Nitric Oxide: A Potential Mediator of Amino Acid-Induced Renal Hyperemia and Hyperfiltration

Andrew J. King, Julia L. Troy, Sharon Anderson, Julia R. Neuringer, Mark Gunning, and Barry M. Brenner

A.J. King, J.L. Troy, S. Anderson, J.R. Neuringer, M. Gunning, B.M. Brenner, Renal Division and Department of Medicine, Brigham and Women's Hospital, and The Harvard Center for the Study of Kidney Diseases, Harvard Medical School, Boston, MA (J. Am. Soc. Nephrol. 1991; 1:1271-1277)

ABSTRACT
The role of nitric oxide in the modulation of systemic and renal hemodynamics was examined by using N'-monomethyl-L-arginine (l-NMMA, 110 µg/kg/min), a competitive inhibitor of the conversion of l-arginine to nitric oxide. l-NMMA or saline vehicle (9.6 µL/min) was infused intravenously into anesthetized euvolemic Munich-Wistar rats. After 30 min, l-NMMA resulted in a uniform increase in mean arterial blood pressure (111 ± 1 to 128 ± 2 mm Hg; P < 0.05) and a modest reduction in renal plasma flow rate (4.4 ± 0.2 to 4.2 ± 0.1 mL/min; P < 0.05), without change in glomerular filtration rate (1.16 ± 0.03 to 1.15 ± 0.03 mL/min); vehicle had no effect on these renal parameters. These rats were then subdivided to receive an intravenous infusion (37 L/min) of either 10% glycine, 11.4% mixed amino acids, or equiosmolar dextrose. l-NMMA pretreatment markedly attenuated glycine-induced hyperfiltration (10 ± 6 versus 33 ± 5%, l-NMMA versus vehicle; P < 0.05) and obliterated the renal hyperemic response (-7 ± 6 versus 16 ± 4%, l-NMMA versus vehicle; P < 0.05). l-NMMA also caused modest blunting of the mixed amino acid-induced hyperfiltration (18 ± 4 versus 30 ± 4%; l-NMMA versus vehicle; P = 0.056) but failed to curtail the renal hyperemia (16 ± 6 versus 20 ± 4%). Dextrose had no effect on glomerular filtration rate or renal plasma flow. These results with mixed amino acids differed from those with glycine alone, presumably because the former was rich in l-arginine, the metabolic precursor for nitric oxide. For glycine-treated rats, urinary 3',5'-cGMP excretion rates increased significantly in both vehicle- and l-NMMA-treated rats (5.1 ± 1.1 to 15.7 ± 2.4 and 7.9 ± 0.6 to 14.6 ± 0.6 pmol/min, respectively). In summary, nitric oxide appears to influence basal systemic, and to a lesser extent, basal renal vascular tone. Furthermore, the renal effects of mixed amino acids and glycine are, at least in part, sensitive to l-NMMA, suggesting that nitric oxide formation contributes to the renal vasodilation and hyperfiltration responses to acute amino acid infusion.

Key Words: EDRF, N'-monomethyl-L-arginine, glycine, hemodynamics, kidney

Over the past decade, it has been recognized that endothelial cells produce several vasoactive mediators which modulate vascular smooth muscle tone in response to a variety of stimuli (1,2). One such mediator, endothelium-derived relaxing factor (EDRF), has been identified to be at least in part nitric oxide (NO) derived from the guanido nitrogen atom(s) of l-arginine (l-Arg) (3). NO is a potent stimulator of soluble guanylate cyclase leading to an increase in vascular smooth muscle cGMP and subsequent relaxation (1). However, the molecular composition of EDRF remains controversial, and nitrosothiols have recently been suggested to be the primary form of EDRF (4). Studies that used a competitive analog of l-Arg, (N'-monomethyl-L-arginine, l-NMMA), have shown in rabbits, guinea pigs, rats, and humans that the endothelium maintains a
steady-state production of NO (5–8). Infusion of L-NMMA results in a sustained pressor effect which is rapidly reversed by subsequent infusion of L-Arg (5).

