-day recovery period from norepinephrine followed. Renal function was determined on days 1, 3, 8, 11, 14, and 20 of the study. GFR and RPF were determined using the single-injection technique as previously described by Hall et al.21

**Statistical Analysis**

All values are reported as mean±SE. Basal values were averaged to give one value for statistical comparison. ANOVA for repeated measures was used to determine statistical significance within groups. Dunnett’s test was used when ANOVA proved significant. Significant differences between groups were calculated with an unpaired Student’s t test. Values of P<0.05 were taken as statistically significant.

**Results**

Fig 1 illustrates MAP and heart rate before, during, and after intrarenal infusion of norepinephrine in dogs pretreated with saline vehicle or L-NAME. In vehicle-pretreated dogs, MAP averaged 100±3 mm Hg under basal conditions, 98±4 mm Hg during 7 days of intrarenal nor-epinephrine infusion, and 95±4 mm Hg during the recovery phase of the experiment. In contrast to the vehicle-pretreated dogs, MAP in dogs pretreated with L-NAME increased significantly in response to 7 days of intrarenal norepinephrine MAP in dogs pretreated with L-NAME averaged 104±5 mm Hg during basal conditions and increased to 110 to 113 mm Hg (P<0.05) during norepinephrine infusion. MAP then decreased to 103±8 mm Hg after norepinephrine infusion was stopped. In both groups of dogs, heart rate remained relatively constant throughout the experiment. Heart rate was an average of 73±5 bpm during basal conditions, 74±7 bpm during norepinephrine infusion, and 76±6 bpm during recovery in dogs pretreated with saline vehicle. In dogs pretreated with L-NAME, heart rate was an average of 62±5 bpm during control conditions, 58±5 bpm during norepinephrine infusion, and 63±5 bpm in recovery.
Fig 2 shows the renal hemodynamic response during basal conditions, in response to an intrarenal infusion of norepinephrine, and during recovery in both groups of dogs. In saline-pretreated dogs, GFR was 49 ± 4 mL/min during basal conditions, tended to decrease to 41 ± 5 mL/min in response to norepinephrine infusion, and then began to return toward basal values (43 ± 3 mL/min) during recovery. In contrast to the control dogs, norepinephrine caused a marked and significant decrease in GFR in dogs pretreated with L-NAME. During basal conditions, GFR in the L-NAME-pretreated dogs was 47 ± 3 mL/min and decreased by 32% to 32 ± 5 mL/min (P < 0.05) during the norepinephrine infusion period. GFR then returned toward basal levels (43 ± 3 mL/min) during recovery. Similarly, the RPF response to norepinephrine was markedly and significantly enhanced in dogs pretreated with L-NAME. In saline-pretreated dogs, RPF was 134 ± 10 mL/min during basal condition, 122 ± 14 mL/min (P < 0.05) in response to norepinephrine, and 121 ± 13 mL/min during the recovery period. In dogs pretreated with L-NAME, RPF was 129 ± 8 mL/min under basal conditions, decreased by 37% to 81 ± 16 mL/min (P < 0.05) after norepinephrine infusion, and returned toward basal values during the recovery period (115 ± 7 mL/min). The RVR response to norepinephrine was also markedly enhanced in dogs pretreated with L-NAME. In saline-pretreated dogs, RVR was 1.24 ± 0.32 mm Hg mL⁻¹ min⁻¹ during basal conditions, 1.45 ± 0.32 mm Hg mL⁻¹ min⁻¹ in response to norepinephrine, and 1.16 ± 0.21 mm Hg mL⁻¹ min⁻¹ during the recovery period. In dogs pretreated with L-NAME, RVR was 1.43 ± 0.15 mm Hg mL⁻¹ min⁻¹.

Fig 3 depicts the renal excretory response before, during, and after intrarenal infusion of norepinephrine in dogs pretreated with vehicle or L-NAME. In vehicle-pretreated dogs, norepinephrine infusion caused a slight reduction in sodium excretion from a basal level of 85 ± 7 mEq/d to 76 mEq/d. Sodium excretion then returned toward basal values (83 ± 10 mEq/d) after norepinephrine infusion was stopped. The sodium-retaining action of norepinephrine was potentiated in dogs pretreated with L-NAME. In this group of dogs, norepinephrine tended to cause a greater reduction in sodium excretion from 94 ± 9 mEq/d to 72 ± 17 mEq/d. In control dogs, urine volume was 10.05 ± 0.10 L/d under basal conditions, 11.0 ± 1.7 L/d during the norepinephrine infusion period, and 1.00 ± 0.12 L/d during the recovery period. Urine flow in dogs pretreated with L-NAME averaged 1.98 ± 0.07 L/d under basal conditions and tended to decrease to 0.94 ± 0.13 L/d during norepinephrine infusion.

