Protein intake regulates the vasodilatory function of the kidney and NMDA receptor expression

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Slomowitz, Larry A., Francis B. Gabbai, Ser J. Khang, Joseph Satriano, Sonia Thareau, Aihua Deng, Scott C. Thomson, Roland C. Blantz, and Karen A. Munger. Protein intake regulates the vasodilatory function of the kidney and NMDA receptor expression. Am J Physiol Regul Integr Comp Physiol 287: R1184–R1189, 2004. First published July 8, 2004; doi:10.1152/ajpregu.00169.2003.—Glycine infusion in normal rats causes an increase in renal plasma flow and glomerular filtration rate (GFR). Although the renal response to glycine infusion is well characterized, the mechanism initiating this vasodilation is unknown. We recently observed functionally active N-methyl-d-aspartate (NMDA) receptors in the kidney, located primarily in tubular structures. The mechanisms regulating activity of the NMDA receptor within the kidney are also unknown, as is its normal day-to-day functional role. Therefore, we hypothesize that dietary protein may impact the functional response to glycine infusion in both untreated rats and rats pretreated with angiotensin-converting enzyme (ACE) inhibitor and, furthermore, that renal NMDA receptors may be involved in the glycine response. Surprisingly, 2 wk of low-protein diet (8% protein vs. 21% protein in control diet) totally inhibited the glycine-induced vasodilation and GFR response. Associated with the absence of renal vasodilation, a significant reduction in proximal tubular reabsorption was observed during glycine infusion in low-protein-diet rats. In contrast to the disease models previously studied in our laboratory, administration of ACE inhibitors did not restore the glycine response in rats treated with low-protein diet. Western blots of normal- and low-protein-diet kidneys demonstrate that the newly described renal NMDA receptor is downregulated in rats fed a low-protein diet. Low-protein feeding results in loss of glycine-induced vasodilation and GFR responses associated with decreased renal NMDA receptor expression. Kidney NMDA receptor expression is conditioned by protein intake, and this receptor may play an important role in the kidney vasodilatory response to glycine infusion and protein feeding in rats.

glycine; N-methyl-d-aspartate receptor; renal hemodynamics; dietary protein

Glycine infusion profoundly increases renal blood flow by mechanisms that are not completely understood. Absence of a vasodilatory response in various experimental conditions [nitric oxide (NO) blockade in normal rats, experimental models of hypertension, diabetes mellitus, chronic glomerulonephritis, cyclosporine administration] is characterized by a significant decrease in proximal tubular reabsorption during amino acid infusion (5, 6, 8, 9). We and others have been interested in the mechanism by which protein feeding and amino acid infusion increase glomerular filtration rate (GFR) and renal blood flow (1, 5, 14, 15). We showed that angiotensin and NO are involved in the response, but these factors do not entirely explain the phenomenon. We know that diabetes, which is a state of high-protein intake for the rat, abolishes the glycine-induced renal vasodilation. Periodic eating of protein is known to result in increases in GFR, and it has been postulated that constant eating of high protein is associated with glomerular hyperfiltration (18). We also showed that whether the amino acid is a branched chain or nonbranched chain, amino acid affects the renal hemodynamic response (17). Therefore, type of proteins, the extent of hypertrophy and underlying disease state, and the activity of the NO and angiotensin II systems all contribute to the overall effect on renal hemodynamics and the response to amino acid infusions.

The N-methyl-d-aspartate (NMDA) receptor is a diheteromeric receptor complex that functions as a membrane calcium channel. Research in this area has been primarily conducted in central nervous system tissue, in which NMDA activation results in calcium entry and the stimulation of neuronal NO synthase (nNOS) activity. Glutamate and glycine are the major agonists leading to activation and channel opening. Recently, we demonstrated the presence of renal NMDA receptors and observed significant functional effects of inhibition of the NMDA receptor in the kidney that are not related to central nervous effects (11). Inhibition of these receptors caused marked renal vasoconstriction and a reduction in renal blood flow. The renal blood flow/GFR response to one of the normal agonists, glycine, which normally increases renal blood flow as discussed above, was nearly abolished in rats pretreated with two different types of NMDA receptor antagonists (11).