Currently, little is known of the role the endothelium plays in the modulation of renal hemodynamics. Several endothelium dependent vasodilators, such as acetylcholine and bradykinin, are known to induce a marked reduction in renal vascular resistance (9). Indeed, a variety of vasoactive hormones influence glomerular filtration, in part by modulation of the relative resistances of the pre- and postglomerular microcirculation (10). Cultured glomerular endothelial cells are capable of producing EDRF in response to agents which increase cytosolic free calcium, further supporting a role for NO in the control of renal microcirculatory hemodynamics (11).

The purpose of the study presented here was to use L-NMMA to examine the role of NO in the maintenance of basal systemic and renal hemodynamics in the anesthetized rat. In addition, studies were performed to assess whether NO plays a role in the renal hyperemia and hyperfiltration associated with acute amino acid infusion.

METHODS

Adult male Munich-Wistar rats (240 to 290 g) were studied in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Rats were anesthetized with Inactin (100 mg/kg i.p.) and placed on a temperature-controlled table. The right femoral artery was cannulated and a baseline sample of blood was collected for determination of hematocrit. This arterial catheter was used for all subsequent blood sampling and for the estimation of mean arterial pressure (MAP) via an electronic transducer connected to a direct-writing recorder. After tracheostomy, bilateral jugular catheters were inserted for infusions of rat plasma, 10% inulin with 0.8% para-aminohippurate (PAH) in 0.9% NaCl (1.2 mL/h), and experimental infusions. The left femoral vein was then cannulated for subsequent infusion of L-NMMA (Calbiochem, San Diego, CA) or vehicle. The left ureter was catheterized for urine collections.

Rats prepared in this fashion have been shown to have a 20% reduction in plasma volume (12); thus, the following protocol was used to maintain euvolemia. Isocotic rat plasma was infused at 0.1 mL/min in a total amount equal to 1% of the body weight, followed by a reduction in infusion rate to 0.60 mL/kg/h.

The experimental protocols are summarized in Figure 1. After a 1-h equilibration period, two 10-min urine collections with concurrent arterial blood samples (0.21 mL) were obtained for baseline determinations of hematocrit and plasma protein concentration (Cp), and for baseline measurements of glomerular filtration rate (GFR) (inulin clearance) and estimated renal plasma flow rate (RPF) (PAH clearance) (baseline). All rats then received a continuous i.v. infusion of either L-NMMA (3 mg/mL; 9.6 μL/min), or sodium acetate vehicle (V), which were continued for the remainder of the experiment. Thirty minutes into the infusion, measurements of GFR and RPF were repeated (Period 1). Subsequently, with continued infusion of L-NMMA or V, rats were also infused i.v. (37 μL/min) with either a 10% glycine solution (N = 16), an 11.4% mixed amino acid solution (Novamine,4 KabiVitrum, Alameda, CA; N = 16) (AA), or an equiosmolar solution of dextrose (1,330 mosmol; N = 12). Final clearance measurements were made after 30 min (Period 2). At the end of the experiment, samples of renal vein blood were drawn for determination of PAH extraction. No difference in PAH extraction was found comparing rats which received V or l-NMMA (88 ± 1 versus 87 ± 2%; not significant [NS]) or comparing the different experimental infusions (87 ± 2, 85 ± 2, and 91 ± 1% [glycine, mixed AA, and dextrose, respectively]; NS). In the groups given glycine with and without l-NMMA, urinary cGMP excretion rates were measured in each urine collection period. In a second group, rats were prepared as described above, given V for l-NMMA and infused with either L-Arg HCl (37 μL/min of 1.330 mM [N = 4] or 660 mM [N = 5]) or D-Arg HCl (660 mM [N = 4]).

Analytical

Inulin concentrations in plasma and urine were measured by a macro-anthrone method (13), and PAH concentrations were measured by the method of Smith et al. (14). Plasma protein concentrations were measured by using refractometry. Urinary cGMP levels were measured by radioimmunoassay.

