Mechanisms Involved in the Cardiovascular-Renal Actions of Nitric Oxide Inhibition

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Abstract The roles of the sympathetic nervous system, angiotensin II, and arginine vasopressin in the cardiovascular-renal responses to nitric oxide synthesis inhibition were examined in eight conscious dogs equipped with arterial and venous catheters and a nonoccluding bladder catheter. Nitric oxide inhibition was achieved by intravenous infusion of N\(^{\text{G}}\)-nitro-L-arginine methyl ester (L-NAME) at 37.1 mmol/kg per minute for 140 minutes in the control group. The same dogs, after a 1-week recovery, were pretreated for 2 days with either prazosin for \(\alpha_1\) blockade, prazosin plus propranolol for \(\alpha_1\) plus \(\beta\) blockade, L-158,809 for angiotensin receptor blockade, or d(CH\(_2\))Tyr(Me)arginine vasopressin for vasopressin-V\(_1\) blockade, and the L-NAME infusion was repeated. After 140 minutes of L-NAME infusion into the control group, mean arterial pressure and renal vascular resistance had increased 16% and 71%, and renal blood flow, glomerular filtration rate, urine flow, and urinary sodium excretion had decreased 33%, 16%, 61%, and 64%, respectively. The decrement in renal blood flow and glomerular filtration during L-NAME administration was unaffected by any of the neurohumoral blockers. During \(\alpha_1\) blockade L-NAME resulted in only a 3% increase in arterial pressure, attenuation of the renal vascular resistance response, and almost total elimination of the decrease in urine flow. During angiotensin blockade the L-NAME-induced increase in arterial pressure was markedly attenuated, and the decrease in urinary sodium excretion was attenuated in the \(\alpha_1\) plus \(\beta\) blockade group. In conclusion, short-term renal and cardiovascular effects of nitric oxide synthesis inhibition in conscious dogs may be mediated, in order of importance, by arginine vasopressin, angiotensin II, and the sympathetics. (Hypertension. 1994;23[part 2]:951-956.)

Key Words • vasopressins • nitric oxide • endothelium • hypertension, renovascular • sympatholytics • angiotensins

A number of recent studies have shown that nitric oxide (NO) synthesis inhibition causes both acute and chronic increases in arterial pressure.\(^1\)-\(^8\) In addition, several studies have shown that administration of the NO synthesis inhibitor N\(^{\text{G}}\)-nitro-L-arginine methyl ester (L-NAME) causes acute\(^9\) and chronic\(^1,\(^2\) renal vasoconstriction in rats. Several investigators have also shown that inhibition of NO synthesis enhances the vasoconstrictor actions of a number of neurohumoral systems.\(^10\)-\(^13\) Therefore, it is conceivable that some of these pressor and renal vasoconstrictor effects of NO synthesis inhibition could be mediated by other neurohumoral factors.

NO synthesis inhibition in anesthetized rats has been shown to cause at least a short-term increase in renal nerve activity.\(^10\) Also, application of L-NAME to isolated afferent arterioles amplified the acute vasoconstrictor effects of angiotensin II (Ang II).\(^11\) In addition, acute NO synthesis inhibition amplified the pressor effects of arginine vasopressin (AVP).\(^12,\(^13\) Therefore, NO release may oppose the effects of several vasoconstrictor systems.

A recent study in anesthetized rats showed that the pressor and renal vasoconstrictor effects of L-NAME were not attenuated during individual or combined blockade of all the major vasoconstrictor systems.\(^3\) However, another study in anesthetized rats showed that neither \(\alpha_1\)-adrenergic nor angiotensin receptor blockade alone attenuated the pressor effects of L-NAME, but a combination of these blockades partially attenuated these effects.\(^4\) Therefore, it is not clear whether the major vasoconstrictor systems mediate the arterial pressure and renal vasoconstrictor effects of L-NAME.

Very little information about the interactions between NO and vasoconstrictor systems is available from studies in dogs. Therefore, our goal was to study the mediatory roles of the sympathetic nervous system, renin-angiotensin system, and AVP in the arterial pressure, renal hemodynamic, and renal excretory changes that occur during NO synthesis inhibition in conscious dogs.