The current studies were devised to determine whether a chronic low-protein diet (LPD) would modify the glycine response and whether the renal NMDA receptor would be regulated by this maneuver. We examined the effects of prolonged restriction of protein intake to determine 1) whether the kidney response to glycine was altered by dietary protein intake, 2) whether this response is modified by angiotensin-converting enzyme (ACE) inhibitors as previously demonstrated by us (5, 6), and 3) whether NMDA receptor expression was altered by this maneuver. Therefore, we took a two-pronged approach by examining the expression of NMDA receptors by Western blot analysis and tested and evaluated the functional response to glycine in animals on normal diet and LPD and animals on the latter diet treated with an ACE inhibitor.

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MATERIALS AND METHODS

Male Wistar rats (Harlan) weighing 250–350 g were used in this study. Rats were placed on a LPD (8% protein, Low Renal Load Diet, no. 960331, ICN Biomedicals, n = 8). This diet has added trimethionine (0.3%) to maintain the needed levels of this amino acid in rats. Control rats were fed the normal rat chow (21% protein, Harlan TecLab, n = 7). After 2 wk of diet, renal function was evaluated by clearance and micropuncture methods. A second group of rats on LPD received the ACE inhibitor captopril (75 mg/l; Sigma, St. Louis, MO) in the drinking water for 5 days before micropuncture studies. Kidney tissues were harvested from separate groups of rats receiving the identical diets for measurement of NMDA receptor protein by Western blot.

Micropuncture Protocol

The rats were anesthetized with Inactin (10 mg/100 g body wt ip BYK; Konstanz, Germany) and surgically prepared as previously described in our laboratory (7, 16). Briefly, the rats underwent tracheostomy and cannulation of the left jugular vein, left femoral artery, and bladder. The left kidney was exposed via a flank incision and placed in a Lucite cup. The cup surrounding the kidney was packed with cotton and 2% agar and the surface was covered with heated (37°C) NaCl-NaHCO3 solution. The femoral artery catheter was used for periodic blood samples and monitoring of mean arterial pressure (MAP) with a transducer (model P23db, Statham Instruments, Gould Division, Hato Rey, Puerto Rico) connected to a desktop computer loaded with the WinDaq software (DATAQ Instruments, Akron, OH). All studies were performed in euvolemic state by infusing 1% body wt donor plasma over a 1-h period followed thereafter by 0.15% body wt donor plasma/h iv. All rats received two additional infusions of NaCl-NaHCO3 solution, one containing [3H]inulin at a rate of 110 μCi/h in a volume of 0.8 ml/h iv and the other (1.4 ml/h) serving as control for the solution containing glycine administered during the second period. Both solutions were initiated at the end of the surgical preparation and were maintained throughout the experiment.

The experiments were designed as two period experiments with baseline measurements in both control diet rats and those receiving LPDs for 2 wk. After the baseline measurements, the rats were infused with glycine, given as a 20% solution in NaCl-NaHCO3 at 1.4 ml/h iv, and after 30 min of infusion, micropuncture measurements were repeated.

Hydrostatic pressures in tubules in free flow (Pf) and stop flow (Ps) and efferent arterioles were measured with a glass micropipette in series with a servo-nulling pressure measurement device (IPM, San Diego, CA) as previously described (7, 16). Efferent arteriolar blood flow samples for determination of efferent protein concentration (Ca) were collected with glass micropipettes, and these were bracketed by two collections from the femoral artery for measurement of afferent protein concentration (Ca). Late surface segments of proximal tubules were identified by intratubular injection of diluted F&DC dye contained in a pipette of 3- to 5-μm outer diameter, and three to five timed tubular fluid collections were randomly obtained from these sites.

