Role of angiotensin II in modulating the hemodynamic effects of nitric oxide synthesis inhibition

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Hernández, Isabel, Luis F. Carbonell, Tomas Quesada, and Francisco J. Fenoy. Role of angiotensin II in modulating the hemodynamic effects of nitric oxide synthesis inhibition. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R104–R111, 1999.—This study examined the role of ANG II in modulating the increase of hematocrit and vascular permeability that follows nitric oxide (NO) synthesis blockade. 

MATERIALS AND METHODS

Systemic Hemodynamic Studies

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IN THE LAST YEARS, a functional interaction between nitric oxide (NO) and ANG II has been reported to be related to the regulation of blood pressure and renal function (1, 19). The changes observed after NO synthesis blockade are due, at least in part, to the fact that physiological NO buffers the influence of endogenous vasoconstrictor systems. In this regard, Sigmon and Beierwaltes (16) observed that the angiotensin AT1-receptor-antagonist losartan attenuated the decrease in cardiac output (CO) and the increase in total peripheral resistance (TPR) produced by inhibition of NO synthase (NOS) with \( N\text{-nitro-L-arginine methyl ester (L-NAME) in anesthetized rats. This experiment was compatible with the idea that vasoconstriction may increase shear stress and NO production, which in turn acts as a regulation system by restraining the constrictor action of a variety of hormones, such as ANG II (1, 11, 12). }

NO synthesis inhibition increases arterial pressure and lowers CO, and it has been frequently suggested that the rise in systemic vascular resistance that follows NOS blockade is the major determinant of the hemodynamic response observed (5). However, the mechanism mediating the fall in CO that follows NOS inhibition remains obscure. Filep and Filep (4) described that inhibition of NOS increased hematocrit, lowered plasma volume, and induced albumin extravasation in several vascular beds. This transvascular loss of fluid after NOS inhibition has been reported to be due to increased albumin leakage in postcapillary venules (3, 9) and to a potentiation of the vascular permeability effect of platelet-activating factor (3). Taken together, these data indicate that factors other than vasoconstriction, such as the hemococoncentration due to decreased plasma volume, may also contribute to lower CO after NOS blockade.

There are increasing reports suggesting a role for ANG II in the pathogenesis of vascular injury and permeability, via mechanisms that are independent of its pressor activity. Exogenous administration of ANG II altered coronary microvascular structure and increased permeability to macromolecules (14). Furthermore, Williams et al. (23) reported that ANG II induced vascular permeability factor mRNA expression in human vascular smooth muscle cells. Therefore, it seems reasonable to postulate that NOS inhibition may increase hematocrit by unrestraining the intravascular fluid leakage produced by ANG II. In preliminary studies in our laboratory, we found that ANG II receptor blockade prevented the increase in hematocrit observed after the administration of L-NAME. This suggested that ANG II may contribute to some of the hemodynamic effects induced by NOS inhibition through a mechanism that regulates plasma volume. However, no data are currently available about the role of ANG II on the microvascular permeability response to NO synthesis blockade. Therefore, the purposes of the present study were to evaluate the role of ANG II in modulating the systemic hemodynamic response to NO synthesis blockade and also to study whether ANG II participates in the L-NAME-induced increases in microvascular permeability. To achieve these goals, we examined the effect of NO synthesis blockade with L-NAME on systemic hemodynamics and microvascular permeability in selected vascular beds, in control untreated rats, and also in rats pretreated with the AT1 angiotensin-receptor-antagonist losartan.

The experiments were performed on conscious, chronically catheterized female Sprague-Dawley rats weighing 300–350 g. The rats were anesthetized with an intramuscular injec-
tion of ketamine (30 mg/kg) and xylazine (20 mg/kg), and catheters were placed in the abdominal aorta and vena cava through the left femoral artery and vein, respectively, for measurement of mean arterial pressure (MAP), heart rate (HR), and intravenous infusions. A right atrial catheter and a thoracic aortic thermocouple were implanted via the right external jugular vein and right carotid artery, respectively. The catheters were brought out through the skin on the dorsal side of the neck. Finally, the distal ends of these lines were threaded through a lightweight flexible spring connected to a swivel. All surgical procedures were performed under aseptic techniques. Rats were placed in plastic cages with the swivels mounted above, allowing complete freedom of movement and free access to chow and tap water. Two full days were permitted for recovery from surgery and acclimatization to the environment, and hemodynamic studies were conducted while rats rested quietly in their home cages.

