Effects of Amino Acid Infusion on Renal Hemodynamics
Role of Endothelium-Derived Relaxing Factor
Jonathan P. Tolins and Leopoldo Raij

Ingestion of protein or intravenous infusion of amino acids acutely elevates glomerular filtration rate (GFR) and renal plasma flow (RPF) by unknown mechanisms. Endothelium-derived relaxing factor (EDRF), now known to be nitric oxide derived from metabolism of L-arginine, participates in local regulation of vascular tone. To investigate the hypothesis that EDRF may participate in the renal vasodilatation and increased GFR after amino acid infusion, we characterized the effect of inhibition of EDRF synthesis with N\textsuperscript{G}-monomethyl L-arginine (LNMMA) on basal renal hemodynamics and the response to infusion of a 10% mixed amino acid solution (1 ml/hr i.v.) in the rat. Renal arterial infusion of LNMMA (500 \mu g/kg/min) resulted in a significant increase in mean arterial pressure, decreases in GFR (20%) and RPF (44%), and a significant increase in filtration fraction. Pretreatment with the angiotensin II receptor antagonist Sar-Gly-angiotensin II did not prevent the increase in blood pressure but blunted the decreases in GFR (11%) and RPF (27%) after LNMMA infusion. Amino acid infusion in the untreated, fasted rat resulted in no change in blood pressure but significant increases in GFR and RPF; these effects were completely inhibited by intrarenal LNMMA but not an equihypertensive intravenous infusion of phenylephrine. In summary, EDRF participates in regulation of basal renal hemodynamics. Furthermore, amino acid-induced hyperfiltration and renal vasodilatation are completely prevented by inhibition of EDRF synthesis. We conclude that EDRF may participate in the renal hemodynamic response to amino acid infusion. (Hypertension 1991;17:1045–1051)
sults from release of nitric oxide by stimulated endothelial cells.11,12 Furthermore, Palmer et al.13,14 demonstrated that nitric oxide originates from the terminal guanidino nitrogen atom of the amino acid, L-arginine. Although not all of the steps involved in the synthesis of nitric oxide are clear, there is evidence for the involvement of a soluble enzyme15 that can be inhibited by the L-arginine analogue, N\(^{-}\)O-monomethyl L-arginine (LNMMA; generous gift of S. Moncada, Wellcome Research Laboratories, Beckenham, Kent, UK), preventing the generation of nitric oxide by endothelial cells.14 This inhibitor of EDRF synthesis has been demonstrated to be active in vivo—infusion in the rat results in hypertension and inhibits the renal hemodynamic response to the endothelium-dependent vasodilator acetylcholine.10

We hypothesized that amino acid infusion results in alterations of amino acid metabolism by the kidney3 with subsequent generation of nitric oxide, and that EDRF is responsible, at least in part, for the observed renal vasodilatation and increase in GFR. To investigate this hypothesis, we characterized the effect of inhibition of EDRF synthesis with LNMMA on the renal hemodynamic response to amino acid infusion in the rat.

**Methods**

We first characterized the effects of inhibition of EDRF synthesis on basal renal hemodynamics by using an intrarenal infusion of LNMMA at various doses (study 1). Subsequently, the effect of inhibition of EDRF synthesis on the renal hemodynamic response to an intravenous infusion of mixed amino acids was evaluated (study 2).

Studies were performed in male Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, Ind.) weighing 250–350 g. Rats in study 1 were maintained on standard rat chow (Ralston-Purina, St. Louis) and water ad libitum until 15–20 hours before experimental studies, when only water was provided. Animals were fasted to eliminate variability in renal hemodynamic responses resulting from recent protein ingestion. Rats in study 2 were conditioned by providing a 6% protein diet (ICN Nutritional Biochemical Lab., Geveland, Ohio) for 7 days before experimental studies; also, they were fasted for 15–20 hours before clearance studies. Conditioning with a low protein diet was done to maximize hemodynamic responses to subsequent amino acid infusion.

**Clearance Studies**

After overnight fast, rats were surgically prepared for determination of mean arterial pressure, GFR, and RPF by clearance techniques, as previously described.10,16 A solution of normal saline containing [methoxy-\(^{3}H\)]inulin (4 \(\mu\)Ci/ml) and \(p\)-aminohippurate (PAH, 20 mg/ml; Merck Sharp & Dohme, West Point, Pa.) was infused at a rate of 1.2 ml/hr after a priming dose of 0.5 ml. To maintain euvoemia, 5% bovine serum albumin in normal saline (Sigma Chemical Co., St. Louis) was infused at 0.5 ml/hr after a priming dose of 1% of body weight. Timed urine collections from the left ureter were performed, with blood collected at the midpoint of each clearance period. Radioactivity in urine and serum was quantitated by liquid scintillation counting. PAH levels in serum and urine were determined by AutoAnalyser (Technicon, Tarrytown, N.Y.). GFR and RPF were calculated with standard formulas. In studies in which significant differences in weight existed between experimental groups, GFR and RPF were normalized for body weight. Renal blood flow was calculated by dividing RPF by (1 minus hematocrit), and renal vascular resistance (RVR) was calculated by dividing mean arterial pressure by renal blood flow.