Methods

Animal Preparation

Experiments were performed on eight conscious male dogs with an average body weight of 22.6±0.8 kg and were approved by the Institutional Animal Committee. With the use of aseptic surgery, preanesthesia with acepromazine maleate (22.6 \(\mu\)mol IM, Tech America), and anesthesia with thiopental sodium (Pentothal, 94.6 mmol/kg IV, Abbott) and methoxyflurane (Penthrane, Abbott), catheters were implanted in the aorta and vena cava through the femoral artery and vein, as previously described.\(^5\) Also, a nonoccluding bladder catheter was implanted through a paramedial abdominal incision. Water intake was ad libitum, and sodium intake was fixed at 75 mmol/d by an intravenous infusion of 450 mL/d isotonic saline plus feeding the dogs 894 g/d H-D dog food (Hill's Pet Products).

During a 2-week recovery period, the dogs were trained to lie quietly in their cages. The dogs were fitted with a backpack.
containing a Statham P23 ID transducer that was used to measure mean arterial pressure (MAP) continuously. The transducer was connected to a model 7D recorder (Grass Medical Instruments), and the output was sent to a digital computer. Pressure data were sampled each minute throughout the day.

**Experimental Protocol**

The mediating role of several vasoconstrictor systems in the cardiovascular-renal responses to NO synthesis inhibition was determined in conscious dogs infused intravenously with 37.1 nmol/kg per minute L-NAME (Sigma Chemical Co) for 140 minutes. Experiments were run in a control group and repeated in the same dogs after they were pretreated with either α₁-adrenergic blockade with prazosin (0.24 μmol/kg as an initial intravenous bolus plus 11.9 μmol/kg per day orally in two divided doses) (Sigma and Lederle), α₁- and β-adrenergic blockade with prazosin and propranolol (6.8 μmol/kg plus 0.034 μmol/kg per minute IV, Sigma), Ang II receptor antagonism with L-158,80914 (0.244 μmol/kg plus 0.122 μmol/kg per minute IV, supplied by Merck Research Laboratories), or AVP blockade with d(CH₂)Tyr(Me)AVP (8.7 nmol/kg plus 1.5 nmol/kg per hour IV, Sigma). Each blockade began 48 hours before the experimental period and continued throughout that period. At least 1 week of recovery was allowed before the next blockade experiment began. The degree of blockade during α, α plus β, Ang II, and AVP blockades was confirmed by the maximum arterial pressure responses to intravenous boluses of phenylephrine (Elkins-Sinn), phenylephrine and isoproterenol (Elkins-Sinn), Ang II (Sigma), and AVP (Peninsula Laboratories), respectively. Dosages are shown in the Table.

Glomerular filtration rate (GFR) and renal plasma flow were determined from the clearances of iothalamate and iodohippurate, respectively. On each experimental day, a bolus injection of 250 nCi/kg [¹³¹I]iothalamate (Glofil, Isotex Diagnostics) was followed by a constant infusion of 4 nCi/kg per minute throughout the experiment. A bolus of 1 μCi/kg [¹³¹I]iodohippurate (Hippuran, ER Squibb & Sons) was followed by a constant infusion of 10 nCi/kg per minute. These infusions continued throughout a 1-hour stabilization period before the beginning of the experiment. Renal blood flow, renal vascular resistance, and fractional excretion of sodium were calculated using standard techniques.

The experiment was initiated with three consecutive 20-minute control periods; then the intravenous L-NAME infusion began, and 4 minutes later seven consecutive 20-minute L-NAME clearance periods were begun. Arterial blood samples were withdrawn at the midpoint of each period for determination of hematocrit and isotope activity. At the end of each clearance period, the bladder was washed with 20 mL sterile water, and this was included with the urine collected for determination of aldosterone and cortisol concentrations. At the end of each period, the bladder was washed with 20 mL sterile water, and this was included with the urine collected for determination of aldosterone and cortisol concentrations. Samples for plasma renin activity and plasma aldosterone and cortisol concentrations were withdrawn during clearances periods 2 and 10, and their values were determined by radioimmunoassay.

