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Research Article

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Nitric Oxide and Angiotensin II

Glomerular and Tubular Interaction in the Rat

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Abstract

Nitric oxide (NO) has been proposed to modulate the renal response to protein as well as basal renal hemodynamics. We investigated whether NO and angiotensin II (AII) interact to control glomerular hemodynamics and absolute proximal tubular reabsorption (APR) during glycine infusion and in unstimulated conditions. In control rats, glycine increased single nephron GFR and plasma flow with no change in APR. The NO synthase blocker, N^G-monomethyl L-arginine (LNMMA), abolished the vasodilatory response to glycine, possibly through activation of tubuloglomerular feedback due to a decrease in APR produced by LNMMA + glycine. Pretreatment with an AII receptor antagonist, DuP 753, normalized the response to glycine at both glomerular and tubular levels. In unstimulated conditions, LNMMA produced glomerular arteriolar vasoconstriction, decreased the glomerular ultrafiltration coefficient, and reduced single nephron GFR. These changes were associated with a striking decrease in APR. DuP 753 prevented both glomerular and tubular changes induced by LNMMA. In conclusion, NO represents a physiological antagonist of AII at both the glomerulus and tubule in both the basal state and during glycine infusion; and inhibition of NO apparently enhances or uncovers the inhibitory effect of AII on proximal reabsorption. (*J. Clin. Invest.* 1992. 89:1248–1256.) Key words: N^G-monomethyl L-arginine • DuP 753 • saralasin • glycine • micropuncture

Introduction

An increase in GFR and plasma flow during amino acid infusion or after a protein load has been demonstrated in both humans and experimental animals (1–4). The mechanism underlying the renal response to proteins is still ill defined; various humoral factors and a suppression of tubuloglomerular feedback (TGF)[1] have been proposed as possible mediators of amino acid–induced hyperfiltration (5–10). More recently, significant evidence has accumulated related to the possibility that endothelial-derived relaxing factor could play an important role in the renal hyperemia associated with amino acid infusion (11, 12). The widespread interest raised by this physiological phenomenon is magnified and is potentially related to studies demonstrating a crucial role for chronic glomerular hyperfiltration in the progression of renal damage (13–16).

Two different groups have recently shown that endothelial-derived relaxing factor or nitric oxide (NO) is involved not only in the regulation of basal renal hemodynamics but also in the renal response to amino acids, since normal rats pretreated with the NO synthase inhibitor, N^G-monomethyl L-arginine (LNMMA), did not demonstrate an increase in GFR and renal plasma flow during amino acid or glycine infusion (11, 12). Although these studies have demonstrated the importance of NO in the renal response to amino acids, the mechanism by which LNMMA prevented the normal response to glycine or amino acids has not been clarified.

The absence of changes in GFR and renal plasma flow observed in normal rats treated with LNMMA is similar to the findings demonstrated in our laboratory in rats with renovascular hypertension (17), experimental diabetes (18), and chronic glomerulonephritis (unpublished observation). In these pathophysiological states, the lack of response to glycine was associated with a significant reduction in proximal tubular reabsorption. Administration of converting enzyme inhibitors restored proximal tubular reabsorption and a normal increase in GFR after glycine, suggesting a potential role for angiotensin II (AII). Our findings in pathophysiological conditions have led us to evaluate whether decreases in absolute proximal tubular reabsorption (APR) may constitute an important mechanism that might explain the lack of response to glycine in normal rats treated with LNMMA. Furthermore, since previous in vitro and in vivo studies have suggested an important interaction between AII and NO at the glomerular level (11, 19), we have investigated the possibility of similarly important interactions in the proximal tubule.

The present study was designed to answer the following questions: (*a*) What is the specific role of NO in the glomerular and tubular response to glycine infusion? (*b*) Do NO and AII interact to modulate these responses? (*c*) Is there an interaction between NO and AII influencing the basal state of glomerular and tubular function?