For renal artery infusions, a PE-10 catheter with a curved, attenuated tip was advanced in a retrograde fashion from the right femoral artery and placed in the orifice of the left renal artery under direct visualization. In this fashion, dissection and manipulation of the renal artery were unnecessary and thus denervation and potential vascular damage were avoided. To maintain patency in the renal artery catheter, heparinized saline was infused at a rate of 0.5 ml/hr when other agents were not being administered.

**Study 1: Effect of Inhibition of EDRF Synthesis on Renal Hemodynamics**

The experimental protocol followed in study 1 is shown schematically in Figure 1A. After overnight fast, rats were prepared for clearance studies as described above. Rats were allowed to equilibrate for 30 minutes after surgery; then, two baseline clearances (10 minutes) were performed. In vehicle rats
(n=4), intrarenal normal saline infusion was continued into the renal artery for 15 minutes followed by three additional 15-minute clearances. Three additional groups of rats were prepared in an identical fashion. In these groups, after baseline clearances, the renal artery infusion was changed to LNMMA at 5, 50, or 500 μg/kg/min (n=4 in each group). After a 15-minute equilibration period, three additional 15-minute clearances were performed. In a final group (n=6) prepared as described above, an infusion of the angiotensin II receptor antagonist Sar-Gly-Angiotensin II (Sar-Gly-Ang II; 15 μg/kg/min i.v.; Sigma) was started during the equilibration period and continued during the remainder of the experiment. This dosage of Sar-Gly-Ang II completely inhibited the pressor response to Ang II (100 ng i.v. bolus) in similarly prepared rats. After baseline clearances, LNMMA (500 μg/kg/min) was infused into the left renal artery, and three additional 15-minute clearances were performed.

Table 1. Effect of Intrarenal Infusion of LNMMA on Renal Function

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>MAP (mm Hg)</th>
<th>GFR (ml/min/300 g body wt)</th>
<th>RPF (ml/min/300 g body wt)</th>
<th>Filtration fraction (mm Hg • min/ml)</th>
<th>RVR (mm Hg • min/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (n=4)</td>
<td>±8</td>
<td>±3 ±4</td>
<td>1.14 ±0.08</td>
<td>4.19 ±0.45</td>
<td>0.28 ±0.02</td>
<td>16.95 ±3.37</td>
</tr>
<tr>
<td>±8</td>
<td>±3 ±4</td>
<td>±0.08 ±0.01</td>
<td>±0.01 ±0.49</td>
<td>±0.02 ±0.02</td>
<td>±1.89 ±1.29</td>
<td>±2.15 ±3.69</td>
</tr>
<tr>
<td>LNMMA (n=4)</td>
<td>±15 ±4</td>
<td>±6 ±5</td>
<td>±0.12 ±0.03</td>
<td>±0.44 ±0.31</td>
<td>±0.01 ±0.04</td>
<td>±2.15 ±3.69</td>
</tr>
<tr>
<td>LNMMA plus</td>
<td>±6 ±5</td>
<td>±0.12 ±0.03</td>
<td>±0.44 ±0.31</td>
<td>±0.01 ±0.04</td>
<td>±2.15 ±3.69</td>
<td>±2.18 ±2.22</td>
</tr>
<tr>
<td>Sar-Gly-Ang II</td>
<td>±8 ±4</td>
<td>±0.11 ±0.10</td>
<td>±0.33 ±0.20</td>
<td>±0.02 ±0.04</td>
<td>±2.18 ±2.22</td>
<td>±2.15 ±3.69</td>
</tr>
<tr>
<td>(n=6)</td>
<td>±4 ±5</td>
<td>±0.10 ±0.10</td>
<td>±0.33 ±0.20</td>
<td>±0.02 ±0.04</td>
<td>±2.18 ±2.22</td>
<td>±2.15 ±3.69</td>
</tr>
</tbody>
</table>

LNMMA, Nω-monomethyl L-arginine; MAP, mean arterial pressure; GFR, glomerular filtration rate; RPF, renal plasma flow; RVR, renal vascular resistance; Sar-Gly-Ang II, Sar-Gly-angiotensin II.