CO was measured by thermodilution as previously described in our laboratory (7). The thermodilution curve and the pressure signal were processed with a microcomputer system (Cardionax II; Columbus Instruments). Hemodynamic values were the mean of three determinations. CO was measured by thermodilution as previously described in our laboratory (7). The thermodilution curve and the pressure signal were processed with a microcomputer instrument. The laser-Doppler flowmeter of the instrument was adjusted to obtain a flow signal of 250 units as a "motility standard." The light was reflected back to the probe and was analyzed in the signal processor of the instrument. The laser-Doppler flowmeter was calibrated with a colloidal suspension of latex particles; the Brownian motion of these particles (at standard temperature, 22°C) was used as a "motility standard." The probe was placed in the suspension, and the gain of the instrument was adjusted to obtain a flow signal of 250 units (±5%).

After surgery and a 1-h equilibration period, MAP, blood flow, systemic hematocrit, and plasma proteins were measured in control conditions. Next, L-NAME (10 mg/kg, group X; n = 5) was administered intravenously as a bolus, and the same measurements were repeated 15, 30, and 60 min after NO synthesis inhibition. An additional group of rats (group XI; n = 5) was pretreated with losartan (10 mg/kg) 20 min

Laser-Doppler Blood Flow Measurements

The rats were fasted overnight, and, on the day of the experiment, the rats were anesthetized with an intramuscular injection of ketamine (30 mg/kg) and an intraperitoneal injection of inactin (thiobutabarbital, 50 mg/kg) and were placed on a heated table to maintain body temperature at 36.5°C. Catheters were placed in the jugular vein for infusions and in the femoral artery for measurement of arterial pressure. The rats received 1–2 ml of a 6% solution of albumin in 0.9% NaCl through a catheter intravenously and in the femoral artery for measurement of arterial pressure. The rats received 1–2 ml of a 6% solution of albumin in 0.9% NaCl through a catheter intravenously and in the femoral artery for measurement of arterial pressure. The rats received 1–2 ml of a 6% solution of albumin in 0.9% NaCl through a catheter intravenously and in the femoral artery for measurement of arterial pressure. The rats received 1–2 ml of a 6% solution of albumin in 0.9% NaCl through a catheter intravenously and in the femoral artery for measurement of arterial pressure. The rats received 1–2 ml of a 6% solution of albumin in 0.9% NaCl through a catheter intravenously and in the femoral artery for measurement of arterial pressure. The rats received 1–2 ml of a 6% solution of albumin in 0.9% NaCl through a catheter intravenous
before the control measurements were performed; next, L-NAME was administered, and the same protocol was followed thereafter.

Data Analysis

All values are reported as means ± SE. A two-way ANOVA for repeated measures was used to examine changes within and between groups, and a post hoc Fisher’s least-significant difference (LSD) test was used to determine differences between means. The results from the protein extravasation study were evaluated by a one-way ANOVA, followed by a Fisher’s LSD test. Changes were considered significant at \(P < 0.05\) (2-tailed test).

RESULTS

Hemodynamic Effects Induced by L-NAME

The hemodynamic determinations performed in groups I-III, before and after the administration of L-NAME, vehicle, or phenylephrine, are presented in Fig. 1. The hemodynamic effects of L-NAME + albumin in group IV (albumin + L-NAME) are also shown in Fig. 2. No significant differences were observed in basal hemodynamic values between groups I, II, III, and IV.