Glossary

AII	angiotensin II
APR	absolute proximal tubular reabsorption
AR	afferent arteriolar resistance
C_A	afferent protein concentration
C_E	efferent protein concentration
DuP 753	2-*n*-butyl-4-chloro-5-hydroxymethyl-1-[2′-(1*H*-tetrazole-5-biphenyl-4-yl)methyl] imidazole
EFP	mean effective filtration pressure

Portions of this work were presented at the 24th meeting of the American Society of Nephrology, Baltimore, MD, 00–00 1991, and have been published in abstract form (1991. *J. Am. Soc. Nephrol.* 2:504–505).

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ER	efferent arteriolar resistance
FR	fractional tubular reabsorption
GFR	left kidney glomerular filtration rate
Hct	hematocrit
HP_E	efferent arteriole hydrostatic pressure
LNMMA	N^G-monomethyl L-arginine
LpA	ultrafiltration coefficient
MAP	mean arterial pressure
NO	Nitric oxide
ΔP	transcapillary hydrostatic pressure gradient
πA	afferent oncotic pressure
P_G	glomerular capillary hydrostatic pressure
P_{US}	urinary space pressure
SNFF	single nephron filtration fraction
SNPF	single nephron plasma flow
TF	tubular fluid inulin concentration
TF/P	tubular fluid to plasma inulin activity
TGF	tubuloglomerular feedback

Methods

Experimental design

The studies were conducted in 39 male Munich-Wistar rats (200–270 g body wt) obtained from Simonsen Laboratories (Gilroy, CA). Eight groups of rats were divided into two different protocols as demonstrated in Fig. 1.

Protocol I. Role of NO and AII in the normal response to glycine infusion. After euvolemic replacement, rats were submitted to basal measurements of glomerular hemodynamics and proximal tubular reabsorption. After completion of basal measurements, glycine was dissolved in isotonic NaCl–$NaHCO_3$ at a concentration of 2.66 M (20 g%) and infused at ~ 1.4 ml/h throughout the remainder of the experiment. 30 min after beginning glycine, measurements of glomerular hemodynamics and proximal tubular reabsorption were repeated (experimental measurements).

Four groups of rats were studied. Group Ia (n = 6) comprised control unmanipulated rats, which only received glycine during the experimental period. In group Ib (n = 6), LNMMA (Calbiochem, Corp., La Jolla, CA), dissolved in isotonic NaCl–$NaHCO_3$, was infused simultaneously with glycine at a rate of 0.5 mg/kg per min and was continued throughout the remainder of the study. Both solutions were contained in the same syringe and started at the same time. This dose of LNMMA does not modify GFR as demonstrated by Tolins et al. (20). In group Ic (n = 6), the nonpeptide AII receptor antagonist, 2-*n*-butyl-4-chloro-5-hydroxymethyl-1-[2′-(1*H*-tetrazole-5-biphenyl-4-yl)methyl] imidaz ole, DuP 753 (21), was administered at the end of the surgical preparation as an intravenous bolus (10 mg/kg dissolved in 2.7 ml of Ringer solution). Measurements were obtained in basal conditions and during concurrent infusion of glycine and LNMMA. In group Id, we evaluated whether the changes in glomerular and tubular function observed during LNMMA infusion in group Ib were specific to inhibition of NO synthesis. Group Id comprised three rats pretreated with L-arginine, the natural precursor of NO, using a 10-fold excess concentration of L-arginine to LNMMA. In this group, after completion of the surgical preparation, L-arginine was dissolved in the isotonic NaCl–$NaHCO_3$ solution containing [^{3}H]inulin and infused at a rate of 5 mg/kg per min. Single nephron GFR (SNGFR) and proximal tubular reabsorption were measured in basal period and during infusion of LNMMA + glycine as in group Ib.

Protocol II. Glomerular and tubular interaction between NO and AII in unstimulated conditions. In this study, measurements of glomerular and tubular function were obtained after euvolemic replacement as in the previous protocol (BASAL). After completion of basal measurements, LNMMA infusion (0.5 mg/kg per min) was begun and continued throughout the experiment. 30 min later, experimental measurements of glomerular and tubular function were obtained.

