Amino acids induce indicators of response to injury in glomerular mesangial cells

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Meek, Rick L., Sheryl K. Cooney, Stephanie D. Flynn, Robert F. Chouinard, Maria H. Poczatek, Joanne E. Murphy-Ullrich, and Katherine R. Tuttle. Amino acids induce indicators of response to injury in glomerular mesangial cells. Am J Physiol Renal Physiol 285: F79–F86, 2003. First published March 18, 2003; 10.1152/ajprenal.00419.2002.—High-protein diets exacerbate glomerular hyperfiltration and the progression of diabetic nephropathy. The purpose of this study was to determine whether amino acids also produce nonhemodynamic injury in the glomerulus. When rat mesangial cells were cultured with an amino acid mixture designed to replicate the composition in plasma after protein feeding, production of mRNA (Northern blot analysis) and/or protein (ELISA or Western blot analysis) for transforming growth factor-β1 (TGF-β1), fibronectin, thrombospondin-1 (TSP-1), and collagen IV were enhanced in a manner comparable to a culture with high glucose (30.5 mM). The bioactive portion of total TGF-β (NKK assay) increased in response to amino acids. The TSP-1 antagonist LSKL peptide reduced bioactive TGF-β and fibronectin, indicating the dependence of TGF-β1 activation on TSP-1. DNA synthesis ([3H]thymidine incorporation), an index of cellular proliferation, increased in response to amino acids and was further enhanced by culture with increased levels of both amino acids and glucose. TGF-β1 and matrix proteins increased when mesangial cells were cultured with excess l-arginine (2.08 mM) alone. Although l-arginine is the precursor of nitric oxide (NO), such responses to amino acids do not appear to be mediated through increased NO production. NO metabolites decreased in the media, and these responses to mixed amino acids or l-arginine were not prevented by NO synthase inhibition. In conclusion, amino acids induce indicators of response to injury in mesangial cells, even when hemodynamic stress is absent. In conditions associated with increased circulating amino acids, such as diabetes and/or a high-protein diet, direct cellular effects could contribute to glomerular injury.

transforming growth factor-β; thrombospondin-1; extracellular matrix proteins; mesangial cell proliferation; hyperglycemia

HIGH-PROTEIN DIETS, RESULTING in increased circulating amino acids, produce glomerular hyperfiltration and hypertension, major mechanisms of progressive renal injury (5, 47). In the clinical setting of diabetes, this is a particular concern because high-protein diets have been promoted to modulate hyperglycemia or to promote weight loss. Furthermore, poor glycemic control also increases plasma amino acid concentrations (10). As such, hyperaminoacidemia is common in diabetes and could exacerbate renal injury. The glomerular hyperfiltration associated with increased circulating amino acids is similar to that induced by hyperglycemia (5, 47). In clinical studies, we have demonstrated that people with poorly controlled type 1 or type 2 diabetes have an augmented glomerular hyperfiltration response to amino acids, which can be corrected by strict glycemic control (41–43). The mechanism(s) of the interaction between hyperglycemia and amino acids is unknown. Hormonal mediators have been proposed, but none has been identified (43). Whether amino acids have direct cellular effects that enhance or cause injury is unexplored.

The mesangial cell is a key cell involved in the control of glomerular hemodynamics, as well as in the response to injury. Recognized causes of injury, such as hyperglycemia, have both hemodynamic and direct cellular effects. In vitro models utilizing mesangial cell culture have shown that cellular stretch (a model of glomerular hypertension) or high-glucose media (a model of hyperglycemia) induce production of transforming growth factor-β (TGF-β) (19, 31, 48). TGF-β is a profibrotic cytokine that is a seminal cellular mediator of response to injury (36). TGF-β is secreted in an inactive form, which can be activated by diverse mechanisms, including interactions with the matrix protein thrombospondin-1 (TSP-1) (20). In mesangial cells cultured with high-glucose media, TSP-1 expression is also elevated and enhances TGF-β bioactivity (28, 39). The potential influence of amino acids on these processes has not been evaluated.