In all the studies, urine was collected in preweighed containers under oil, and periodic plasma samples were obtained for [3H]inulin concentration to compute whole, two-kidney, GFR, and single-nephron (SN)GFR.

Analytic Methods

[3H]Inulin activity in plasma, urine, and tubular fluid was monitored on a model B4530 Tri/Carb liquid scintillation counter (Packard Instrument, Downers Grove, IL). GFR, SNGFR, absolute (APR) and fractional (FR) proximal tubular reabsorption were determined as described in previous studies (7). Protein concentration in systemic and efferent arteriolar plasma samples was measured by a microadaptation of the Lowry method (21).

Calculations

Glomerular capillary hydrostatic pressure (Pc) was calculated as 

\[ P_c = \text{SFP} + \pi_a \]

where \( \pi_a \) corresponds to the oncocotic pressure at the level of the afferent arteriole (systemic oncocotic pressure). Single-nephron plasma flow (SNPF), afferent arteriolar resistance (AR), efferent arteriolar resistance (ER), oncocotic pressure (\( \pi_c \)), and the glomerular ultrafiltration coefficient (LpA) were calculated as previously described (7, 16).

Western Blot Protein Analysis

After kidney extraction, a total kidney aliquot was homogenized in lysis buffer [50 mM Tris-HCl, 0.3% SDS, 2 mM EDTA, and complete protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany)]. After three 15-min cycles of freezing-thawing (−70 to 37°C) to lyse the cells, the mixture was spun down (10 min at 14,000 rpm) at 4°C. The pellet was then discarded and the supernatant was kept as kidney lysate. Brain tissue, from the entire rat brain, was processed in a parallel fashion. Protein concentration was determined with a commercial kit (DC Protein Assay, Bio-Rad, Hercules, CA). A total of 80 μg of protein was separated in a 3–8% SDS-polyacrylamide gel and then transferred to a nitrocellulose membrane (Invitrogen, Carlsbad, CA). Brain tissue protein (50 μg) was run as a positive control. The membrane was blocked overnight with 5% nonfat dry milk, washed, and incubated in a solution containing 1:400 anti-NMDA Ab (Mouse anti-NMDA-NR1, Santa Cruz Biotechnologies, Santa Cruz, CA) for 2 h. After being washed, the membrane was incubated with 1:30,000 horseradish peroxidase-labeled anti-goat IgG (Santa Cruz Biotechnologies). The membrane was then developed with ECL plus (Amersham Pharmacia Biotech, Piscataway, NJ), and densitometry was performed on the resultant positive bands (National Institutes of Health Image J). Blots were then stripped and probed for β-actin as a housekeeping protein.

Statistics

Paired data in a single experiment were compared with the paired t-test. ANOVA followed by Bonferroni correction was performed to obtain statistical significance between groups of animals. The level of statistical significance was defined as \( P < 0.05 \). Not significant comparisons are indicated by “ns.” All results are expressed as means ± SD.

RESULTS

Two weeks of LPD did not affect total body weight (control rats 344 ± 17 g vs. LPD 327 ± 20 g; ns). However, rats fed the LPD had significantly smaller kidneys after 2 wk (1.31 ± 0.15 vs. 1.07 ± 0.19 g, control vs. LPD left kidney weight, \( P < 0.05 \)).

