Role of Nitric Oxide in Long-term Angiotensin II-Induced Renal Vasoconstriction

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In vitro studies have indicated that nitric oxide may play an important role in modulating the renal vascular actions of angiotensin II (Ang II). However, the physiological importance of this interaction in the long-term regulation of renal hemodynamics is unknown. Therefore, the goal of this study was to determine if long-term Ang II-induced renal vasoconstriction was potentiated by nitric oxide synthesis inhibition. The intrarenal effects of Ang II were examined in eight unilaterally nephrectomized, conscious dogs before and after systemic inhibition of nitric oxide synthesis. Ang II infusion into the renal artery at 0.5 ng/kg per minute resulted in decreases in renal plasma flow of 15% and 9% after 3 and 5 days, respectively. During this time, glomerular filtration rate decreased 12% after 3 days of angiotensin but was not significantly changed after 5 days. After 4 days of recovery from Ang II, nitric oxide synthesis was inhibited with intravenous 10 mg/kg per minute of L-NAME for 5 days, and this caused a significant decrease in renal plasma flow but no change in glomerular filtration rate. Infusion of Ang II into L-NAME-pretreated dogs for an additional 5 days further decreased renal plasma flow and glomerular filtration 14% and 11%, respectively. However, the effects of Ang II and L-NAME on renal plasma flow were only additive on days 3 and 5 of this period, and the effects on glomerular filtration were additive on day 3 but were potentiated on day 5. Neither iothalamate space, plasma renin activity, plasma aldosterone concentration, nor plasma cortisol concentration was changed during the experiment, and a marked decrease in the acetylcholine depressor response during L-NAME indicated significant nitric oxide synthesis inhibition. In conclusion, long-term inhibition of nitric oxide synthesis did not potentiate the Ang II-induced reduction in renal plasma flow, but by the end of the angiotensin and nitric oxide inhibition periods, the effects of angiotensin on glomerular filtration were potentiated. (Hypertension 1993;21:949–955)

KEY WORDS • endothelium • endothelium-derived relaxing factor • arginine • glomerular filtration rate

Endothelium-derived relaxing factor has been recently identified as nitric oxide (NO) or a related compound. Acute blockade of NO synthesis has resulted in increases in arterial pressure in anesthetized and conscious animals, suggesting that there is a basal release of NO.

In addition to its effects on basal vascular resistance, several recent studies have indicated that NO may acutely counteract the constrictor effects of several pressor systems. Previous studies have shown that removal of the vascular endothelium enhances the acute vasoconstrictor effects of angiotensin II (Ang II) and phenylephrine in isolated vascular rings and arginine vasopressin in the perfused mesenteric vascular bed of the rat. NO has also been suggested to counteract the effects of vasoconstrictors in the renal circulation. Ito et al found that the NO synthesis inhibitor N\textsuperscript{G}-nitro-L-arginine-methyl ester (L-NAME) amplified the effects of Ang II in isolated afferent arterioles. Also, in preliminary studies in dogs, acute L-NAME infusion into the renal artery markedly amplified the renal vasoconstrictor effects of Ang II. Therefore, a number of short-term studies indicate that NO may oppose the effects of vasoconstrictors in several vascular beds, including the kidney.

If NO has a long-term modulatory role on Ang II-induced renal vasoconstriction, this modulatory effect could significantly influence renal hemodynamics, excretory function, and arterial pressure during conditions of increased Ang II release. However, whether NO modulates the long-term renal vasoconstrictor actions of Ang II is unknown. Therefore, the goal of this study was to determine if NO modulates the long-term Ang II-induced effects on renal plasma flow (RPF) and glomerular filtration rate (GFR) in conscious dogs. In addition, the importance of this interaction in altering the long-term regulation of extracellular fluid volume, plasma renin activity, and arterial pressure was examined.

