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

Joey Granger, Christine Schnackenberg, Jackie Novak, Brett Tucker, Todd Miller, Stephen Morgan, Salah Kassab

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±5 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 (Hypertension. 1997;29[part 2]:205-209.)

Key Words • endothelial factors • kidney • renal hemodynamics • arterial pressure

Renal adrenergic stimulation via intrarenal infusion of norepinephrine results in vasoconstriction, increased tubular reabsorption of sodium, enhanced renin, and hypertension.1,2 The effects of norepinephrine on renal hemodynamics, sodium excretion, and arterial pressure are achieved directly by activation of α-adrenergic receptors and indirectly by enhanced angiotensin II formation.3-5 Indeed, a recent study by Renthart et al. demonstrated that angiotensin II plays a critical role in mediating the chronic hypertension associated with levels of adrenergic stimulation that have little chronic influence on renal hemodynamics.6-9

The renal hemodynamic and possibly hypertensive responses to chronic renal adrenergic stimulation may also be influenced by vasodilator counterregulatory mechanisms. Although adrenergic stimulation results in rather large short-term reductions in renal blood flow and GFR, these changes in renal hemodynamics are not sustained chronically despite continuous intrarenal administration of norepinephrine.7 Although the exact mechanisms for the escape from adrenergically mediated renal vasoconstriction may be multiple, it is likely that local vasodilator factors such as nitric oxide may be involved.8-12 Consistent with this suggestion are results from previous studies from our laboratory indicating that nitric oxide plays an important role in modulating the short-term vasoconstrictor actions of angiotensin II and norepinephrine.14-16 However, whether nitric oxide plays a role in modulating the long-term actions of norepinephrine is uncertain, since we have recently reported that the long-term actions of angiotensin II on renal function and arterial pressure are not potentiated by chronic nitric oxide synthesis blockade.17-18 Therefore, the objective of this study was to quantitate the long-term role of nitric oxide in modulating the chronic renal hemodynamic and hypertensive actions of renal adrenergic stimulation via intrarenal norepinephrine infusion. To achieve this objective, we determined the long-term effects of intrarenal norepinephrine infusion on renal function and arterial pressure regulation in dogs with or without renal nitric oxide synthesis blockade with L-NAME.

Methods

Animal Preparation

Experiments were performed in 15 conscious, chronically instrumented female mongrel dogs weighing 18 to 21 kg. Surgery and care of animals were conducted according to the National Institutes of Health guidelines for the care and use of animals and with the approval of the Institutional Animal Care and Use Committee. Surgery was performed under aseptic conditions. The preanesthetic agent sodium thiopental (5%) and atropine (1.5 mg IM) were given initially, followed by isoflurane (1% to 2%) continued throughout surgery. Tygon catheters were implanted in the abdominal aorta, distal to the origins of the renal arteries, via the femoral arteries for arterial pressure monitoring and blood sampling. Tygon catheters were also implanted in the vena cava via the femoral veins for intravenous infusion of various substances. A unilateral nephrectomy was then performed on the right kidney. In the left renal artery, a Tygon catheter was implanted as previously described by Herd and Barger.19 All catheters were tunneled subcutaneously, exteriorized between the scapulae, and placed in neck collars. During surgical recovery, dogs were given Buprenorphine (10 mg IM) for pain and Flo-Cillrin (62000 U IM) for prophylaxis.
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 keep the dogs pretreated with saline vehicle (0) or with the nitric oxide synthesis inhibitor L-NAME (3 μg · kg⁻¹ · min⁻¹) (©). In dogs pretreated with saline vehicle, MAP 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.

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.22 Significant differences between groups were calculated with an unpaired Student’s t test. Values of P<0.05 were taken as statistically significant.
NO-Norepinephrine Interactions and Renal Hemodynamics

Fig 2 GFR and RPF responses before, during, and after intrarenal infusion of norepinephrine (0.10 µg kg⁻¹ min⁻¹) in dogs pretreated intrarenally with saline vehicle (shaded bar) or with the nitric oxide synthesis inhibitor L-NAME (3 g kg⁻¹ min⁻¹) (open bar)

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 under basal conditions, increased by 91% to 2.73 ± 0.105 mm Hg mL⁻¹ min⁻¹ (P < 0.05) after norepinephrine infusion, and returned toward basal values during the recovery period (1.45 ± 0.02 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 return 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, 10.0 ± 1.7 L/d during the norepinephrine-infusion period, and 10.0 ± 0.12 L/d during the recovery period. Urine flow in dogs pretreated with L-NAME averaged 9.8 ± 2.7 L/d under basal conditions and tended to decrease to 9.4 ± 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 adrenoceptors 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., 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. Ito and colleagues were one of the first to demonstrate that nitric oxide modulates the vasoconstrictor actions of angiotensin II in isolated preglenomlar 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. Interstingly, Ito et al. 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. These findings led us to propose that the escape from adrenergically mediated renal vasoconstriction during continuous long-term intrarenal infusion of norepinephrine may, in part, be due to nitric oxide. However, whether nitric oxide played a role in modulating the long-term actions of norepinephrine was uncertain, since we have previously reported that the acute but not long-term actions of angiotensin II on renal function and arterial pressure were potentiated by chronic nitric oxide synthesis blockade. In contrast to the angiotensin II experiments, we found in the present study that the long-term renal hemodynamic actions of norepinephrine are markedly potentiated by nitric oxide synthesis inhibition. Thus, nitric oxide appears to play a greater role in modulating the chronic renal adaptation to the long-term effects of norepinephrine than angiotensin II. The exact reason why L-NAME does not potentiate the long-term actions of angiotensin II is unclear but may be due to compensatory activation of other vaso dilatory systems such as renal prostaglandins.

Chronic renal adrenergic stimulation via intrarenal norepinephrine infusion has been shown to cause hypertension through its effect to reduce renal sodium excretion or renal pressure natriuresis. Although the chronic hypertension induced by norepinephrine could be mediated through direct α-receptor stimulation or indirectly via enhanced renin release, a recent study by Reinhart et al. demonstrated that angiotensin II plays a major role in mediating the hypertension induced by intrarenal infusion of norepinephrine at a rate of 0.1 μg kg⁻¹ min⁻¹. They found that when the renin-angiotensin system was clamped by converting enzyme inhibition plus angiotensin II replacement, the chronic hypertensive effect of intrarenal norepinephrine was completely abolished. In our study, we 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 infusion of L-NAME at a rate of 3 μg kg⁻¹ min⁻¹. We previously reported that this intrarenal dose of L-NAME is sufficient to abolish endothelium-dependent vasodilatation induced by Bradykinin in conscious dogs. 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. 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 chronic 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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