Renal Micropuncture Results

Control rats. Glycine infusion did not affect MAP (112 ± 10 vs. 108 ± 7 mmHg, ns). Similar to our previous findings, glycine administration in control rats resulted in a significant increase in GFR (2.8 ± 0.9 vs. 3.3 ± 0.9 ml/min, 2-kidney values, \( P < 0.038 \)) and SNGFR (27.6 ± 6.9 vs. 40.1 ± 11.7 ml/min, \( P < 0.05 \); Fig. 1 and Table 1) (4–7, 9, 10). Urinary flow rate increased (5 ± 4 to 28 ± 18 μl/min, \( P < 0.005 \)). Single-nephron blood flow (SNBF) was also increased from 143 ± 38 to 181 ± 57 ml/min (\( P < 0.05 \)). The increase in SNBF was due to a statistically significant decrease in AR (38.9 ± 16 to 23.4 ± 9.4 Gdyne·s·cm⁻², \( P < 0.05 \)) and a numerical decrease in ER (27.8 ± 9.9 to 22.8 ± 11.8 Gdyne·s·cm⁻², ns). Glycine infusion did not modify the trans-

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capillary hydrostatic pressure gradient (36.8 ± 1.5 to 37.1 ± 3.5 mmHg, ns) because a significant increase in $P_G$ (48.0 ± 1.5 vs. 59.5 ± 2.8 mmHg, $P < 0.05$) was paralleled by a significant increase in tubular pressure (11 ± 0.8 vs. 22 ± 2.6 mmHg, $P < 0.05$). The $LpA$ increased from 0.030 ± 0.009 to 0.042 ± 0.015 nl·s$^{-1}$·mmHg$^{-1}$, but this increase failed to achieve statistical significance ($P = 0.066$). (Note: these $LpA$ values for control animals are on the low range of normal. This finding is probably incidental and related to this group of rats. No historical controls were used. See Refs. 12 and 23 for similar values.) Afferent (systemic) protein concentration and oncotic pressure were mildly but significantly reduced during glycine infusion (4.6 ± 0.5 vs. 4.4 ± 0.4 g/dl and 14.1 ± 2.1 vs. 13.2 ± 1.9 mmHg, respectively, $P < 0.05$). No differences were observed in efferent arteriolar protein concentration and therefore oncotic pressure (7.4 ± 0.6 vs. 7.5 ± 0.7 g/dl and 28.2 ± 3.5 vs. 28.9 ± 4.7 mmHg, respectively, ns) between baseline and glycine.

Figure 1 presents the results of measurements of proximal tubular reabsorption. APR was measured in a subset of nephrons during baseline and then during glycine infusion. Increases in SNGFR (26.9 ± 7.5 to 34.8 ± 7.9 nl/min, $P < 0.009$) during glycine infusion were coupled with increases in APR (8.8 ± 2.3 vs. 9.4 ± 3.3 nl/min, ns) such that FR remained constant (0.34 ± 0.06 vs. 0.28 ± 0.08 nl/min, ns). (Note: absolute values for SNGFR are slightly different in Fig. 1 compared with Table 1 because Fig. 1 represents only the means of nephrons used to measure APR. The relative changes are the same. Statistics and $n$ are based on the same animals as Table 1.)

**LPD**

Administration of LPD did not significantly modify baseline hemodynamics when compared with control rats. Table 1 and Fig. 1 present the results obtained in rats receiving LPD. Again, glycine did not increase systemic arterial pressure (122 ± 9 to 121 ± 5 mmHg, ns). In contrast with the control diet group, glycine infusion in rats receiving LPD did not increase GFR (2.6 ± 0.5 to 2.2 ± 0.3 nl/min, $P < 0.015$) and did not modify SNGFR (30.9 ± 4.5 vs. 33.2 ± 5.6 nl/min, ns), SNBF (149 ± 24 vs. 136 ± 38 nl/min, ns), $\Delta P$ (35 ± 3 vs. 35 ± 3 mmHg, ns), or either one of the glomerular arteriolar resistances (AR or ER). Urinary flow rate increased (6 ± 2 to 37 ± 13 μl/min, $P < 0.005$), in a ratio identical to rats on control diet, even though GFR remained constant. Significant increases were observed in $P_G$ (51 ± 5 to 63 ± 8 mmHg, $P < 0.05$) and tubular hydrostatic pressure (16 ± 5 vs. 28 ± 7 mmHg) similar to the changes found in the control group, and a mild but significant increase was also observed in $LpA$.