Statistical

Reported values represent means ± SE. Individual baseline and experimental hemodynamic values of

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4 Amino acid content of Novamine includes (per deciliter): lysine, 900 mg; leucine, 790 mg; phenylalanine, 790 mg; valine, 730 mg; isoleucine, 570 mg; methionine, 570 mg; threonine, 570 mg; tryptophan, 190 mg; alanine, 1,65 g; arginine, 1,12 g; aspartic acid, 1,320 mg; glutamic acid 570 mg; serine, 480 mg; asparagine, 330 mg; tyrosine, 30 mg.
TABLE 1. Systemic and renal hemodynamic parameters

<table>
<thead>
<tr>
<th></th>
<th>(\overline{AP}) (mm Hg)</th>
<th>GFR (mL/min)</th>
<th>RPF (mL/min)</th>
<th>FF (%)</th>
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<tbody>
<tr>
<td>Glycine + V (N = 8)</td>
<td></td>
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<tr>
<td>Baseline</td>
<td>113 ± 2</td>
<td>1.17 ± 0.05</td>
<td>4.99 ± 0.30</td>
<td>23 ± 1</td>
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<tr>
<td>Period 1</td>
<td>119 ± 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.09 ± 0.06</td>
<td>4.93 ± 0.34</td>
<td>24 ± 1</td>
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<tr>
<td>Period 2</td>
<td>123 ± 4&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>1.14 ± 0.08&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>5.67 ± 0.34&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>25 ± 1</td>
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<tr>
<td>Glycine + (l)-NMMA (N = 8)</td>
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<tr>
<td>Baseline</td>
<td>112 ± 2</td>
<td>1.11 ± 0.04</td>
<td>4.45 ± 0.21</td>
<td>25 ± 1</td>
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<td>Period 1</td>
<td>126 ± 3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.13 ± 0.07</td>
<td>4.21 ± 0.17</td>
<td>26 ± 1</td>
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<tr>
<td>Period 2</td>
<td>135 ± 5&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>1.21 ± 0.04</td>
<td>3.97 ± 0.28</td>
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<td>Mixed AA + V (N = 8)</td>
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<tr>
<td>Baseline</td>
<td>113 ± 2</td>
<td>1.18 ± 0.04</td>
<td>4.54 ± 0.10</td>
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<tr>
<td>Period 1</td>
<td>118 ± 4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.10 ± 0.06</td>
<td>4.58 ± 0.23</td>
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<td>1.46 ± 0.08&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>5.50 ± 0.35&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>27 ± 1</td>
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<td>Mixed AA + (l)-NMMA (N = 8)</td>
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<tr>
<td>Baseline</td>
<td>108 ± 2</td>
<td>1.22 ± 0.05</td>
<td>4.59 ± 0.35</td>
<td>27 ± 2</td>
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<tr>
<td>Period 1</td>
<td>123 ± 0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.19 ± 0.05</td>
<td>4.16 ± 0.24</td>
<td>29 ± 2</td>
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<tr>
<td>Period 2</td>
<td>120 ± 5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.40 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.78 ± 0.23</td>
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<tr>
<td>Baseline</td>
<td>110 ± 2</td>
<td>1.16 ± 0.02</td>
<td>4.43 ± 0.19</td>
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<tr>
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<td>4.75 ± 0.25</td>
<td>24 ± 1</td>
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<tr>
<td>Period 2</td>
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<td>1.13 ± 0.07</td>
<td>4.44 ± 0.16</td>
<td>28 ± 1</td>
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<tr>
<td>Dextrose + (l)-NMMA (N = 6)</td>
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<tr>
<td>Baseline</td>
<td>116 ± 2</td>
<td>1.12 ± 0.05</td>
<td>4.15 ± 0.25</td>
<td>27 ± 1</td>
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<tr>
<td>Period 1</td>
<td>135 ± 4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.12 ± 0.06</td>
<td>4.01 ± 0.19</td>
<td>28 ± 1</td>
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<tr>
<td>Period 2</td>
<td>134 ± 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.21 ± 0.04</td>
<td>4.37 ± 0.19</td>
<td>28 ± 1</td>
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<sup>a</sup> Values are means ± SE. FF, filtration fraction.