*p<0.05 versus vehicle.
**p<0.01 versus baseline.
†p<0.05 LNMMA versus Sar-Gly-Ang II.
‡p<0.01 versus baseline.
§p<0.05 versus baseline.

After 7 days on a 6% protein diet and overnight fasting, all rats were prepared for clearance studies as described above. The experimental protocol followed in study 2 is shown schematically in Figure 1B. After baseline clearances, an intravenous infusion of a 10% solution of mixed amino acids (10% FreAmine III; [g/dl] isoleucine 0.69, leucine 0.91, lysine 0.73, methionine 0.53, phenylalanine 0.56, threonine 0.40, tryptophan 0.15, valine 0.66, alanine 0.71, arginine 0.95, histidine 0.28, proline 1.12, serine 0.59, and glycine 1.40; Kendall McGaw Laboratories, Inc., Irvine, Calif.) was begun at 1 ml/hr. L-Arginine represented 9.8% of the total amino acids present in the infused solution. After 15 minutes, three additional clearance periods were performed. Three groups of rats were studied. In vehicle rats (n=6), normal saline was infused into the left artery at 0.5 ml/hr throughout the experiment. In LNMMA rats (n=7), an equal volume of saline containing LNMMA (250 μg/kg/min) was infused into the left renal artery beginning 30 minutes before the baseline clearances. This dosage of LNMMA was used because, based on data from pilot studies, this was the maximum dosage at which baseline renal hemodynamics were not significantly changed compared with vehicle-infused rats. Because infusion of LNMMA into the renal artery at this dosage increased systemic blood pressure slightly but significantly, it was important to demonstrate that the effect of LNMMA on the renal hemodynamic response to amino acid infusion was specific and not a result of a general increase in vascular tone or renal autoregulatory responses to increases in systemic blood pressure. Therefore, an additional group of rats was studied. Phenylephrine (n=6) received an equal volume of saline infused into the left renal artery throughout the experiment. An intravenous infusion of phenylephrine (300 μg/kg/hr, Sigma) was started 30 minutes before the baseline clearances. This dose of phenylephrine was designed to increase the systemic blood pressure to a level identical to that seen in LNMMA rats, thus serving as an additional control.

Statistical Analysis

Data are presented as mean±SEM. Differences between baseline and final conditions within one group are compared by paired Student's t test. Differences between groups were compared by single-factor analysis of variance and subsequent Scheffe test. Differences were considered significant at a probability of less than 0.05. All statistical analyses were done with STATVIEW 512 software (Brainpower, Calabasas, Calif.).

Results

Effect of Inhibition of EDRF Synthesis With LNMMA on Basal Renal Hemodynamics

As shown in Table 1, renal artery infusion of saline in vehicle rats did not affect renal hemodynamic parameters during the course of the experiment. Infusion of LNMMA at 5 or 50 μg/kg/min did not
significantly alter mean arterial pressure, GFR, RPF, or RVR compared with baseline levels (data not shown). However, renal artery infusion of LNMMMA at 500 µg/kg/min induced significant changes in these parameters (Table 1). Rats receiving this dose of LNMMMA demonstrated a significant increase in mean arterial pressure compared with both baseline levels and vehicle rats. As shown in Figure 2, GFR decreased by 20%, and RPF decreased dramatically (44%). Filtration fraction was significantly increased by renal artery infusion of LNMMMA. Given the increase in systemic blood pressure and the decrease in RPF, it can be seen that RVR increased markedly. Thus, infusion of LNMMMA, an agent that specifically inhibits EDRF synthesis, resulted in a decrease in GFR and marked renal vasoconstriction.

Because these renal hemodynamic effects are similar to those expected after infusion of Ang II, we investigated whether the renal hemodynamic response to inhibition of EDRF synthesis with LNMMMA resulted from increased generation and/or unopposed effects of Ang II. As shown in Table 1, pretreatment with the Ang II receptor blocker Sar-Gly-Ang II did not prevent systemic hypertension during LNMMMA infusion. Furthermore, GFR and RPF still decreased significantly compared with baseline levels. However, these changes were blunted in magnitude compared with rats receiving LNMMMA alone (Figure 2). In rats pretreated with Sar-Gly-Ang II, GFR and RPF decreased by only 11% and 27%, respectively.