Either a mixture or certain individual amino acids could have renal effects. l-Arginine is a plausible candidate because it is the precursor of nitric oxide (NO). NO has many important functions in the kidney, in-
cluding promoting vasodilation, as which may enhance
glomerular hyperfiltration, as well as direct cellular
effects, which may be protective or injurious. Depend-
ing on the experimental system, increased NO has
been variably reported to either decrease or increase
TGF-β production and fibrosis (9, 21, 37). In mesangial
cells cultured with a high-glucose concentration, stim-
ulation of NO production through endogenous (inter-
leukin-1β) or exogenous (NO donor) methods pre-
vented TGF-β induction (9). Furthermore, suppression
of NO by hyperglycemia has been reported to increase
TGF-β activation and mesangial matrix production
(40, 44). In mesangial cells, it is unclear to what extent
L-arginine functions as an NO precursor without cyto-
kine stimulation of inducible NO synthase, and possi-
ble relationships among amino acids, NO, and TGF-β
have not been studied.

The overall goal of this study was to determine
whether amino acids, in the absence of hemodynamic
stress, produce mesangial cell responses associated
with the type of glomerular injury observed in dia-
betes. A mixture of amino acids, designed to replicate
the composition in plasma after protein feeding, was evalu-
ated for effects on expression of TGF-β1 and matrix
proteins as well as on mesangial cell proliferation and
viability (other indicators of response to injury). These
effects were explored with and without a concomitant
high-glucose concentration. Whether TSP-1 influences
amino acid-induced TGF-β bioactivity was assessed by
use of an inhibitory peptide. The effects of L-arginine
on NO and expression of TGF-β1 and matrix proteins
were also determined.

METHODS

Mesangial Cell Isolation and Culture

Rat mesangial cells were recovered from glomeruli isolated
by sieving the cortex of kidneys excised from 6-month-old female
Sprague-Dawley rats. Mesangial cells were grown in DMEM
(Life Technologies, Gaithersburg, MD) supplemented with penicillin-streptomycin (100 U/ml) and 10% heat-inactivated
fetal bovine serum (Summit Technologies, Ft. Collins, CO) in
a humidified atmosphere of 5% CO2 at 37°C. Cells were
passaged at confluence using 0.025% trypsin (Life Technolo-
gies) in PBS and used for experiments between passages 4–6
and 14–19.

Experimental Conditions

Increased levels of amino acids and glucose. Mesangial
cells were seeded into 100-mm dishes or 24-well plates (Nun-
clon, Cambridge, MA) at 10,000 cells/cm2. When cells reached
confluence, they were made quiescent in serum-free DMEM
for 48 h. Cells were then exposed to experimental conditions
for 48 h (Table 1): 1) control, serum-free DMEM; 2) increased
amino acids, serum-free DMEM supplemented with 10% 
Travasol mixed amino acid solution (Baxter, Deerfield, IL)
and L-arginine; addition of Travasol and L-arginine, which
increased individual amino acid concentrations 1.5- to 6-fold
and raised osmolarity 13.3 mosM, relative changes compara-
able to those observed in plasma after a protein meal (3); 3) high-glucose, serum-free DMEM containing 30.5 mM glu-
cose; 4) a combination of increased amino acids and high

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Control/High Glucose, mM</th>
<th>Increased Amino Acids/Combination, mM</th>
<th>Increased L-arginine, mM</th>
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</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>5.6/30.5</td>
<td>5.6/30.5</td>
<td>5.6</td>
</tr>
<tr>
<td>L-Alanine</td>
<td>0</td>
<td>3.101</td>
<td>0</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>0.40</td>
<td>2.48</td>
<td>2.08</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>0.32</td>
<td>0.32</td>
<td>0.32</td>
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<tr>
<td>L-Glutamine</td>
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<td>4.00</td>
<td>4.00</td>
</tr>
<tr>
<td>L-Glycine</td>
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</tr>
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<td>0.20</td>
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<td>L-Isoleucine</td>
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<td>1.41</td>
<td>0.80</td>
</tr>
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<td>L-Leucine</td>
<td>0.80</td>
<td>1.54</td>
<td>0.80</td>
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<td>0.80</td>
<td>1.22</td>
<td>0.80</td>
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<td>L-Methionine</td>
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<tr>
<td>L-Phenylalanine</td>
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<td>L-Proline</td>
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<td>0</td>
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<td>L-Serine</td>
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<td>L-Threonine</td>
<td>0.80</td>
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<td>L-Tryptophan</td>
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<tr>
<td>L-Tyrosine</td>
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<tr>
<td>L-Valine</td>
<td>0.80</td>
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</table>

Amino acid concentrations in control and high-glucose conditions
were those found in standard DMEM.