Statistical analysis of plasma renin activity and plasma aldosterone and cortisol concentrations was performed using a paired t test comparing L-NAME values with control values.
Manning et al
Nitric Oxide and Renal Function 953

Results
Cardiovascular and Humoral Responses to Blockade
The Table shows that the MAP response in each blockade group was attenuated when challenged with the appropriate agonist. In addition, during every blockade these arterial pressure responses were decreased in magnitude as indicated, but also the duration of the responses was only a few seconds compared with a 3- to 5-minute response during the control period. Therefore, if each response were integrated over time, every blockade would be nearly 100% complete. Also, significant decreases in plasma aldosterone concentration within each blockade group. Analysis of other data was performed using ANOVA for repeated measures to determine overall significance. Significance during the individual L-NAME clearance periods for each blockade group was compared with the corresponding clearance period of the control group using Dunnett's test for multiple comparisons with the control.13 Significance was assumed at a value of P<.05. All data are reported as mean±SEM.

Changes in Mean Arterial Pressure
Fig 1 shows that after 130 minutes of L-NAME infusion MAP had increased 16±3% in the control group. In both sympathetic blockade groups, the MAP changes were similar to control. However, by the end of the L-NAME period in the Ang II and AVP blockade groups arterial pressure was changed only 6±3% and 3±2%, respectively.

Changes in Renal Blood Flow and Glomerular Filtration Rate
Fig 2 shows that renal blood flow had decreased 33±2% by the end of the L-NAME period. There were no significant differences in this response in any of the blockade groups. As seen in Fig 3, GFR decreased 16±3% in the control group by the end of the L-NAME period. Except for a transient attenuation of the decrease in GFR in the Ang II and AVP groups, there were no other significant differences.

Fig 1. Line graphs show percent changes in mean arterial pressure (MAP) during intravenous infusion of 37.1 nmol/kg per minute N°-nitro-L-arginine methyl ester (L-NAME) in eight conscious dogs. All blockade groups were pretreated with their respective blockers for 48 hours before the experiment. α Blockade: prazosin; α + β blockade: prazosin plus propranolol; angiotensin II (All) receptor blockade: L-158,809; arginine vasopressin (AVP) V, blockade: d(CH2)5Tyr(Me)AVP. MAP values during the control period for the control, α, α + β, All, and AVP groups were 94±3, 85±2, 76±3, 92±3, and 96±3 mm Hg, respectively. *P<.05 compared with the same time in the control group.

Fig 2. Line graphs show changes in renal blood flow (RBF) during intravenous infusion of N°-nitro-L-arginine methyl ester (L-NAME). α Blockade: prazosin; α + β blockade: prazosin plus propranolol; angiotensin II (All) receptor blockade: L-158,809; arginine vasopressin (AVP) V, blockade: d(CH2)5Tyr(Me)AVP. Values for RBF during the control period for the control, α, α + β, All, and AVP groups were 197±21, 193±20, 203±22, 226±17, and 205±17 mL/min, respectively. *P<.05.
Changes in Renal Vascular Resistance

As seen in Fig 4, renal vascular resistance increased 71 ± 6% in the control group by the end of the L-NAME period. Significant attenuation in the renal vascular resistance response was evident only in the AVP blockade group by the end of the L-NAME period.

Changes in Urine Flow and Urinary Sodium Excretion

Fig 5 shows that urine flow decreased 61 ± 5% in the control group by the end of L-NAME administration. A marked attenuation in this response was seen during almost all of the L-NAME period in the AVP blockade group.

As seen in Fig 6, urinary sodium excretion had decreased 64 ± 5% in the control group by the end of the L-NAME period. At this time, the change in sodium excretion was significantly attenuated in the α plus β blockade group.