<sup>b</sup> \(P < 0.05\) versus baseline.

<sup>c</sup> \(P < 0.05\) versus period 1.

Results given \(V\) or \(l\)-NMMA were compared by paired \(t\) test, whereas group values were compared by unpaired \(t\) test. Baseline and experimental values within groups presented in Table 1 were analyzed by one factor analysis of variance with repeated measures; treatment means were compared with the Scheffé \(F\) test. Comparisons of percent changes among groups were performed by single factor analysis of variance with the Scheffé \(F\) test. Where values were not normally distributed a nonparametric rank testing with the Kruskal-Wallis test was performed. Statistical significance was defined as \(P < 0.05\).

**RESULTS**

**Effects of \(l\)-NMMA on Basal Renal and Systemic Hemodynamics**

Infusion of \(l\)-NMMA led to a uniform increase in \(\overline{AP}\) which was significantly greater than that in \(V\) controls (111 ± 1 to 128 ± 2 versus 112 ± 1 to 117 ± 1 mm Hg, respectively; \(P < 0.05\)) (Figure 2). The rise in \(\overline{AP}\) generally achieved a plateau by 15 to 30 min and remained constant for the duration of the experiment (120 to 150 min). \(l\)-NMMA had no effect on \(GFR\) (1.16 ± 0.03 to 1.15 ± 0.03 mL/min; NS) but induced a slight but significant fall in \(RPF\) (4.4 ± 0.2 to 4.2 ± 0.1 mL/min; \(P < 0.05\)) (Figure 2). Values for \(GFR\) (1.17 ± 0.02 to 1.22 ± 0.04 mL/min; NS) and \(RPF\) (4.7 ± 0.1 to 4.7 ± 0.2 mL/min; NS) remained stable in \(V\)-infused controls. Because perfusion pressure, but not \(RPF\), increased with \(l\)-NMMA, the calculated renal vascular resistance rose significantly (Figure 2).

**Modulation of Response to Amino Acid Infusion by \(l\)-NMMA**

Glycine infusion led to marked increases in \(RPF\) (16 ± 4%) and \(GFR\) (33 ± 5%) (both \(P < 0.05\). Table 1 and Figure 3), in the absence of change in renal perfusion pressure or \(C\_A\) (5.2 ± 0.1 to 5.2 ± 0.1 g/dL; NS). Equimolar dextrose infusion had no significant effect on \(AP\), \(RPF\), or \(GFR\) in the presence or absence of \(l\)-NMMA (Table 1). However, \(l\)-NMMA obliterated the hyperemic response (7 ± 6 versus 16 ± 4% \(l\)-NMMA versus \(V\); \(P < 0.05\)) to glycine and, thus, markedly blunted the hyperfiltration response.
Renal Effects of L-NMMA

Figure 2. Effects of L-NMMA or vehicle infusion on AP, GFR, RPF, and renal vascular resistance (RVR). Results represent baseline values and values after a 30-min infusion of L-NMMA (N = 22) or V (N = 22). Values are means ± SE. *P < 0.05 versus baseline; †P < 0.05 versus V.

Figure 3. Percent change in AP, GFR, RPF, and filtration fraction (FF) after a 30-min infusion of AA or glycine after pretreatment with vehicle or L-NMMA. Values are means ± SE. *P < 0.05, L-NMMA versus V; †P < 0.05, glycine versus AA.

Glycine had no significant effect on the hypertensive response to L-NMMA (Table 1 and Figure 3) and did not change C_a (5.4 ± 0.1 to 5.3 ± 0.1 g/dL, Period 1 to 2; NS).