Increased levels of amino acids with TSP-1 inhibitory pep-
tide. In separate experiments, LSKL peptide (AnaSpec, San
Jose, CA), a selective antagonist of TSP-1, and SLLK peptide
(AnaSpec), an inert control, were used to evaluate effects of
TSP-1 on TGF-β bioactivity (30). Confluent mesangial cells
were cultured with serum-free DMEM for 48 h. Cells were
then exposed to the following conditions for 48 h: 1) control,
serum-free DMEM; 2) control plus LSKL peptide (5 μM); 3)
control plus SLLK peptide (5 μM); 4) increased amino acids
(Table 1); 5) increased amino acids plus LSKL peptide (5 μM);
and 6) increased amino acids plus SLLK peptide (5 μM).
The conditioned media were removed and 2 μg/ml each of the
protease inhibitors pepstatin, leupeptin, antipain, and apro-
tinin (Sigma, St. Louis, MO) were added. The media were
then stored at −70°C until assayed for TGF-β bioactivity and
fibronectin protein.

Increased level of L-arginine and NO synthase inhibition.
Confluent mesangial cells were made quiescent in serum-free
DMEM for 48 h. Cells were then placed in experimental
conditions for 48 h (Table 1): 1) control, serum-free DMEM; 2)
increased amino acids; and 3) L-arginine (2.08 mM). Each of
these conditions was also prepared with the addition of the
NO synthase inhibitor Nω-monomethyl-L-arginine (L-
NMMA; 1 mM) to examine whether NO synthesis contrib-
uted to the experimental outcomes.

Outcome Measurements

mRNA. Total RNA was isolated from mesangial cell mono-
layers utilizing Tri Reagent (Molecular Research Center,
Cincinnati, OH) and quantified by UV spectrophotometry
(Beckman DU 660, Beckman Coulter, Fullerton, CA).

Probes specific for rat sequences published in the GeneBank
database were obtained by RT-PCR. TGF-β1; cDNA is from
nucleotides 818–1315 of sequence X52498. Fibronectin cDNA
is from nucleotides 5372–5636 of sequence X15906 (EIIa
splice variant associated with TGF-β bioactivity). α1(IV) Col-
lagen is from nucleotides 11–421 of sequence AA924749.
Collagen IV protein was not measured because an antibody capable of recognizing the rat protein was not available. Multiple antibodies to human or mouse collagen IV from commercial sources were tested, but none had cross-reactivity with rat collagen IV in our ELISA and Western blot systems.

**TGF-β bioactivity.** Both total and bioactive TGF-β in conditioned media were measured by colony formation of normal rat kidney cells (NRK-49F, American Type Culture Collection, Manassas, VA) in soft agar assay, as previously described (33). Baseline colony formation was measured in wells incubated with 2.5 ng of epidermal growth factor in fresh media.

Mesangial cell proliferation. Incorporation of [3H]thymidine was used to measure DNA synthesis as an index of mesangial cell proliferation. Cells were grown in 24-well plates and exposed to 4 μCi [3H]thymidine/ml of media (NEN Life Sciences, Boston, MA) for the last 4–8 h of treatment. Cells were washed three times and incubated with ice-cold 10% trichloroacetic acid for 15 min. Fixed cell layers were solubilized with 0.4 M NaOH at 60°C for 10 min and neutralized with 0.2 M glacial acetic acid. Each 100-μl sample was diluted in 0.2 M NaOH and vacuum blotted onto a nylon membrane (Immobilon NY+, Millipore, Bedford, MA), air-dried, and exposed to a 1H-sensitive phosphor screen. Data were collected from the screen with the Cyclone Storage Phosphor System and analyzed using Optiquant software (Packard Instruments). Total cellular protein was measured with a DC Protein Assay Kit (Bio-Rad). Absorbance was measured on a Bio-Tek FL-600 plate reader (Bio-Tek Instruments). Data were reported in digital light units per square millimeter per microgram of cell protein.

**NO metabolites.** As an index of NO in conditioned media, nitrite and nitrate were measured using a fluorometric NO assay kit (Calbiochem, San Diego, CA). Fluorescence was determined with a Bio-Tek FL-600 fluorescence plate reader (Bio-Tek Instruments) using an excitation wavelength of 365 nm and an emission wavelength of 460 nm. Nitrate/nitrite were expressed as the ratio to total media protein (nmol/μg).