Four groups of rats were studied. Group IIa (n = 6) consisted of control rats. Group IIb (n = 6) received DuP 753, 10 mg/kg, as an intravenous bolus at the end of the surgical preparation. In group IIc, we used saralasin Sar^1-Ala^8-AII (Sigma Chemical Co., St. Louis, MO) instead of DuP 753 to rule out the possibility that AII-independent effects of DuP 753 account for its effect observed in group IIb. Group IIc consisted of three rats in which saralasin was initiated at the time of equilibration and continued throughout the experiment at the rate of 8 μg/kg per min. In this group, only SNGFR and proximal tubular reabsorption were measured during the basal and experimental periods. To test the capacity of L-arginine to reverse the changes in SNGFR and proximal reabsorption observed during LNMMA in group IIa, an additional group of rats (group IId, n = 3) was studied in basal conditions and during concurrent administration of L-arginine (10-fold excess) and LNMMA.

Micropuncture studies

The animals were maintained with ad lib. access to tap water and normal rat chow until the morning of the experiment. Rats were anesthetized with Inactin (BYK, Konstanz, Germany) (10 mg/100 g body wt, i.p.) and placed on a temperature-regulated micropuncture table. Rats were surgically prepared according to standard procedures. Briefly, the rats underwent tracheostomy and cannulation of the left jugular vein, left femoral artery, left ureter, 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–$NaHCO_3$ solution. The femoral artery catheter was used for periodic blood samples and monitoring of the mean arterial pressure (MAP) with a transducer (model P23db; Statham Instruments, Gould Division, Hato Rey, PR) and recorded on a Statham (Statham Instruments) chart recorder. All studies were performed in a euvolemic state by infusing 1% body wt donor plasma over a 1-h period followed thereafter by 0.15% body wt donor plasma/h. All rats also received two additional infusions of NaCl–$NaHCO_3$ solutions, one containing [^{3}H]inulin at a rate of 110 μCi/h in a volume of 0.8 ml/h, the other infusion (1.4 ml/h) served as a control for the solution containing glycine and/or LNMMA administered during the second period. Both solutions were initiated at the end of the surgical preparation and were maintained throughout the experiment. Hydrostatic pressures in the glomerular capillaries (P_G), urinary space (P_{US}), and efferent arterioles (HP_E) were measured with a glass micropipette (1–3 μm in external tip dia, filled with 1.2 M saline) in series with a servo-nulling pressure measurement device (IPM, Inc., San Diego, CA) as previously described (22). Glass pipettes of 13–16 μm were used to collect at least three efferent arteriolar blood samples for determination of efferent protein concentration (C_E) and these were bracketed by two collections from the femoral artery for measurement of afferent protein concentration (C_A). Late surface segments of proximal tubules were identified by intratubular injection of diluted FD & C dye contained in a pipette of 3–5-μm o.d. Four-to-five timed tubular fluid collections were randomly obtained from these sites to determine the SNGFR and the ratio of tubular fluid to plasma inulin activity (TF/P). In all the studies, urine was collected in preweighed containers under oil, and periodic plasma samples were obtained for [^{3}H]inulin concentration to compute whole-kidney GFR, SNGFR, and TF/P.

Analytic methods

[^{3}H]Inulin activity in plasma, urine, and tubular fluid was monitored on a model B4530 Tri/Carb Packard liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, IL). SNGFR and GFR were determined as described in previous studies from this laboratory (23). Protein concentration in systemic and efferent arteriolar plasma samples was measured by a microadaptation of the Lowry protein method (24).