### Table 1. Glomerular hemodynamic findings in control, LPD, and LPD + CAP rats

<table>
<thead>
<tr>
<th></th>
<th>SNGFR, nl/min</th>
<th>SNBF, nl/min</th>
<th>$P_T$, mmHg</th>
<th>$P_G$, mmHg</th>
<th>$P_{sc}$, mmHg</th>
<th>$\Delta P$, mmHg</th>
<th>$AR \times 10^9$, dyn cm$^{-2}$</th>
<th>$ER \times 10^9$, dyn cm$^{-2}$</th>
<th>$\pi_A$, mmHg</th>
<th>$\pi_E$, mmHg</th>
<th>$LpA$, nl·s$^{-1}$·mmHg$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal diet ($n = 7$)</td>
<td>27.6 ± 6.9</td>
<td>143 ± 38</td>
<td>11.0 ± 0.8</td>
<td>48.0 ± 1.5</td>
<td>34.0 ± 2.6</td>
<td>36.8 ± 1.5</td>
<td>38.9 ± 16</td>
<td>27.8 ± 9.9</td>
<td>14.1 ± 2.1</td>
<td>28.2 ± 3.5</td>
<td>0.030 ± 0.009</td>
</tr>
<tr>
<td>+Glycine ($n = 7$)</td>
<td>40.1 ± 11.7*</td>
<td>181 ± 57</td>
<td>22.0 ± 2.6*</td>
<td>59.5 ± 2.8*</td>
<td>46.2 ± 3.4*</td>
<td>37.1 ± 3.5</td>
<td>23.9 ± 14</td>
<td>22.8 ± 11.8</td>
<td>13.2 ± 1.9</td>
<td>28.9 ± 4.7</td>
<td>0.042 ± 0.015</td>
</tr>
<tr>
<td>LPD ($n = 8$)</td>
<td>30.9 ± 4.5</td>
<td>149 ± 24</td>
<td>16 ± 5</td>
<td>51.5 ± 5</td>
<td>37.5</td>
<td>35.3</td>
<td>37.7 ± 5.8</td>
<td>24.0 ± 5.2</td>
<td>14.5 ± 1.4</td>
<td>25.9 ± 2.4</td>
<td>0.041 ± 0.018</td>
</tr>
<tr>
<td>+Glycine ($n = 8$)</td>
<td>33.2 ± 5.6</td>
<td>136 ± 38</td>
<td>28 ± 7*</td>
<td>63 ± 8*</td>
<td>50 ± 8*</td>
<td>36.2</td>
<td>37.5 ± 10</td>
<td>29.7 ± 8.0</td>
<td>13.0 ± 1.4</td>
<td>31.0 ± 6.0</td>
<td>0.049 ± 0.022*</td>
</tr>
<tr>
<td>LPD + CAP ($n = 6$)</td>
<td>36.9 ± 9.8</td>
<td>156 ± 40</td>
<td>14.9 ± 0.9</td>
<td>46 ± 2</td>
<td>34 ± 1</td>
<td>31.2 ± 2.8†</td>
<td>36.3 ± 10</td>
<td>22 ± 5.5</td>
<td>11.9 ± 0.9</td>
<td>26.8 ± 0.8</td>
<td>0.055 ± 0.023†</td>
</tr>
<tr>
<td>+Glycine ($n = 6$)</td>
<td>41 ± 15</td>
<td>177 ± 62</td>
<td>22.7 ± 4.8*</td>
<td>58 ± 7*</td>
<td>45 ± 3*</td>
<td>34.7 ± 3.1*</td>
<td>26.2 ± 10.2</td>
<td>20.8 ± 8.0</td>
<td>13.6 ± 1.1</td>
<td>29.4 ± 1.8</td>
<td>0.052 ± 0.026</td>
</tr>
</tbody>
</table>