After 60 min of infusion of L-NAME, MAP increased by 22.4 ± 5.6% (group I, Fig. 1A). This pressor response was accompanied by a fall in HR (−8.6 ± 4.4%, 60 min after L-NAME). After 3 h of NOS inhibition, MAP returned to near baseline values (from a control value of 120.2 ± 4.1, it reached a peak value of 146 ± 4 after 1 h of L-NAME and fell to 128 ± 6 mmHg after 3 h of L-NAME), and this was associated with normalization of HR (from a baseline of 351 ± 12, it fell to 319 ± 10 after 1 h of L-NAME and returned to 350 ± 16 beats/min after 3 h of L-NAME; Fig. 1D). CI decreased by 50.8 ± 4.0 and 59.3 ± 6.2% 1 and 3 h after L-NAME, respectively (group I, Fig. 1B; \(P < 0.05\)). TPR increased (\(P < 0.01\)) by 154.2 ± 14.0 and 186.2 ± 44.1% 1 and 3 h after the administration of L-NAME. SV fell by 38.9 ± 2.8 and 59.3 ± 4.7% \((P < 0.01)\) 1 and 3 h, respectively, after L-NAME was given (Fig. 1E).

The infusion of phenylephrine produced an increase in blood pressure that was similar to the hypertension observed after the administration of L-NAME (Fig. 1A). However, CI fell less during the infusion of phenylephrine than after L-NAME \((P < 0.01)\). CI decreased from a basal value of 38.3 ± 1.5 to 18.7 ± 1.4 and 15.5 ± 2.2 ml·min⁻¹·100 g⁻¹ after 1 and 3 h, respectively, whereas CI only fell from a control value of 41.5 ± 2.2 to 29.3 ± 2.0 and 26.6 ± 2.1 ml·min⁻¹·100 g⁻¹ after 1 and 3 h, respectively, of...
infusion of phenylephrine. Furthermore, the changes observed in SV and TPR were smaller (P < 0.01) after phenylephrine than after the administration of L-NAME (Fig. 1). TPR only increased 73 and 96% after 1 and 3 h of infusion of phenylephrine, respectively, whereas it rose by 154.2 and 186% 1 and 3 h after L-NAME, respectively.

When the replacement of plasma volume loss was performed with albumin infusion in group IV (Fig. 2), a significant inhibition of the hemodynamic effects induced by L-NAME was observed. The infusion of albumin did not modify the hypertension and bradycardia produced by L-NAME (Fig. 2, A and D, respectively). However, the fall in CI (Fig. 2B) and SV (Fig. 2E) observed after L-NAME was significantly attenuated (P < 0.05) by volume replenishment. Albumin infusion was also effective in blunting the rise in TPR (Fig. 2C) produced by L-NAME.

Effect of Losartan on L-NAME-Induced Hemodynamic Changes

The group pretreated with losartan (group V) had lower baseline MAP than time control rats (group II, 104.2 ± 4.9 vs. 120.4 ± 4.6 mmHg, respectively), although there were no significant differences in CI (42.6 ± 2.5 vs. 39.2 ± 3.8 ml·min⁻¹·100 g⁻¹ in groups V and II, respectively) or TPR (2.5 ± 0.2 vs. 3.2 ± 0.5 mmHg·ml⁻¹·min⁻¹·100 g⁻¹ in groups V and II, respectively).

In the presence of losartan, some of the hemodynamic changes induced by L-NAME were attenuated (group V; Fig. 3). Pretreatment with losartan did not reduce the hypertension (+33 mmHg) and bradycardia (-98 beats/min) produced by L-NAME (Fig. 3, A and D, respectively). However, the peripheral vasoconstriction observed after L-NAME was significantly attenuated (P < 0.05) by pretreatment with losartan (Fig. 3C). Losartan was also effective in inhibiting the fall in CI (Fig. 3B) and SV (Fig. 3E) produced by L-NAME. CI fell 50.8 ± 4 and 59.1 ± 6.2% 1 and 3 h after L-NAME, whereas it only decreased by 36.7 ± 4 and 31.3 ± 6.8% 1 and 3 h after losartan + L-NAME (P < 0.05). Furthermore, in rats given losartan, L-NAME had no significant effect on SV.