**Cell viability.** Cell viability was assessed using an In Vitro Toxicology Assay Kit (Sigma) for lactate dehydrogenase (LDH). Cells were grown in 24-well plates and treated as previously described. Conditioned media were collected at the end of the experimental periods, and the remaining cell layer was lysed with kit reagent. LDH values obtained from the media and the lysed cell layer were combined for total LDH. Media LDH was expressed as a percentage of total LDH.

**Statistics.**

Data are expressed as means ± SE. One-way ANOVAs were used to evaluate the various outcome measurements in response to experimental conditions. Two-way ANOVAs were used to evaluate differences between the group with increased amino acids and control and TSP-1 peptide groups for total and bioactive TGF-β. Specific tests between conditions were conducted using a priori contrasts. Statistics were computed using SPSS, version 10 (SPSS, Chicago, IL). Probabilities <5% (P < 0.05) were considered statistically significant.

**RESULTS.**

**TGF-β1 and Matrix Proteins in Response to Increased Amino Acids, High-Glucose, or the Combination Condition.**

Increased amino acids enhanced expression of all genes in a manner similar to high glucose (Fig. 1). The
combination condition of increased amino acids and high glucose increased mRNA for TGF-β1, α1(IV) collagen, and TSP-1. A similar trend was noted for fibronectin, but the change from control did not reach statistical significance (P = 0.06). The effects of the combination condition were not significantly different from either elevated levels of glucose or amino acids alone. Mannitol treatment, designed to produce levels of osmolarity similar to experimental conditions, did not increase mRNA for the various proteins (data not shown). TGF-β1 (ELISA), fibronectin, and TSP-1 proteins increased in parallel with the mRNA results (Table 2).

Table 2. Transforming growth factor-β1 and matrix proteins in response to experimental conditions

<table>
<thead>
<tr>
<th>Protein</th>
<th>Increased Amino Acids</th>
<th>High Glucose</th>
<th>Combination</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGF-β1 (n = 4)</td>
<td>1.28 ± 0.05*</td>
<td>1.17 ± 0.08*</td>
<td>1.14 ± 0.08</td>
</tr>
<tr>
<td>Fibronectin (n = 11)</td>
<td>1.58 ± 0.02*</td>
<td>1.60 ± 0.22†</td>
<td>1.38 ± 0.12†</td>
</tr>
<tr>
<td>TSP-1 (n = 6–8)</td>
<td>1.36 ± 0.08*</td>
<td>1.32 ± 0.08*</td>
<td>1.82 ± 0.29*</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed as fold-change from control. *P < 0.05, †P < 0.01: increase in experimental groups over control.

TSP-1-Induced Activation of TGF-β by Increased Amino Acids

Both total and bioactive TGF-β (NRK assay) were elevated in the conditioned media of the increased amino acids group (Fig. 2, A and B). Total TGF-β increased 1.88 ± 0.34-fold (P = 0.006), whereas bioactive TGF-β increased 2.68 ± 0.21-fold (P < 0.001). As a result, bioactive TGF-β was proportionally greater in the group that received increased amino acids compared with control [48.9 ± 6.3 vs. 30.5 ± 1.7% (P = 0.021)]. A parallel increase in media fibronectin protein was observed [4.77 ± 0.30 and 2.67 ± 0.20 ng/μg (P < 0.001)] in the increased amino acids and control groups, respectively. The TSP-1 antagonist LSKL peptide decreased the increment in bioactive TGF-β without changing total TGF-β in the increased amino acids group.
Effects of the amino acid mixture or L-arginine alone, with or without nitric oxide synthase inhibition, on TGF-β₁ and matrix proteins

### Table 3.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Increased Amino Acids</th>
<th>L-Arginine</th>
<th>Increased amino acids</th>
<th>L-Arginine</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGF-β₁ mRNA (n = 8–11)</td>
<td>1.47 ± 0.11†</td>
<td>1.58 ± 0.16*</td>
<td>1.66 ± 0.27*</td>
<td>1.08 ± 0.06</td>
</tr>
<tr>
<td>Protein (n = 8–12)</td>
<td>1.41 ± 0.11†</td>
<td>1.31 ± 0.06†</td>
<td>1.27 ± 0.09*</td>
<td>1.21 ± 0.06†</td>
</tr>
<tr>
<td>Fibronectin mRNA (n = 8–11)</td>
<td>1.31 ± 0.13*</td>
<td>1.25 ± 0.13*</td>
<td>1.26 ± 0.07†</td>
<td>1.12 ± 0.11</td>
</tr>
<tr>
<td>Protein (n = 8)</td>
<td>1.58 ± 0.20*</td>
<td>1.56 ± 0.14†</td>
<td>1.28 ± 0.09*</td>
<td>1.36 ± 0.13†</td>
</tr>
<tr>
<td>TSP-1 mRNA (n = 8–11)</td>
<td>1.19 ± 0.06†</td>
<td>1.33 ± 0.08†</td>
<td>1.09 ± 0.10</td>
<td>1.63 ± 0.12*</td>
</tr>
<tr>
<td>Protein (n = 7–8)</td>
<td>1.85 ± 0.29†</td>
<td>1.61 ± 0.14†</td>
<td>2.28 ± 0.58*</td>
<td>2.37 ± 0.35</td>
</tr>
<tr>
<td>α₁(IV) Collagen mRNA (n = 6–10)</td>
<td>1.63 ± 0.17*</td>
<td>1.75 ± 0.24*</td>
<td>1.54 ± 0.08*</td>
<td>1.36 ± 0.11*</td>
</tr>
</tbody>
</table>