Values are means ± SE. LPD, low-protein diet; CAP, captopril; SNGFR, single-nephron glomerular filtration rate; SNBF, single-nephron blood flow; $P_T$, hydrostatic pressure with free flow; $P_G$, glomerular capillary hydrostatic pressure; $\Delta P$, transcapillary hydrostatic pressure gradient; $\pi_A$, afferent oncotic pressure; $\pi_E$, efferent oncotic pressure; $P_{sc}$, hydrostatic pressure with stop flow; AR, arteriolar resistance; ER, efferent arteriolar resistance; $LpA$, glomerular ultrafiltration coefficient. * $P < 0.05$ baseline vs. glycine. † $P < 0.05$ ANOVA, baseline values different from normal diet.
Analysis of proximal tubular reabsorption in 30 nephrons revealed that LPD was associated with significantly higher APR (13.8 ± 3.3 vs. 8.8 ± 3.2 nl/min, P < 0.05) and FR (0.46 ± 0.07 vs. 0.34 ± 0.06, P < 0.05) values compared with control in baseline conditions. Glycine infusion significantly reduced proximal tubular reabsorption (Fig. 1). In contrast to the changes observed in control rats and despite absence of changes in SNGFR (30.7 ± 4.6 vs. 32.5 ± 5.7 nl/min, ns), glycine infusion was associated with a significant reduction in proximal reabsorption expressed as absolute (13.8 ± 3.3 vs. 9.7 ± 4.1 nl/min, P < 0.01) or fractional values (0.46 ± 0.07 vs. 0.30 ± 0.13 nl/min, P < 0.01).

LPD + Captopril

The addition of captopril, an ACE inhibitor, did not produce significant changes in baseline glomerular hemodynamics compared with LPD with the exception of lower values for ΔP compared with both control and LPD rats (P < 0.025) and πA (P = 0.011). LpA values were significantly higher in LPD + captopril compared with control rats (P = 0.019). Arterial pressure remained constant during glycine infusion (114 ± 2 to 110 ± 1 mmHg). Glycine did not significantly modify GFR (3.2 ± 0.7 to 3.3 ± 0.5 ml/min), SNGFR (36.9 ± 9.8 vs. 41 ± 15 nl/min), and SNBF (156 ± 40 vs. 177 ± 62 nl/min). Again, the urinary flow rate increased significantly with glycine infusion (7 ± 1 to 41 ± 25 μl/min, P < 0.005). These fivefold increases in urinary flow rate are comparable in all three groups despite differences in baseline values and differences in GFR. Significant increases were observed in PbG (46.1 ± 0.9 vs. 57.5 ± 2.4 mmHg), Pfr (14.9 ± 0.9 vs. 22.7 ± 4.8 mmHg), and AP (31.2 ± 2.8 vs. 34.7 ± 3.1 mmHg). A significant reduction was observed in AR (36.3 ± 10.0 vs. 26.2 ± 10.2 Gdynes·s·cm⁻³) in the absence of significant changes in ER (22.1 ± 5.5 vs. 20.8 ± 8.0 Gdynes·s·cm⁻³).

At the level of the proximal tubule, neither absolute nor fractional tubular reabsorption was modified by glycine infusion in this group of rats (Fig. 1).

**NMBA Protein Expression**

Figure 2 shows a representative Western blot for NMDA. The expression of NMDA is significantly lower in kidneys from rats fed the LPD for 2 wk (lanes 5–7; 2,104 ± 191 relative density units) when compared with control-diet animals (lanes 1–4; 3,444 ± 175 relative density units, P < 0.005). β-Actin levels were not different between groups (Fig. 2, bottom). Therefore, when normalized to β-actin, values are 4,484 ± 845 for control, and 2,642 ± 827 for LPD (P < 0.02). Brain levels of NMDA did not change between groups (data not shown).