The changes observed in hematocrit in groups I-VI are presented in Table 1. L-NAME markedly increased hematocrit (from a basal value of 47.63 ± 0.6 to 53.1 ± 0.9 and 53.6 ± 1.0% 1 and 3 h after L-NAME, respec-
tively; \( P < 0.05 \)), whereas phenylephrine infusion had no effect on hematocrit, despite the fact that the degree of hypertension achieved with both compounds was similar. In group IV, as expected, the infusion of 2% albumin prevented the increase of hematocrit induced by L-NAME. In addition, in losartan-pretreated rats, L-NAME had no effect on hematocrit (from a basal value of 46.8\( \pm \)1.6 to 46.3\( \pm \)1.6 and 46.4\( \pm \)2.1% 1 and 3 h after L-NAME, respectively). In control rats receiving vehicle (saline, a bolus of 0.2 ml\( \pm \)0.5 ml/h) and also in the rats given losartan, the hematocrit remained unaltered over the 3 h of observation.

Table 1. Effect of treatments on hematocrit

<table>
<thead>
<tr>
<th>Groups</th>
<th>Baseline</th>
<th>1 Hour</th>
<th>3 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (group II)</td>
<td>46.6( \pm )2.2</td>
<td>45.6( \pm )2.2</td>
<td>44.2( \pm )2.1</td>
</tr>
<tr>
<td>L-NAME (group I)</td>
<td>47.6( \pm )0.6</td>
<td>52.1( \pm )0.9*</td>
<td>53.6( \pm )1.0*</td>
</tr>
<tr>
<td>Phenylophrine (group III)</td>
<td>42.7( \pm )3.4</td>
<td>43.2( \pm )2.9</td>
<td>43.0( \pm )3.4</td>
</tr>
<tr>
<td>L-NAME + albumin (group IV)</td>
<td>41.7( \pm )1.8</td>
<td>40.2( \pm )2.3</td>
<td>40.0( \pm )1.9</td>
</tr>
<tr>
<td>Losartan + L-NAME (group V)</td>
<td>46.8( \pm )1.6</td>
<td>46.3( \pm )1.6</td>
<td>46.4( \pm )2.1</td>
</tr>
<tr>
<td>Losartan + saline (group VI)</td>
<td>44.9( \pm )1.9</td>
<td>44.7( \pm )1.9</td>
<td>44.4( \pm )1.9</td>
</tr>
</tbody>
</table>

Values are means\( \pm \)SE. Units are \% L-NAME. N\(^{-}\)-nitro-L-arginine methyl ester. Values were obtained before treatments (baseline) and 1 and 3 h after treatments were started. *\( P < 0.05 \) vs. baseline.

Plasma renin activity in the control group and in rats given L-NAME was 2.4\( \pm \)1.5 and 2.2\( \pm \)2.1 ng ANG II\( \cdot \)ml\(^{-1}\)\cdot h\(^{-1}\), respectively.

\( ^{125} \)I-Albumin Extravasation

The administration of L-NAME (group VII) produced an increase of 163 and 80% in \((^{125} \)HSA\) content in the heart and small intestine, respectively, compared with group VIII, which was treated with saline, whereas no significant changes were detected in the abdominal muscle and kidney (Fig. 4). When losartan was injected 30 min before L-NAME (group IX), the increase in protein extravasation was markedly attenuated (\( P < 0.05 \)) in heart and small intestine (1.8\( \pm \)0.3 and 1.8\( \pm \)0.02% injected counts\( \cdot \)min\(^{-1}\)\cdot 100 mg tissue\(^{-1}\), respectively). The \((^{125} \)HSA\) content of muscle and kidney did not change after treatments (Fig. 4).

Laser-Doppler Blood Flow Experiments

The effects of NO synthesis blockade in control and losartan-pretreated anesthetized rats on intestinal laser-Doppler blood flow signal, hematocrit, and plasma protein concentration are presented in Figs. 5 and 6.
L-NAME raised arterial pressure by 24% in control animals (Fig. 5), and this was associated with a significant increase in hematocrit ($P < 0.05$) of $3\%$ and in plasma proteins of $0.6 \text{ g/100 ml}$ 15 and 30 min after L-NAME ($P < 0.05$; Fig. 6). However, in rats given losartan, MAP rose by 43%, but hematocrit remained unaltered, and there was only a slight increment in plasma proteins ($0.28\%$) 30 min after the administration of L-NAME (Fig. 6). The laser-Doppler flow signal rose by 17% 15 min after L-NAME alone and returned to baseline thereafter (Fig. 5). However, in rats given losartan, the laser-Doppler flow signal values remained unaltered after 30 min of L-NAME infusion.