Methods

Animal Preparation and Experimental Protocol

Experiments were performed on eight conscious dogs with an average body weight of 21.8±1.1 kg. Using aseptic techniques and anesthesia with thiopental sodium (Pentothal, 25 mg/kg i.v., Abbott, North Chicago)
and methoxyflurane (Penthrane, Abbott), we implanted catheters in the aorta and inferior vena cava through the femoral artery and vein as previously described. An intrarenal artery catheter was also implanted using the technique of Herd and Barger, except that the intravascular portion of the catheter was Silastic. The contralateral kidney was then surgically removed. Water intake was ad libitum, and sodium intake was fixed at approximately 42 mEq/day. The dogs were also fed psyllium hydrophilic mucilloid (Metamucil, 2.5 g twice a week, Procter & Gamble, Cincinnati, Ohio) to prevent constipation, which sometimes occurs during long-term L-NAME administration. Sodium and water balances were calculated by first taking the differences between water and the water content of the food, the average variance for repeated measures to determine overall significance for repeated measures to determine overall

Experimental Methods and Instrumentation

Dogs were fitted with a backpack containing a Statham P23 1D transducer that was used to measure arterial pressure. This in turn was connected to a model 7D recorder (Grass Medical Instruments, Quincy, Mass.), and the output was sent to a digital computer. Data were sampled each minute throughout the day to determine mean arterial pressure and heart rate.

The depressor response to acetylcholine (Sigma Chemical Co., St. Louis, Mo.) (250 or 375 μg i.v. bolus) was determined to confirm NO synthesis inhibition with L-NAME. The absolute decrease in arterial pressure due to acetylcholine was normalized by dividing by the average daily mean arterial pressure.

GFR and RPF were determined from the clearances of [123I]iothalamate (Glofil, Isotex Diagnostics, Friendswood, Tex.) and [131I]iodohippurate (Hippuran, E.R. Squibb and Sons, Princeton, N.J.) using the single-injection technique described by Hall et al. and assuming an extraction ratio of 1.0 for Hippuran. The volume of distribution of sodium iothalamate, an index of extracellular fluid volume, was determined using the technique of Sapirstein et al. Renal vascular resistance (RVR), filtration fraction, and the fractional excretion of sodium were calculated using standard techniques.

Plasma renin activity and plasma concentrations of aldosterone and cortisol were determined by radioimmunoassay. Plasma and urine sodium and potassium concentrations were determined by flame photometry.

Statistical analysis was performed using analysis of variance for repeated measures to determine overall

Results

Changes in Mean Arterial Pressure, Heart Rate, and Renal Vascular Resistance

Figure 1 shows that mean arterial pressure did not change significantly from its control value of 81.6±2.7 mm Hg during intrarenal Ang II infusion at 0.5 ng/kg per minute. However, during L-NAME administration, arterial pressure increased an average of 20%, but there was no further significant increase in mean arterial pressure during Ang II+L-NAME. Heart rate, also shown in Figure 1, decreased significantly from its control value of 81.6±2.7 beats per minute during the L-NAME period and on the first day of Ang II+L-NAME.
Changes in Renal Plasma Flow, Glomerular Filtration Rate, and Filtration Fraction

Figure 2. Bar graphs show effects of infusion of angiotensin II (ANGII) and N\(^\text{G}\)-nitro-L-arginine-methyl ester (L-NAME) on renal plasma flow, glomerular filtration rate, and filtration fraction. *p<0.05 compared with control. Absolute control values (C) are indicated.

There were no significant increases in RVR from its control value of 0.54±0.04 mm Hg/mL per minute during the Ang II period as shown in Figure 1, but during Ang II+L-NAME, RVR increased significantly with respect to day 0 of the control period. However, approximately one half of this increase in RVR during Ang II+L-NAME was due to the effects of L-NAME. Further analysis of the effects of Ang II on RVR will follow in Figure 3 and its discussion.

Changes in Plasma Renin Activity and Plasma Aldosterone and Cortisol Concentrations

Figure 4 shows that Ang II had no significant effects on plasma renin activity or plasma aldosterone and plasma cortisol concentrations. In addition, neither L-NAME by itself nor L-NAME+Ang II caused any significant changes in these variables.