Calculations

Superficial nephron filtration fraction (SNFF), single nephron plasma flow (SNPF), afferent arteriolar resistance (AR), efferent arteriolar resis-

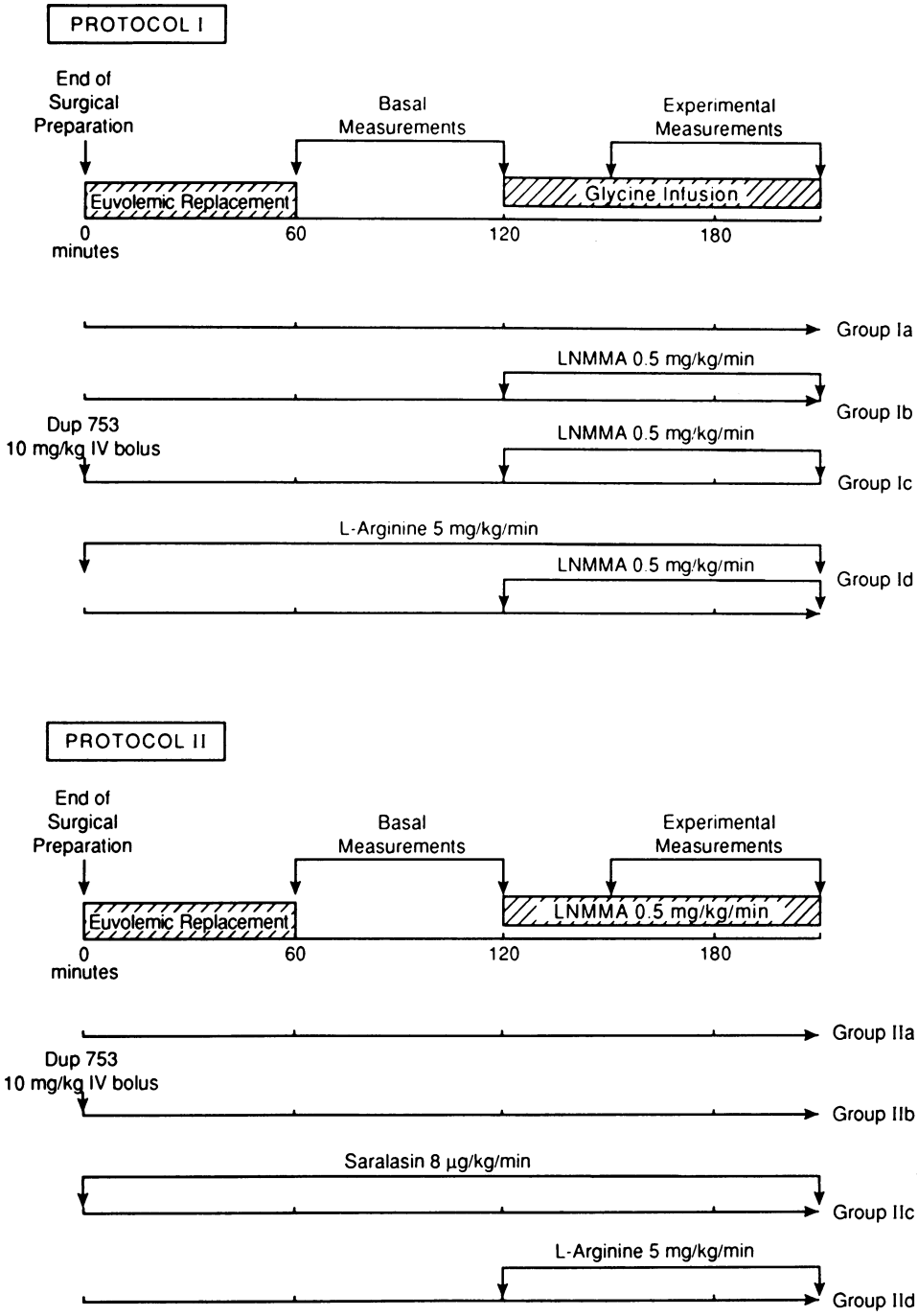


Figure 1. Experimental design.

tance (ER), and oncotic pressure (π) from protein concentration were calculated as described in previous publications (22, 25).

To calculate proximal reabsorption, the volume of the samples collected from late proximal tubules was transferred to a previously calibrated, constant-bore capillary tube. The volume of tubular fluid was determined from length measurements in this capillary tube. From the total radioactivity count rate and the known volume, the tubular fluid inulin concentration (TF) can be determined, and from the plasma inulin radioactivity (P) (corrected for plasma water), the TF/P. Fractional tubular reabsorption (FR) at the late proximal tubule site can be determined from the TF/P ratio from the following relationship: FR = (1 − P/TF) × 100%. Absolute tubular fluid reabsorption in the proximal tubule (APR) can be determined from: APR = SNGFR × FR (23).