Values are means ± SE for mRNA and proteins displayed as fold-change from control. *P < 0.05; †P < 0.01; ‡P < 0.001 compared with the respective values in the group that received only increased amino acids. No. of measurements: L-NMMA, N⁶-monomethyl-L-arginine. Experiments with and without L-NMMA had separate control groups.

Increased L-arginine raised mRNA and protein levels for TGF-β₁ (ELISA), fibronectin, and TSP-1, and mRNA for α₁(IV) collagen in a manner comparable to the amino acid mixture group (Table 3). Both conditions also resulted in decreased NO metabolites in the conditioned media: 160 ± 18 vs. 125 ± 10 and 101 ± 10 nmol/µg (P = 0.015 and P = 0.025) in control, amino acid mixture, and L-arginine groups, respectively. Addition of the NO synthase inhibitor L-NMMA did not consistently influence TGF-β₁ or matrix proteins in either the amino acid mixture or L-arginine condition (Table 3). When the amino acid mixture group also received L-NMMA, mRNA and protein for TGF-β₁ and fibronectin and α₁(IV) collagen mRNA remained above control levels. For TSP-1, the protein level was greater than control, but the mRNA level was not. None of these values was significantly different from those in the amino acid mixture group that did not receive NO synthase inhibition. When L-NMMA was added to the L-arginine group, TGF-β₁ and fibronectin proteins were greater than control, but mRNA levels were not. To the contrary, TSP-1 and α₁(IV) collagen mRNA remained greater than control. Although TSP-1 protein was numerically larger than control, it was not statistically significant (P = 0.074). None of these was significantly different from the respective values in the group treated with only L-arginine. In summary, no overall pattern of response to support a role for increased NO production was observed.

**Effects of Amino Acids on Mesangial Cell Proliferation and Viability**

The culture of mesangial cells with increased amino acids raised [³H]thymidine incorporation in a manner comparable to that induced by high glucose (Fig. 3). The combination condition increased [³H]thymidine incorporation more than either condition alone. The mixture of amino acids did not adversely affect mesangial cell viability, as determined by LDH release into the media [11 ± 4 vs. 6 ± 1% in control (P = 0.142)]. However, the high-glucose and combination conditions did increase media LDH compared with control [23 ± 2 and 24 ± 5%, respectively (P < 0.001 for both values vs. control)].
DISCUSSION

The present data demonstrate that amino acids have adverse effects beyond the renal hemodynamic changes long associated with excess protein in the diet. In glomerular mesangial cells, production and activation of TGF-β and expression of matrix proteins (fibronectin, collagen IV, and TSP-1) occurred in response to increased amounts of amino acids designed to replicate those in plasma after protein feeding. The effects of increased amino acids were similar in magnitude to those induced by the high-glucose condition. L-Arginine alone enhanced expression of TGF-β1 and matrix proteins to the same extent as the amino acid mixture and high-glucose conditions. Even though L-arginine is an NO precursor, NO metabolites decreased in the conditioned media of both the L-arginine alone and amino acid mixture groups. In addition, increased levels of amino acids augmented DNA synthesis as much as observed in response to a high-glucose level and, with the combination condition, this index of cellular proliferation was even more pronounced.