Changes in the Acetylcholine Depressor Response

During the control period, the average decrease in mean arterial pressure after an intravenous acetylcholine bolus was -37.7±6.3%. No change in this response occurred during Ang II alone, but on the fifth day of L-NAME, the response had decreased to -12.4±5.9% (p<0.05). This depressor response remained significantly decreased during the Ang II+L-NAME period.

Changes in Sodium and Water Balance, Fractional Excretion of Sodium, and Iothalamate Space

Table 1 shows that urinary sodium excretion decreased and sodium balance increased significantly on the first day of L-NAME. Volume intake, which is the total of water intake by drinking and the volume of the solutions infused, remained close to its control value during the entire experiment. Urinary volume output tended to decrease on the first day of L-NAME, but the change did not reach significance. Volume balance increased on the first day of L-NAME but decreased the following day. Fractional excretion of sodium and iothalamate space remained close to their control values throughout the experiment.
Changes in Plasma Electrolyte Concentrations

Plasma sodium concentration changed very little from its control value of 142.1±0.5 mEq/L during the experiment and decreased significantly only on the fifth day of L-NAME to 140.6±0.7 mEq/L. Plasma potassium concentration increased slightly from a control value of 4.2±0.1 to 4.6±0.1 mEq/L (p<0.05) on the fifth day of L-NAME and remained at this slightly elevated value during the Ang II+L-NAME period.

Discussion

The goal of this study was to examine the hypothesis that NO chronically modulates Ang II–induced alterations in renal hemodynamics. Previous acute studies have found that Ang II–induced contraction of rings of rat aorta and bovine coronary artery was markedly enhanced by destruction of the endothelium or by administration of hemoglobin or methylene blue to rings with endothelium. Any of these interventions would prevent NO release or markedly attenuate its effects. In another study, Ito et al14 found that NO synthesis inhibition significantly potentiated the effects of Ang II on isolated rabbit afferent arterioles, suggesting that NO acutely protects the afferent arterioles from excessive vasoconstriction. Conrad and Whittemore16 recently found that the acute pressor response to Ang II was increased after N(G)-monomethyl L-arginine was acutely administered to conscious rats. On the other hand, in the same study, administration of an Ang II antagonist did not prevent the pressor response to N(G)-monomethyl L-arginine,16 which argues against an enhanced role of Ang II in the control of arterial pressure during NO synthesis inhibition. In agreement with this study, Pucci et al17 found that captopril treatment of anesthetized rats did not prevent the pressor effects of L-NAME. Therefore, these studies may indicate that Ang II may not be increased enough during acute NO synthesis inhibition to be an important mediator of the cardiovascular effects of this inhibition. However, several acute studies have suggested that Ang II–induced vasoconstriction is opposed by NO in a number of vascular beds, but whether NO plays a role in the long-term regulation of renal hemodynamics by counteracting the renal vasoconstrictor effects of Ang II is unknown.

In preliminary short-term studies on conscious dogs,9 we have shown that Ang II–induced effects on RPF, RVR, and GFR were markedly potentiated by administration of L-NAME. However, in the present study in L-NAME–pretreated dogs, the effects of Ang II on GFR were potentiated only on the fifth day of Ang II+L-NAME, and the effects of Ang II and L-NAME on RPF and RVR were only additive. Therefore, the...
acute renal protective effect of NO may not be sustained during long-term elevation of renovascular Ang II concentrations.