Effect of Inhibition of EDRF Synthesis With LNMMMA on the Renal Hemodynamic Response to Amino Acid Infusion

Intravenous infusion of a 10% mixed amino acid solution at 1 ml/hr had no effect on systemic blood pressure but induced significant increases in GFR and RPF (Table 2). RVR decreased numerically, but this change was not statistically significant. In the present study, LNMMMA was infused into the renal artery at 250 µg/kg/min, a dosage that did not change baseline GFR or RPF compared with vehicle rats but did result in a significant increase in mean arterial pressure (Table 2). In rats pretreated with intrarenal LNMMMA, amino acid infusion had no effect on GFR or RPF (Figure 3). After amino acid infusion, LNMMMA rats demonstrated a final RPF that was significantly lower and a final RVR that was significantly higher than those observed in vehicle rats.

As an additional control, the vasoconstrictor phenylephrine was given intravenously to a third group of rats at a dose designed to increase mean arterial pressure to a degree similar to that observed in rats receiving LNMMMA (Table 2). In this group, amino acid infusion increased GFR and RPF and decreased RVR to extents similar to those observed in vehicle rats.

Table 2. Effect of Amino Acid Infusion on Renal Function

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>MAP (mm Hg)</th>
<th>GFR (ml/min/300 g body wt)</th>
<th>RPF (ml/min/300 g body wt)</th>
<th>RVR (mm Hg · min/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Final</td>
<td>Baseline</td>
<td>Final</td>
<td>Baseline</td>
</tr>
<tr>
<td>Vehicle (n=6)</td>
<td>253±10</td>
<td>126±3</td>
<td>128±5</td>
<td>1.10±0.07</td>
<td>1.32±0.06†</td>
</tr>
<tr>
<td>LNMMMA (n=7)</td>
<td>274±10</td>
<td>149±3*</td>
<td>154±4*</td>
<td>1.21±0.07</td>
<td>1.17±0.06</td>
</tr>
<tr>
<td>Phenylephrine (n=6)</td>
<td>250±6</td>
<td>155±2*</td>
<td>147±3*‡</td>
<td>1.19±0.07</td>
<td>1.46±0.12‡</td>
</tr>
</tbody>
</table>

MAP, mean arterial pressure; GFR, glomerular filtration rate; RPF, renal plasma flow; RVR, renal vascular resistance; LNMMMA, N\textsuperscript{6}-monomethyl l-arginine.

*\(p<0.05\) versus vehicle.
†\(p<0.05\) versus baseline.
‡\(p<0.01\) versus baseline.
§\(p<0.05\) LNMMMA versus phenylephrine.
rats (Figure 3). Thus, the inhibitory effect of LNMMA on amino acid-induced renal hyperfiltration and vasodilatation was not related to nonspecific increases in renal vascular tone or hemodynamic changes secondary to renal autoregulatory responses to increases in systemic blood pressure.

Discussion

It has recently become apparent that apart from its role in regulation of vascular permeability and control of hemostasis, the vascular endothelium participates in regulation of vascular tone. The characterization of LNMMA as an inhibitor of the enzymatic synthesis of nitric oxide from L-arginine has facilitated the evaluation of the role of EDRF in various vascular beds. Previous research in our laboratory has demonstrated that intravenous infusion of LNMMA in the rat is associated with an increase in systemic blood pressure and that this hypertensive effect can be reversed by administration of a large molar excess of L-arginine. The systemic and renal hemodynamic effects of the endothelium-dependent vasodilator acetylcholine were also prevented by LNMMA. Furthermore, we recently reported that inhibition of EDRF synthesis with LNMMA enhanced hypoxic pulmonary vasoconstriction in pulmonary artery rings and isolated, perfused rat lungs. Thus, previous in vivo studies have indicated that EDRF may play a role in the regulation of systemic blood pressure as well as regional hemodynamics.

In the present study, infusion of LNMMA into the renal artery had profound effects on basal renal hemodynamics. Systemic blood pressure was increased, indicating some systemic effect of this agent despite intrarenal administration. RVR markedly increased, accompanied by a significant decrease in GFR and an increase in filtration fraction. These data indicate that EDRF is important in the maintenance of basal renal vascular tone and GFR.

The mechanism underlying these renal hemodynamic effects is not elucidated by the present study; however, it is interesting to speculate on possible glomerular hemodynamic events. Removal of a tonic vasodilatory effect on the efferent arteriole by inhibition of EDRF synthesis would be expected to increase postglomerular resistances, leading to a decrease in RPF, relative preservation of GFR, and an increase in filtration fraction. Such an effect would be consistent with our observations. Although Ang II may act on both preglomerular and postglomerular arterioles, it has been reported to have a relative predominance of vasoconstrictor effects on the efferent arteriole. Thus, our observations raised the question of whether increases in intrarenal Ang II levels or actions could result from inhibition of EDRF synthesis. Increases in cyclic GMP (cGMP) levels accompany EDRF-induced relaxation of vascular smooth muscle in vitro and the effects of endothelium-dependent vasodilators in vivo. Therefore, it has been suggested that cGMP is an important intracellular messenger for the effects of EDRF in vivo. Furthermore, vasodilators that increase cGMP levels, including EDRF, have been demonstrated to antagonize the effects of Ang II. Thus, it is possible that decreased synthesis of EDRF allows increased expression of Ang II effects on glomerular hemodynamics. Consistent with this hypothesis was our observation that Ang II receptor blockade blunted the hemodynamic response to LNMMA. Direct effects of inhibition of EDRF synthesis on the contractile state of the glomerular mesangium with subsequent alterations in the glomerular ultrafiltration coefficient, \( K_f \), could also contribute to the observed responses.