**DISCUSSION**

The results of the present study indicate that ANG II plays a role in the hemodynamic effects and intravascular fluid shift induced by NO synthesis blockade. The L-NAME-induced fall in CO and the rise in vascular resistance were markedly attenuated by pretreatment with the AT$_1$-receptor-antagonist losartan. Furthermore, the present study reports for the first time that AT$_1$ receptor blockade with losartan attenuates the marked fluid leakage and the albumin escape from the intravascular space induced by L-NAME. In fact, the effects of L-NAME on hematocrit and albumin escape were abolished by losartan, which demonstrates a key role for ANG II in L-NAME-induced hemoconcentration.

In the present study, the inhibition of NO synthesis resulted in increased blood pressure and TPR, whereas it decreased HR, CO, and SV, as previously described by other authors (5, 10, 21). The increase in TPR that occurs in response to the inhibition of NO synthesis has been suggested to be the major determinant of the fall in CO observed (5). However, it has been reported that, by inhibiting the L-NAME-induced hypertension with
the prostacyclin analog iloprost, the increase in systemic vascular resistance was abolished, but the pretreatment with this vasodilator did not prevent the fall in CO produced by L-NAME (24). These results indicate that the peripheral vasoconstriction originated by NOS inhibition may not be the only cause of the reduction in CO. This point of view is consistent with the finding in the present study that a different vasoconstrictor, phenylephrine (administered at an equipressor dose to L-NAME), lowered CI by only 30%, whereas L-NAME reduced it by 52%. Other factors, such as hemoconcentration, have also been implicated in the decrease in CO that follows NOS blockade (3). By using the nomogram published by Van Beaumont (20), it can be estimated that the increase of hematocrit produced by NO synthesis inhibition (+4.5%) is due to a 17% drop in plasma volume. In the present study, the administration of a 2% albumin solution to rats given L-NAME produced the same degree of hypertension as L-NAME alone with no changes in hematocrit; this treatment reduced CI by only 30%. Similar results have been recently reported (6). Taken together, these data indicate that the fall in CI seen after NO synthesis inhibition (~50%) is mediated by two mechanisms: vasoconstriction, which accounts for about ~30%, and hemoconcentration, which produces the remaining 20% drop in CI. This is in agreement with a previous report showing that an infusion of nitroprusside alone did not restore CO to normal values after L-NAME, and only expanding plasma volume with dextran abolished the hemodynamic effects of NOS blockade (2). Therefore, it seems clear that the hemoconcentration produced after NOS inhibition is necessary for the full expression of the hemodynamic response observed after L-NAME.

The present study clearly demonstrates a role for ANG II in the hemodynamic responses to L-NAME, because L-NAME increased vascular resistance by 186%, whereas, in rats pretreated with losartan, NOS blockade increased TPR by only 90%. Furthermore, pretreatment with losartan also prevented the increase in hematocrit (+5.5% in rats given L-NAME vs. −0.4% in rats given L-NAME + losartan), and it blunted the decrease in CI (−52% in rats given L-NAME vs. −36% in rats given L-NAME + losartan) and SV (−36% in rats given L-NAME vs. −18% in rats given L-NAME + losartan) induced by L-NAME, indicating a close relationship between the hemoconcentration and these two hemodynamic parameters. Another phenomenon that might participate in the fall in CO produced by L-NAME administration is the intense bradycardia observed. However, it has been reported that pretreating conscious rats with atropine abolished the L-NAME-induced bradycardia without affecting the decrease in CO (22), indicating that the reflex changes in HR cannot account for the decrease in CO. This hypothesis is further supported by our results showing that the L-NAME-induced bradycardia was more pronounced, whereas CO fell less in the rats pretreated with losartan than in the group treated with L-NAME alone.