Mixed AA infusion resulted in increases in RPF (20 ± 4%) and GFR (30 ± 4%) (both P < 0.05 versus Period 1; Table 1, Fig. 3). Mixed AA had no significant independent effect on AP (Table 1) or C_a (5.5 ± 0.1 to 5.4 ± 0.1 g/dL). The percent rise in RPF was not significantly different in the L-NMMA group when compared with that in V (16 ± 6 versus 20 ± 4%, respectively; NS) nor was the change in C_a (−4 ± 1 versus −4 ± 1%, L-NMMA versus V; NS). However, the hyperfiltration induced by the amino acids was numerically blunted by pretreatment with L-NMMA (18 ± 4 versus 30 ± 4%, L-NMMA versus V; P = 0.056). This observed effect of L-NMMA may be an underestimate of the effectiveness of blockade (hence, of dependence on NO), because the mixed AA solution contained a substantial quantity of the competing substrate L-Arg (53 mM). Indeed, in three of the eight animals receiving L-NMMA plus mixed AA, AP had decreased to near baseline values by the end of the experiment, whereas none of the rats receiving L-NMMA plus glycine or dextrose had a subsequent decrease in AP.

The effects of L-NMMA on the renal hyperemic responses to glycine and mixed AA were strikingly different (Figure 3). The mixed AA-induced rise in RPF was minimally blunted by L-NMMA, whereas the hyperemic response to glycine was obliterated. The finding that glycine-induced hyperfiltration, albeit markedly blunted by L-NMMA, still persisted suggests that an increased glomerular transcapillary hydraulic pressure difference or a change in the glomerular capillary surface area may have offset the
fall in flow, a possibility supported by the finding of a 19% increase in filtration fraction in the presence of glicine plus L-NMMA (Period 1 to 2; P < 0.05). By contrast, with mixed AA plus L-NMMA, GFR and RPF fell more or less proportionately, as indicated by the resulting near constancy of filtration fraction (3 ± 4%; NS).

To help clarify the role of arginine in the response to the mixed amino acids, studies of L- and D-Arg infusions were performed. Doses of L-Arg which were equimolar to the glycine group (1,330 mM) failed to induce hyperfiltration (1.21 ± 0.13 to 1.21 ± 0.16 mL/min; N = 4; NS) or hyperemia (4.6 ± 0.3 to 4.7 ± 0.3 mL/min; NS). However, this infusion led to extreme diuresis (104 mL/min) potentially superimposing volume contraction; thus, lower doses were examined. L-Arg (660 mM; N = 5) led to no change in $\bar{A}$P (120 ± 4 to 126 ± 4 mm Hg) and a trend upward in GFR (11 ± 5%; 1.18 ± 0.07 to 1.30 ± 0.08 mL/min; P = 0.06) and in RPF (13 ± 6%; 4.7 ± 0.2 to 5.3 ± 0.3 mL/min; P = 0.09). D-Arg (660 mM; N = 4) led to an increase in $\bar{A}$P which was comparable to that induced by L-NMMA (15 ± 3 mm Hg; 122 ± 3 to 137 ± 5 mm Hg; P < 0.05), though it failed to change GFR (1.18 ± 0.05 to 1.18 ± 0.06 mL/min; NS) and RPF (5.2 ± 0.3 to 5.1 ± 0.1 mL/min; NS).

To assess the activation of soluble guanylate cyclase by NO, urinary cGMP excretion rates were measured in the rats given glycine with and without L-NMMA. After glycine, urinary cGMP rose by 274 ± 66% (6.1 ± 1.1 to 15.7 ± 2.4 pmol/min; P < 0.05) in rats pretreated with V as compared with 192 ± 18% (7.9 ± 0.6 to 14.6 ± 0.6 pmol/min; P < 0.05) in the L-NMMA group. Although the percent increase in cGMP excretion was significantly lower in the rats receiving L-NMMA, there was no significant difference in the final absolute excretion rates achieved.