High-protein diets exacerbate progressive renal disease, particularly in diabetes (5, 47). Conversely, diets low in protein reduce the progression and, most importantly, decrease the risk of end-stage renal disease and death in diabetic nephropathy (14, 24, 47). Furthermore, when combined with renin-angiotensin system blockade, a low-protein diet has the additive effect of reducing proteinuria (11, 26, 32). These reports and others indicate that dietary protein has effects other than those related to glomerular hypertension or renin-angiotensin system activation, but the mechanisms are still unclear (11, 23, 26, 32). Our data provide new evidence that amino acids have direct cellular effects associated with glomerular injury. However, we cannot exclude involvement of the renin-angiotensin system in the responses of mesangial cells to amino acids. Mesangial cells can produce angiotensin II, especially when stretched or cultured with an increased amount of glucose (4, 38). In addition, in cultured mesangial cells, we recently found that increased amino acids approximately doubled the amount of mRNA for the angiotensin II type 1 receptor and reduced expression of aminopeptidase A, a metalloprotease that degrades angiotensin II (8). These responses could potentiate effects of angiotensin II, including stimulation of TGF-β production and mesangial cell proliferation (2, 18, 29).

Increased mesangial cell DNA synthesis in response to amino acids is another new finding in this study. However, unlike the high-glucose condition, the amino acid mixture did not adversely affect mesangial cell viability, nor did it worsen the reduced cell viability associated with high glucose. In addition to mesangial matrix expansion, cell growth is a common feature of diabetic nephropathy (45). The augmented DNA synthesis in response to the combination condition suggests that, when high circulating levels of glucose and amino acids coexist, a common occurrence in diabetes, mesangial cell proliferation could be exacerbated in an additive fashion. In a recent preliminary report, similar findings were observed for the single amino acid glutamine, alone or in combination with a high-glucose level (35). In our experimental system, glutamine was not further increased by the addition of mixed amino acids because it is not present in the standard solution for hyperalimentation (Travasol). This solution was chosen to produce an in vitro condition similar to clinical hyperaminoacidemia. Nevertheless, even without further increasing glutamine, increases in other amino acids augmented mesangial cell DNA synthesis. These data suggest that common features among amino acids could lead to this consequence.

TGF-β is secreted in an inactive form due to a noncovalent association with the latency-associated peptide. Although the mechanism of activation is not fully understood, TSP-1 can interact with the latency-associated peptide to liberate active TGF-β (20, 30, 33). Either short- or long-term exposure of mesangial cells to a high-glucose level increases TGF-β bioactivity by such a TSP-1-dependent mechanism (28, 46). In the present study, TSP-1 induced by amino acids also activated TGF-β and enhanced production of the matrix proteins fibronectin. Of particular interest, amino acid-induced TGF-β production and activation and matrix protein (fibronectin, collagen IV, and TSP-1) expression were nearly identical to responses produced by the high-glucose condition. However, the combination of elevated levels of amino acids and glucose did not further enhance expression of TGF-β1 or these matrix proteins. Taken together, the available data suggest that the mechanism(s) leading to amino acid- or glucose-induced responses may be redundant or shared.

High-glucose concentrations have previously been reported to decrease NO and promote TSP-1-dependent TGF-β bioactivity in mesangial cells (40, 44). Similarly, we found that NO metabolites in the conditioned media decreased when mesangial cells were cultured with either the amino acid mixture or L-arginine. This may seem paradoxical because L-arginine is the precursor for NO. In addition, production of TGF-β1 and matrix proteins increased as much in response to L-arginine as with the amino acid mixture. No clear directional effect of L-NMMA on these outcomes was observed, further supporting the contention that the mechanism(s) in this system is not likely to be dependent on increased NO production. The increase in TGF-β itself could conceivably reduce production of NO by inhibiting transcription of inducible NO synthase, as has been observed in vascular smooth muscle cells stimulated by interleukin-1β (25). However, in mesangial cells, the contribution of inducible NO synthase to the production of NO is uncertain in the absence of cytokine stimulation. The quenching of NO by reactive oxygen species could also explain the decrease in NO metabolites in the conditioned media (6, 16). Indeed, glutamine has recently been reported to enhance superoxide production by neutrophils (27). Similarly, in recent experiments performed in our laboratory, we have found an increase in hydrogen perox-
Amino acids and oxidative stress, which could be a mechanism for quenching NO and promoting injury via the TGF-β pathway. We have demonstrated that amino acids may directly cause or enhance mesangial cell responses associated with fibrosis and progressive renal disease. Such effects could add to glomerular injury, especially in diabetes, a condition where high levels of circulating amino acids, as well as hyperglycemia, are common metabolic disturbances.

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