The pressor and hemodynamic actions of L-NAME observed in the present study were associated with hemoconcentration, as evidenced by the marked increase in hematocrit in both conscious and anesthetized rats. Inhibition of NO synthesis may increase hematocrit by increasing vascular permeability and/or by transmission of increased systemic arterial pressure to the capillaries. In our experiments in anesthetized rats, we also observed a transitory increase in plasma protein concentration after L-NAME that was prevented by pretreatment with losartan, indicating that, in the first 30 min after L-NAME, there was a loss of fluid from the intravascular space. However, 1 h after L-NAME, plasma protein concentration returned to normal in rats given L-NAME alone, whereas hematocrit remained elevated thus suggesting that some loss of plasma protein also took place after NO synthesis inhibition. This is also supported by the marked increase in radiolabeled albumin escape after L-NAME observed in the present study. The increases in albumin escape in heart and small intestine are in agreement with previous studies that reported increases in microvascular permeability in the cat intestine (8) and in other vascular beds in the rat after L-NAME administration (3). In addition, in the present study, AT1 receptor blockade with losartan attenuated the marked intravascular fluid shifts and the albumin escape induced by L-NAME, probably because ANG II plays an important role in regulating vascular permeability when NO synthesis is inhibited. ANG II per se may increase vascular permeability, as demonstrated by direct exposure of rabbit aortic endothelium to the peptide (15). Furthermore, recent studies have shown that captoril and losartan pretreatment blunted the rise in hematocrit induced by atrial natriuretic peptide, independent of a consistent effect on systemic pressure (18). In addition, Williams et al. (23) have demonstrated that ANG II induces the vascular permeability factor mRNA expression in human vascular smooth muscle, suggesting a novel mechanism whereby ANG II could powerfully influence vascular endothelial permeability. Although at the present time the mechanisms mediating these effects remain unclear, our results showing that L-NAME-induced effects on hematocrit and albumin escape were abolished by losartan demonstrate a key role for ANG II in L-NAME-induced hemoconcentration.

The observation in the present study that L-NAME increased blood pressure and microvascular blood flow in intestine suggests that these changes may result in a rise of capillary hydrostatic pressure, by transmission of the increased systemic arterial pressure. The mechanism explaining this effect is unknown. However, it has been reported that the response to L-NAME administration may be heterogeneous in different vascular beds. In this regard, Wang et al. (21) reported that small intestine blood flow remained unaltered after NOS blockade. In addition, Nagao et al. (13) reported that L-NAME did not block the response to ACh in the mesenteric artery, indicating a minor contribution of NO to endothelium-dependent relaxation in this vascular bed. Therefore, the transient increase in blood flow seen in intestine after L-NAME administration could be
the result of a redistribution of flow from other vascular beds that are more sensitive to NO synthesis blockade than the intestine (21). Interestingly, pretreatment with losartan prevented the rise of intestinal blood flow seen after L-NAME. The mechanism mediating this effect is also unknown, but it may be related to the fact that losartan reduced the vasoconstriction induced by L-NAME in most vascular beds, as reported in recent studies (12, 17). Therefore, the attenuation of the vasoconstrictor response to L-NAME by pretreatment with losartan in the renal and other circulations (12, 17, 21) may also suppress the redistribution of blood flow to the mesenteric vascular bed, and then it might reduce the plasma protein loss and contribute to minimize the hemodynamic changes after NOS inhibition.

In summary, the results of the present study suggest that the renin-angiotensin system contributes to the hemodynamic effects caused by NO suppression. The fall in CO observed after inhibition of NO synthesis is in part a consequence of unmasking the actions of endogenous ANG II, which promotes a loss of plasma albumin in some organs, such as heart and small intestine.

In recent years, a growing body of evidence has been accumulating regarding the role of NO in the control of systemic hemodynamics. It now seems clear that one of the key functions of NO is to buffer the action of vasoconstrictors and preserve the tissular perfusion. It would be interesting in future experiments to measure NO and investigate more directly the interrelations between NO and other hormonal systems in the regulation of blood flow in different vascular beds in normal and pathophysiological conditions.

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