Changes in SNPF modify the profile of the mean effective filtration pressure (EFP) along the glomerular capillary by affecting the rate at which protein is concentrated and the rate of rise in π along x^*. The SNGFR can therefore be determined as follows: SNGFR = LpA × EFP where LpA is the glomerular ultrafiltration coefficient, which in turn is a product of the hydraulic conductivity (Lp) of the glomerular membrane and A, the effective filtering surface area of the glomerular capillary. LpA is computed by using an iterative procedure as previously described (26).

Statistics

The statistical analysis was performed using individual nephron values for SNGFR, SNPF, AR, and ER for each nephron from which tubular fluid was collected and the single values of the micropressures recorded. The individual values for SNPF, AR, and ER were calculated using individual SNGFR measurements and mean values for pressures, hematocrit (Hct), and protein concentrations recorded during the same period. Values of MAP, GFR, SNFF, Hct, protein concentra-

tions, and LpA were entered as a mean of the single values obtained in each period. Statistical significance of measurements between the two periods was evaluated by either unpaired or paired t test, depending on whether there were multiple or single values obtained per period (27). Tukey multiple comparison analysis was also performed to obtain statistical significance among groups of animals. The level of statistical significance was defined as $P < 0.05$. All results are expressed as mean±SEM.

Results

Protocol I

Role of NO and AII in the normal response to glycine infusion. At the time of the micropuncture experiment, body weight was similar in the Ia (240±11 g), Ib (235±7 g), and Ic (235±6 g) groups of rats. Micropuncture results obtained in untreated control rats, groups Ia and Ib, and DuP 753–treated rats, group Ic, are depicted in Table I. MAP in the basal period was similar in the two groups of untreated rats (Ia and Ib) and numerically higher when compared with DuP-treated rats (Ic) (+8 mmHg, NS). Acute administration of DuP 753 in group Ic did not significantly modify basal measurements of GFR, SNGFR, or any of the determinants of the ultrafiltration process, except for a slight decrease in AR.

Glycine infusion in control rats (Ia) induced a significant increase in GFR; whole-kidney GFR increased 19% and SNGFR increased 33%. The rise in SNGFR was due to a significant increase in SNPF. Since SNPF changed in the same proportion as SNGFR, SNFF remained unchanged. The renal vasodilatory response to glycine was characterized by a parallel reduction in both afferent and efferent glomerular resistances. Numerical increases in both P_G and P_{US} resulted in no change in transcapillary hydrostatic pressure gradient (ΔP).

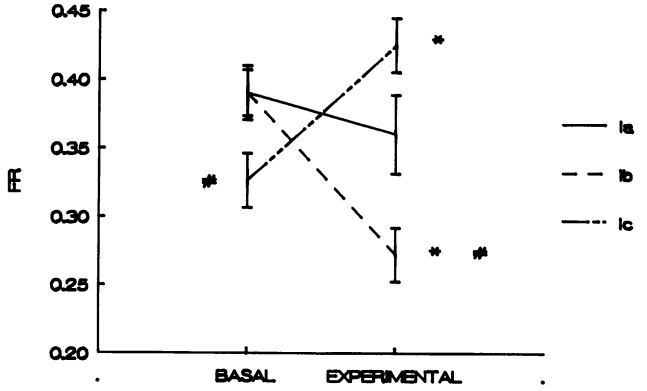
Concurrent infusion of glycine and LNMMA in group Ib was associated with complete elimination of the response to glycine; whole-kidney GFR and SNGFR remained unchanged in the second period. Similarly, SNPF was not significantly modified by the infusion of glycine plus LNMMA. P_G, and consequently ΔP, increased significantly because of the rise in MAP (23%) associated with parallel, but not significant, increases in afferent and efferent glomerular resistances. The ultrafiltration coefficient, LpA, decreased 41% during glycine + LNMMA. The reduced LpA was counterbalanced by increases in ΔP such that SNGFR remained constant.