**DISCUSSION**

The findings in this study suggest that NO production contributes to the maintenance of basal systemic vascular tone in the anesthetized euvoletic normotensive rat and also contributes to AA-induced renal hyperemia and hyperfiltration. The systemic pressor effect induced by L-NMMA in the basal state confirms previous findings in the rat, rabbit, and guinea pig and suggests that there is steady-state output of NO (5–7). This evidence, as well as the short half-life of the mediator, make this EDRF ideally suited for moment-to-moment modulation of systemic vascular resistance. Infusion of L-Arg alone failed to induce a hypotensive effect in the anesthetized rat, suggesting that in the unstimulated state, systemic production of NO is not limited by the availability of substrate. This confirms the findings of Aisaka et al. (15) and Baylis et al. (16), who showed that infusion of high concentrations of L-Arg alone did not induce hypotension in the anesthetized guinea pig or in the conscious rat, respectively. However, excess L-Arg prolonged the hypotensive effect of the endothelium-dependent vasodilator acetylcholine in the guinea pig. Conversely, the hypotensive effects of acetylcholine were abbreviated by L-NMMA, indicating ready access by both L-NMMA and L-Arg to the active NO synthetase (15). By contrast, transient hypotension in response to bolus doses of L-Arg (5 to 200 mg/kg) has been noted in conscious unrestricted rats, an effect which was significantly blunted by N$^\text{0}$-nitro-L-arginine (17). L-Arg-induced hypotension has been reported in humans; however, the role of EDRF in this response remains controversial (18,19). In the studies presented here, L-Arg failed to induce hypotension, whereas a significant rise in systemic pressure was seen with D-Arg infusion. The mechanism of this response remains to be determined.

The modest effects of L-NMMA on basal renal hemodynamics in this study suggest that in the anesthetized rat, the renal vasculature is less NO-dependent in the steady state than other resistance beds. Confirming these findings, others have demonstrated no change in GFR or RPF after bolus i.v. infusion of L-NMMA (15 mg/kg) in the anesthetized rat (20). In the absence of direct measurements, the possibility remains that L-NMMA led to offsetting effects on the determinants of renal perfusion and glomerular ultrafiltration and, thus, resulted in near constancy of RPF and GFR. Preliminary findings in normal rats indicate that L-NMMA raises the glomerular capillary hydraulic pressure by increasing efferent arteriolar resistance without changing the single nephron GFR (21). Indeed, the capacity of the renal vasculature of the rat and dog to respond to known endothelium-dependent vasodilators such as acetylcholine and bradykinin suggests that this vascular bed possesses the capability to produce large quantities of NO (22,23).

Renal hyperfiltration and hyperemia after AA infusion and/or protein loading have been recognized for over half a century (24,25). However, the mechanisms involved in this localized response remain unresolved (26). The study presented here demonstrated that similar molar doses of glicine or mixed AA induced hyperfiltration and renal hyperemia of approximately equivalent magnitude, whereas lower doses of L-Arg induced lesser changes in GFR and RPF. The vasodilatory effect appears to be specific for the renal vasculature, as $\bar{A}$P did not fall with either mixed AA, glycine, or L-Arg. In addition, confirming the findings of others (27,28), equimolar amounts of dextrose failed to induce such changes, arguing against a volume or osmolar effect as the mediator of the observed renal responses. The study presented here indicates that the renal effects of glicine and mixed AA are, at
least in part, sensitive to L-NMMA, suggesting that NO formation is one component of the chain of events which leads to postprandial renal hyperemia and hyperfiltration.