The mechanism by which amino acid infusion or protein loading results in renal vasodilatation and increased GFR remains unclear. Changes in levels of glucagon and other endogenous hormones as well as in prostaglandins have been postulated to be important, but none of these has provided a complete explanation for the observed changes. A preliminary report by Jaffa and coworkers demonstrated that pretreatment with a kinin receptor antagonist blunted the increase in GFR induced by amino acid infusion in the rat. Because bradykinin is a known agonist of EDRF generation by vascular tissues, LNMMA by guest on April 8, 2017 http://hyper.ahajournals.org/ Downloaded from
these data suggest a possible role for EDRF in amino acid–induced renal hemodynamic changes. Furthermore, as noted above,13,14 the amino acid L-arginine has been postulated to be the immediate precursor of the EDRF nitric oxide. In the rat, L-arginine, when given in a large molar excess, rapidly reverses LNMMA–induced hypertension10 and reduces the severity of hypoxic vasoconstriction in the isolated, perfused rat lung.20 Preliminary reports have also demonstrated that exogenous l-arginine can normalize endothelium-dependent vascular relaxations in hypercholesterolemic rabbits, which typically have impaired vasodilatory responses.27 However, this effect of exogenous l-arginine on vascular responses was observed after infusion at doses that would result in levels much higher than are likely to be attained in the present study. Hirschberg et al28 reported that intravenous arginine infusion in humans resulted in renal hemodynamic responses similar to those observed with a mixed amino acid infusion in the present study. Thus, it is clear that exogenous amino acids, L-arginine in particular, can effect vascular responses, perhaps through modulation of EDRF/nitric oxide synthesis. We therefore postulated that increased amino acid levels, perhaps in concert with appropriate hormonal changes, result in modulation of renal amino acid metabolism and that these changes result in increased generation of EDRF from L-arginine. Thus, increased synthesis of EDRF would be responsible, at least in part, for the increase in GFR and renal vasodilatation observed after amino acid infusion.

In the present study, we demonstrated that infusion of the EDRF/nitric oxide synthesis inhibitor LNMMA into the renal artery at a dose that did not significantly affect baseline renal hemodynamics completely inhibited the increase in GFR and renal vasodilatation induced by amino acid infusion, as observed in vehicle-pretreated rats. Furthermore, this effect was specific for LNMMA; catecholamines, an equivalent systemic vasoconstriction with phenylephrine did not prevent these changes in renal hemodynamics after amino acid infusion. We have therefore observed for the first time that renal EDRF/nitric oxide synthesis must be intact for the normal renal hemodynamic response to amino acid infusion to occur. These data are consistent with the hypothesis that increased generation of EDRF plays a role in the effects of amino acid infusion on renal hemodynamics. At present, this conclusion is based on the demonstrated effects of a single inhibitor of EDRF/nitric oxide synthesis and therefore must be viewed with caution. Clearly, these results must be confirmed with additional studies and perhaps with other inhibitors as they become available.

In summary, inhibition of EDRF synthesis by intrarenal infusion of LNMMA was associated with systemic hypertension, decreased GFR, and renal vasoconstriction. This effect may be in part mediated by increased action of Ang II in the setting of decreased levels of EDRF. The renal hemodynamic effects of amino acid infusion, increased GFR, and renal vasodilatation were completely prevented by pretreatment with LNMMA but not by phenylephrine. We conclude that EDRF may play a role in amino acid–induced hyperfiltration and renal vasodilatation.

Acknowledgments
We thank Bich Ha for excellent technical assistance and Diana Jacobson for assistance in manuscript preparation.

References


KEY WORDS • endothelium • nitric oxide • amino acids • hemodynamics • glomerular filtration rate • blood flow
Effects of amino acid infusion on renal hemodynamics. Role of endothelium-derived relaxing factor.
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Hypertension. 1991;17:1045-1051
doi: 10.1161/01.HYP.17.6.1045

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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