**DISCUSSION**

Clearly, amino acids are no longer viewed as merely building blocks for proteins, and they have been known for some years to exert functional effects via specific receptors. In fact, glycine infusion produces major increases in glomerular filtration via renal vasodilation (4, 17). It is also well known that periodic high-protein calorie intake induces vasodilation in the kidney probably for the purpose of excretion of metabolic products of this protein calorie load (24). Previous studies in our laboratory examining the renal response to glycine administration both in normal conditions and in various disease models have established a reproducible pattern suggesting the presence of separate glomerular and tubular components in the response to glycine (4–7, 9, 10). Increases in GFR during glycine require renal vasodilation and parallel increases in absolute proximal tubular reabsorption to match the increase in nephron filtration rate such that FR is well maintained. Absence of a glomerular response has correlated so far with reductions in tubular reabsorption during glycine infusion. We postulated that the decrease in tubular reabsorption during glycine increases distal delivery to the macula densa and activates tubuloglomerular feedback, which prevents any further increases in nephron filtration rate.

The results of the current study support previous findings by us and other investigators demonstrating that glycine infusion increases GFR by a plasma flow-dependent mechanism. Indeed, glycine led to reductions in afferent and efferent arteriolar resistances with a subsequent increase in SNGFR. Glomerular capillary pressures were not modified by glycine.

The mechanism by which glycine decreases glomerular resistances is not completely understood. Previous studies in our laboratory showed that inhibition of NO with nonspecific NOS blockers (N⁵-monomethyl-L-arginine) prevents the vasodilation and increase in GFR associated with glycine, suggesting that NO plays an important role as modulator/effecter of the glycine response. We showed more recently that glycine-induced renal vasodilation is suppressed with NMDA antagonists (11). Taken altogether, these results suggest that activation of the NMDA receptor by glycine in the kidney may lead to vasodilation mediated by NO effects in the kidney or indirectly by modifying ANG II activity as we previously proposed (4).

The findings of the current study provide further evidence supporting the role of the renal NMDA receptor in glycine-induced renal vasodilation by demonstrating that downregulation of the receptor with LPD is associated with loss of the renal vasodilatory response to glycine. Close examination of the determinants of the glomerular hemodynamic process in LPD rats did not demonstrate any significant differences between LPD and control rats. However, these results present a conundrum with the previously published results wherein we demonstrated decreased baseline renal blood flow in rats given acute NMDA antagonists, yet the rats in the current study that have decreased NMDA receptors via LPD did not show a difference in baseline hemodynamics vs. control-diet rats. These findings reveal some of the complexities of this system. The previously published studies were undertaken by administration of the NMDA antagonists in acute experiments in which all the receptors are blocked with a pharmacological agent, whereas studies described herein were chronic studies...
where 50% of receptors were downregulated, at least as far as protein expression, by a LPD. It is likely that there are other factors that change during the 2-wk adaptation to the LPD and these may contribute to the seemingly disparate results.

The results of the current study stress the potential role of the NMDA receptor in modulating proximal tubular reabsorption, because downregulation of the receptor correlates with a decrease in absolute and fractional proximal reabsorption during glycine administration (although the baselines are shifted up). The reduction in proximal reabsorption during glycine in LPD rats is similar to the reduction in proximal reabsorption we previously observed in various experimental conditions characterized by absence of vasodilatory response to glycine including Goldblatt hypertension, experimental diabetes, cyclosporine administration, and chronic glomerulonephritis (6, 7, 9, 10). During low-protein feeding, as in the models previously mentioned, the decrease in proximal reabsorption increases delivery of sodium chloride to macula densa, thereby activating tubuloglomerular feedback and preventing or nullifying any potential vasodilatory response.