Glycine is not a nitrogen donor source for the formation of NO (29). The observed renal hyperemic and hyperfiltration responses must therefore reflect the activation of NO synthetase with the utilization of endogenous renal vascular NO donor sources, presumably L-Arg. Whether the stimulus for NO production indicates a direct effect of glycine on the endothelium or vascular smooth muscle and involves the release of intermediary substances is not yet known. However, infusions of glycine and other metabolizable AA into the isolated perfused kidney are known to induce vasodilation (30). In the study presented here, L-Arg-induced renal effects were modest and stereospecific. Our high dose failed to induce any change in whole kidney hemodynamics, whereas with still higher doses Baylis et al. (16) noted prominent renal hyperemia (+64%) without a significant effect on GFR in conscious rats. Several L-Arg salts (methylester > hydroxyamate > chloride), but not D-Arg, lead to renal vasodilation in the isolated perfused kidney (31). In addition, maneuvers which disrupted the endothelium or inhibited EDRF release significantly impaired this vasodilatory response (31). Taken together, these studies suggest a direct effect of these AA on the renal vasculature. Whether this effect is due to excess substrate for NO synthetase or occurs by another mechanism remains to be determined. Potentially, the renal response to a protein load is enhanced by the synergistic effects of the stimulated NO synthetase and the relative excess of L-Arg substrate.

L-Arg excess reverses the systemic effects of L-NMMA, due to competitive binding to the NO synthetase (5). The rate of L-Arg infusion (2.0 μmol/min) during the mixed AA experiments in this study exceeded that of L-NMMA (0.16 μmol/min) by more than 10-fold, which likely accounts for the lesser degree of blunting of the renal hyperemia and hyperfiltration observed in the mixed AA group as compared with that in the glycine rats. In support of this conclusion, preliminary studies have verified that hyperfiltration and hyperemia induced by a 10% mixed AA solution were obliterated by intrarenal L-NMMA (32).

Despite obliteration of the glycine-induced hyperemic response by L-NMMA, there was persistent, albeit markedly reduced, hyperfiltration. This finding, in conjunction with the observed 19% rise in filtration fraction, suggests that glycine, in the presence of L-NMMA, increased either the glomerular transcapillary hydraulic pressure gradient or the glomerular capillary ultrafiltration coefficient. Indeed, Zatz and de Nucci (21) observed that L-NMMA raises the glomerular capillary hydraulic pressure in normal rats. This raises the intriguing possibility that afferent and efferent arterioles may differ in their ability to produce NO or respond to stimuli for NO production. Indeed, the vasodilatory response to acetylcholine of isolated rabbit afferent arterioles was equivalent to that of efferent arterioles whereas, with another NO stimulator, bradykinin, the efferent arteriole failed to relax (22).

Urinary cGMP (UcGMP) output increased markedly in both the V- and L-NMMA-treated rats given glycine. Although the percent increase was less in the L-NMMA group than in the V rats, the final excretion rates were not different. In the absence of plasma levels it is not possible from our studies to determine with certainty the source of this cGMP. Mesangial cells in co-culture with either glomerular or aortic endothelial cells have been shown to release cGMP (11,33). Currently, it is not known to what extent cGMP produced by the renal microcirculation enters the urinary space. It is of interest that UcGMP excretion rates are 31% higher in humans after a meat meal as compared with that after equivalent sodium and water ingestion (34). However, this increase was not contemporaneous with the hemodynamic changes. Others have noted a threefold increase in UcGMP excretion after i.v. acetylcholine infusion which was prevented by L-NMMA pretreatment; however, plasma levels were not measured (20). Further studies are needed to determine the utility of UcGMP in the assessment of renal vascular tone.

Control of renal vascular smooth muscle tone is modulated by an array of circulating and local mediators. The dynamic nature of the renal microcirculation, which permits the glomerulus to adapt to a wide variety of conditions, depends on the availability of both vasodilating and vasoconstricting mediators, the balance of which ultimately determines the renal vascular resistance. Only recently has the endothelial monolayer been recognized to be an important transducer of both chemical and mechanical signals into appropriate changes in vascular smooth muscle tone. We postulate that AA infusion results in a series of adaptations which alter the balance of vasodilating and vasoconstricting factors leading to a reduction of renal vascular resistance. The present finding that this vasodilation is, at least in part, sensitive to L-NMMA, suggests that NO plays a role in this renal hyperemic and hyperfiltration response.

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REFERENCES