Discussion

The goal of this study was to determine whether the sympathetics, Ang II, or AVP mediate any of the pressor, renal vasoconstrictor, or excretory effects of NO synthesis inhibition in conscious dogs. A previous study by Pucci et al in anesthetized rats showed that the acute effects of L-NAME on MAP and renal vascular resistance were not mediated by any of the endogenous vasoconstrictor systems. In the present study we found similar results for α-adrenergic blockade; however, AVP blockade attenuated the changes in MAP, renal vascular resistance, and urine flow; Ang II attenuated the MAP response; and α plus β blockade attenuated the decrease in sodium excretion.

Several studies indicate that AVP may mediate some of the cardiovascular-renal changes during NO synthesis inhibition. These studies have shown that the effects of AVP on MAP and regional blood flow were enhanced during NO synthesis inhibition. These vasoconstrictor effects of AVP may cause a release of NO through the α1 receptor. High doses of AVP cause vasodilation in some vascular beds that may be mediated by NO. In addition, NO synthase has recently been identified in the posterior pituitary, suggesting that NO release may change AVP synthesis. In fact, Goyer et al found that plasma AVP concentration increased during short-term intravenous infusion of L-NAME into conscious rabbits.

In the present study, AVP V1 receptor antagonism during the L-NAME period resulted in a marked attenuation...
FIG 5. Line graphs show percent changes in urine flow during intravenous infusion of L-NAME. a Blockade: prazosin; a + p blockade: prazosin plus propranolol; angiotensin II (All) receptor blockade: L-158,809; arginine vasoressin (AVP) V1 receptor blockade: d(CH2)Tyr(Me)AVP. Values for urine flow during the control period for the control, a, a + p, All, and AVP groups were 0.71±0.11, 1.16±0.27, 0.71±0.17, 1.1±0.14, and 0.53±0.06 mL/min, respectively. *P<.05.

enhanced sensitivity to V1 agonists in the systemic and renal circulations. Another possible cause of this attenuated decrease in urine flow in the V1-blocked group in the present experiment is that the 48-hour administration of the V1 blocker may have caused V2 blockade, but this is unlikely because short-term studies have shown that d(CH2)Tyr(Me)AVP had little effect on antidiuresis.22 Further experiments will be necessary to determine why this urine flow response occurred. Nevertheless, AVP blockade had remarkable effects on MAP and the urinary excretion of water during L-NAME infusion.

Whether or not Ang II mediates the cardiovascular-renal changes during L-NAME administration is controversial. The increase in MAP after intravenous bolus administration of L-NAME into anesthetized rats was found to be independent of changes in Ang II.6 However, long-term L-NAME-induced hypertension in rats was prevented by Ang II blockers.24 Bolus or long-term administration of L-NAME has also caused variable effects on plasma renin activity, but in the present study renin activity did not significantly increase during short-term L-NAME administration. However, Ang II recep-
tor blockade caused significant attenuation of the L-NAME–induced hypertension, thus suggesting that sensitivity to Ang II may have increased, which agrees with the study of Pollock et al.

Some studies have indicated that the sympathetic nervous system may mediate the cardiovascular-renal actions of NO synthesis inhibition. Sakuma et al. found that short-term intravenous administration of NO inhibitors caused increases in renal nerve activity. Lacolley et al. found that ganglionic blockade caused large decreases in MAP, and during L-NAME this sympathetic blockade prevented increases in both MAP and renal vasoconstriction. Return of MAP to normal with phenylephrine restored these responses. In the present study the MAP and renal vasoconstrictor responses were unchanged by α blockade. Because baseline MAP was changed very little by the chronic α-blockade, our results agree with those of Lacolley et al. and Sakuma et al. When the α plus β sympathetics were blocked, the decrease in urinary sodium excretion normally seen during L-NAME was attenuated, which could have been partially due to the decrease in plasma aldosterone that occurred in this group.

In conclusion, in conscious dogs infused with L-NAME on a short-term basis, the increase in arterial pressure and the decreases in urine flow and urinary sodium excretion may be partially mediated by AVP, Ang II, and the sympathetic nervous system, but the changes in renal blood flow and GFR were independent of changes in these neurohumoral factors.

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References

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