We also examined the effects of administration of ACE inhibitors in rats with LPD. The rationale for such treatments includes 1) the fact from our previous experience that ACE inhibitors restore the glycine response in models of hypertension, experimental diabetes, and patients with diabetes mellitus and chronic glomerulonephritis (2, 26, 27) and 2) the evidence from previous studies in the literature that LPD is associated with increased renal renin content (13). Under normal conditions, glycine does not modify ANG II concentration and increases renal vasodilation. In contrast, the absence of a vasodilatory response to acute glycine infusion is associated with increased kidney tissue ANG II values (10). We also demonstrated in the two-kidney, one-clip hypertension model, and experimental diabetes model, dissociation between the ability of ACE inhibitors and angiotensin receptor blockers (ARB) to restore the normal glycine vasodilatory response (5, 7). Under both experimental conditions, ACE inhibitors restored normal response, whereas the ARBs did not. We previously proposed that differences in NO stimulation between ACEIs and ARBs explain such discrepancies (5, 7). The current study with LPD is the first demonstration that ACE inhibitors do not restore the glycine response. Infusion of glycine in captopril-treated rats was associated with a significant reduction in afferent arteriolar resistance, but no change in efferent resistance. In contrast with the glomerular response, captopril prevented the decrease in APR and FR during glycine infusion, suppressing any potential stimulation of the tubuloglomerular feedback system. These findings in the LPD + captopril group provide some preliminary but potentially very exciting information about the function of the NMDA receptor. These results suggest several possibilities regarding the mechanism(s) of action of this receptor. First, the NMDA receptor may be responsible for modulating not only tubular response to glycine but also glomerular response probably through independent mechanisms because restoring tubular response is not enough to restore glomerular response. Additionally, activation of this receptor may modulate ANG II activity, at least at the tubular level. Finally, these results may also suggest that the activity of the NMDA receptor may be important in the generation of NO. Additional studies will be required to further define these possibilities.

The NMDA receptor has been localized to various regions of the brain but has not been widely described outside of the central nervous system. Recently, we showed the NMDA receptor is constitutively expressed in the rat kidney (11). Leung and co-workers (19) confirmed the presence of NMDA receptors (both NR1 and NR2C subunits) in rat kidney. They also identified NMDA in LLC-PK1 and opossum kidney cells, both derived from proximal tubules, as well as Madin-Darby canine kidney cells, which are derived from distal tubules. Recently, we identified all four known NR2 subunits in rat kidney by Western blot analysis and by RT-PCR (29, 30). After 2-wk LPD, the NR2 A, B, and C subunits were all affected, whereas NR2D is not changed (29, 30). The implications are that diet may have multifaceted effects on regulation of the NMDA receptor and the glycine response and further suggest the importance of the NMDA receptor to kidney function.

The current findings clearly demonstrate that the NMDA NR1 receptor subunit expression is downregulated after 2 wk of low-protein feeding. The mechanism by which diet affects NMDA receptor regulation is unknown; however, it may be related to amino acid content of the diet. Very little is known about the activation of the NMDA receptor complex or its regulation in the kidney. However, there is a large body of knowledge about its activation in the central nervous system, which may provide insights into the role in kidney function (3, 20, 22, 25, 28). In fact, many of the same substrates that are important to central nervous system receptor function are also abundantly present in the kidney. l-Endoglutamate and glycine are the two known coagonists in the brain (the latter concentration limiting), and these amino acids are known to be present in the kidney. The kidney also has very high levels of intracellular arginine, which is converted to agmatine, NO, and polyamines in the kidney, all of which are known to regulate the NMDA receptor complex. Nevertheless, how these endogenous substrates might be presented or delivered to the NMDA receptor is currently unknown. The current studies lend evidence that changes in protein intake are an important regulator of the renal NMDA receptor.

In conclusion, glycine-induced vasodilation was abolished after 2 wk of LPD in rats, possibly due to decreases in absolute proximal reabsorption. In contrast to other models studied, captopril treatment did not completely restore this response. Coincident with the effects of glycine on vasodilation, LPD also downregulated kidney NMDA receptors. These studies indicate that the renal NMDA receptor may be a critical element in protein/amino acid-induced vasodilation.

GRANTS

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