The reason that the effects of Ang II on RVR and RPF were not potentiated by long-term NO synthesis inhibition in the present study is not clear, but several possibilities exist. First, the renal vessels may not release as much NO in response to a long-term Ang II infusion compared with an acute infusion. If increased shear stress is the major stimulus for NO release during renal vasoconstriction, there is a possibility that release of NO during long-term increases in shear stress in the kidney may be attenuated. This concept is partly supported by preliminary results from a study in partially nephrectomized dogs by Brown. The blood flow per nephron is increased in partially nephrectomized dogs, compared with normal nephrons. The increased filtration fraction in the remnant kidney may attenuate the vasoconstrictor response to Ang II given acutely, but not during long-term infusion.
and the response to NO blockade would be expected to result in greater renal vasoconstriction than in normal dogs. However, the percentage decrease in renal blood flow during NO blockade in Brown's study was less in the partially nephrectomized dogs compared with normal dogs. These data suggest that NO release, measured indirectly as the renal blood flow response to NO blockade, was not increased during long-term increases in shear stress in partially nephrectomized dogs. Thus, there is a possibility that long-term increases in shear stress in the kidney due to Ang II infusion may not cause a long-term increase in NO release from the renal vasculature. A second reason for the lack of potentiation of the Ang II effects on RVR and RPF is that L-NAME could have caused an increase in endogenous formation of Ang II, and this would diminish the effects of the intrarenal arterial infusion of Ang II during the Ang II+L-NAME period. However, plasma renin activity did not change in our studies in the experimental period, which would argue against this possibility. This does not preclude, however, the possibility that L-NAME may have caused a small increase in intrarenal release of renin that would not increase systemic plasma renin activity.

During the Ang II+L-NAME period, the renal vasconstrictor effects of Ang II may have been affected by the altered renal hemodynamic baseline due to L-NAME. Ito et al. addressed this question in their recent study by increasing the tone in isolated perfused afferent arterioles with norepinephrine to the same level as in the L-NAME-treated group. The Ang II effect was sustained only in the L-NAME group, suggesting that the enhanced Ang II-induced vasoconstrictor was independent of baseline vascular tone and dependent on the inhibition of NO release.

The potentiation of the Ang II decreases in GFR during NO synthesis inhibition could have been due to enhanced constriction of the afferent arterioles as previously shown or to enhanced mesangial cell contraction, resulting in a decrease in the glomerular capillary filtration coefficient ($K_f$). However, Ang II receptors are located in both preglomerular and postglomerular arterioles, and some studies have suggested that Ang II has a balanced vasoconstrictor effect on the afferent and efferent arterioles, whereas others have suggested that the main effect was on the efferent arteriole. Perhaps the lack of effect of Ang II on the afferent arteriole in some studies is due to release of renal autacoids such as prostaglandins or NO. Studies in which prostaglandin synthesis was blocked with meclofenamate suggested that prostaglandins normally protect the afferent arteriole from the constrictor effects of Ang II without affecting the efferent arteriole. If NO chronically protects the afferent arteriole but not the efferent arteriole from the constrictor effects of Ang II in a similar way to prostaglandins, long-term blockade of NO synthesis would potentiate the effects of Ang II on the afferent arteriole. This could explain why GFR decreased more in our experiments during Ang II+L-NAME relative to Ang II alone. In addition, blockade of the release of NO from the mesangium could have potentiated the effects of Ang II on $K_f$, which would also tend to decrease GFR. However, arguing against the concept that NO protects only the afferent arteriole and mesangium are studies by Baylis et al. This study showed that long-term NO synthesis inhibition in rats resulted in decreases in GFR and $K_f$ and increases in afferent and efferent resistances, implying that NO causes a basal decrease in efferent and afferent resistances and an increase in $K_f$.

In conclusion, after 5 days of Ang II infusion into the renal artery at 0.5 mg/kg per minute, RPF decreased moderately, but GFR and RVR were unchanged. The 5-day intravenous infusion of L-NAME at 10 mg/kg per minute also caused a decrease in RPF, an increase in RVR, but no change in GFR. When the Ang II infusion was combined with L-NAME for an additional 5 days, RPF and GFR decreased further, and RVR increased further. Neither Ang II nor Ang II+L-NAME resulted in any significant change in sodium balance, volume balance, or iothalamate space. The long-term RVR and RPF responses to Ang II and L-NAME were additive, and the effects of Ang II on GFR were potentiated only at the end of the Ang II+L-NAME period. Therefore, long-term NO synthesis inhibition with L-NAME in dogs did not potentiate the Ang II-induced effects on RVR and RPF and did moderately potentiate the effects on GFR, but only after 5 days of infusion of Ang II+L-NAME.

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References


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