Discussion

Renal adrenergic stimulation via intrarenal infusion of norepinephrine at a dose of 0.1 µg kg⁻¹ min⁻¹ results in rather large short-term reductions in renal blood flow and GFR; however, the changes in renal hemodynamics are usually not sustained chronically despite continued administration of norepinephrine. Although the exact mechanisms for the escape from adrenergically mediated renal vasoconstriction may be multiple, the present study was
performed to determine whether local vasodilator factors such as nitric oxide are involved. As reported recently by Reinhart et al.,7 we found that chronic intrarenal infusion of norepinephrine at a rate of 0.1 μg kg⁻¹ min⁻¹ for 7 days resulted in only slight reductions in RPF and GFR. The new and important finding of the present study is that the chronic norepinephrine-induced decreases in renal hemodynamics are markedly enhanced in dogs in which renal nitric oxide synthesis is inhibited. In marked contrast to the control dogs, intrarenal infusion of norepinephrine for 7 days produced sustained decreases in RPF (37%) and GFR (32%) in dogs pretreated with the nitric oxide synthesis inhibitor L-NAME. These findings indicate that nitric oxide plays an important role in protecting the renal vasculature from the long-term vasoconstrictor actions of norepinephrine.

Previous studies have indicated that nitric oxide plays an important role in protecting the renal vasculature from a variety of vasoconstrictors such as angiotensin II and endothelin.8–11 Ito and colleagues8 were one of the first to demonstrate that nitric oxide modulates the vasoconstrictor actions of angiotensin II in isolated preglomerular microvessels of rabbits. We have also reported an important short-term interaction between nitric oxide and angiotensin II in control of renal hemodynamics in conscious dogs.14,15 Interestingly, Ito et al.8 failed to demonstrate a role for nitric oxide in modulating the vasoconstrictor actions of norepinephrine in isolated afferent arterioles of rabbits. In contrast, we found in a recent study that the acute renal hemodynamic actions of norepinephrine were markedly enhanced in conscious dogs pretreated with the nitric oxide synthesis inhibitor L-NAME.16 These findings led us to propose that the escape from adrenergically mediated renin-angiotensin II replacement, the chronic hypertensive effect of intrarenal norepinephrine was completely abolished. In our previous studies, we have also fixed the renin-angiotensin system constant in all of the dogs to examine the long-term interaction between nitric oxide and norepinephrine without the confounding effects of changes in angiotensin II formation. Our results in the control dogs confirm the findings of Reinhart et al. in that we did not observe any hypertensive response to norepinephrine. However, we did find that norepinephrine increased arterial pressure in dogs pretreated with L-NAME. These data indicate that nitric oxide plays a role in modulating the long-term actions of norepinephrine on arterial pressure. The enhanced blood pressure response to intrarenal norepinephrine in the L-NAME-pretreated dogs was most likely related to the larger reductions in GFR and sodium excretion observed in this group of dogs.

In the present study, nitric oxide synthesis within the kidney was inhibited by an intrarenal injection of L-NAME at a rate of 3 μg kg⁻¹ mm⁻¹. We previously reported that this intrarenal dose of L-NAME is sufficient to abolish endothelium-dependent vasodilation induced by bradykinin in conscious dogs.20 Furthermore, we reported that this intrarenal dose of L-NAME in conscious dogs decreased RPF by only 10% to 15% while having no effect on GFR.20 In the present study, however, RPF was not significantly different between the control group and the L-NAME-pretreated group. The lack of statistical significance may be due to the variability of RPF between the two groups of dogs or possibly to the fact that the renal responses to L-NAME may be attenuated in animals without an intact renin-angiotensin system. Despite no significant differences in RPF or GFR between the control and L-NAME-pretreated dogs under basal conditions, intrarenal nitric oxide synthesis inhibition was very effective in enhancing the renal hemodynamic response to norepinephrine.

In summary, we found that intrarenal infusion of norepinephrine for 7 days in control dogs resulted in slight decreases in RPF and GFR while having no effect on MAP. In marked contrast, in dogs pretreated with intrarenal L-NAME, norepinephrine decreased RPF by 37% and GFR by 32%. In addition, the blood pressure response to norepinephrine was also enhanced in dogs with renal nitric oxide synthesis inhibition. The results of this study demonstrate that nitric oxide plays an important role in modulating the long-term actions of norepinephrine on renal function and arterial pressure. The physiological implication of this study is that the kidneys may become very susceptible to enhanced renal adrenergic activity under pathophysiological diseases associated with endothelial dysfunction and decreased renal nitric oxide production.

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