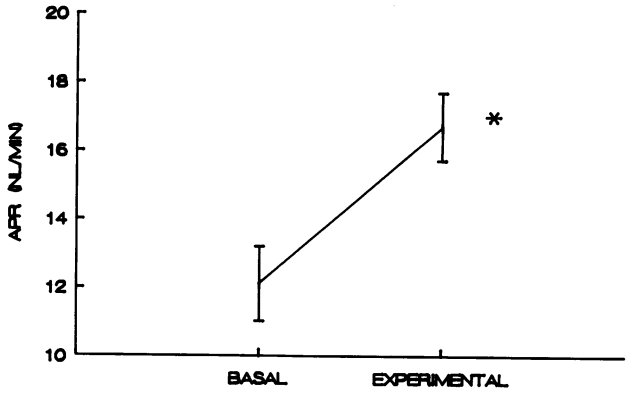
Pretreatment with DuP 753 (group Ic) restored a normal response to glycine during glycine + LNMMA infusion. In this group, both GFR and SNGFR increased in the same proportion as in control rats (group Ia) receiving only glycine (16 and 33%, respectively). The rise in SNGFR was produced by significant increases in SNPF and ΔP. As observed in glycine + LNMMA rats (group Ib), the combined infusion of glycine and LNMMA in DuP 753 rats increased the MAP (37%). Both afferent and efferent glomerular resistances during glycine + LNMMA were significantly lower in DuP 753–treated rats (group Ic) when compared with rats in group Ib, therefore allowing DuP 753 rats to increase SNPF 53% in response to glycine despite the presence of LNMMA. In contrast to untreated rats, infusion of glycine + LNMMA in DuP 753 rats did not change the glomerular ultrafiltration coefficient. Baseline Hct was not different among the three groups of rats: Hct was 52±0.7% in group Ia, 51±0.5% in group Ib, and 49±1% in group Ic. Hct values did not change in the experimental period. Similarly, no difference was detected in basal πA; πA was 15.4±1.4 mmHg in group Ia, 16.2±1.1 mmHg in group Ib, and 16.9±1.2 mmHg in group Ic. Glycine infusion with or without LNMMA did not modify values of πA in any of the three groups; in the experimental period, πA was 14.1±1 mmHg in group Ia, 15.6±1 mmHg in group Ib, and 17.7±1.1 mmHg in group Ic.

Table I. Glomerular Hemodynamics in Untreated Rats (Groups Ia and Ib) and DuP 753–treated Rats (Group Ic) in Basal Period and During Glycine or Glycine + LNMMA Infusion

	MAP	GFR	SNGFR	SNPF	SNFF	P_G	P_{US}	ΔP	EFP	AR	ER	LpA
	mmHg	*ml/min*	*nl/min*			*mmHg*				*$\times 10^9$ dyn·s·cm^{-5}*		*nl/s/mmHg*
Group Ia												
Basal	112.5	0.95	35.4	115.0	0.32	49.6‖	12.6‖	37.0	14.7	22.5	18.4	0.047
	±4.0	±.11	±2.2	±7.0	±.03	±1.0	±0.8	±0.8	±2.6	±1.3	±1.1	±.009
Glycine	119.0	1.13*	47.0*	150.0*	0.32	53.0‖	16.4	36.6‖	17.1	18.4*	11.4*	0.057
	±4.0	±.10	±3.0	±9.0	±.02	±2.3	±2.2	±1.1	±2.9	±1.1	±0.7	±.017
Group Ib												
Basal	112.0	0.92	38.5	127.0	0.31	55.4	17.6	37.8	15.4	19.8	14.7	0.046
	±4.0	±.10	±1.8	±9.0	±.03	±1.5	±0.8	±1.0	±2.3	±1.4	±1.1	±.007
Glycine +	138.0*‡	0.99	40.9	147.0	0.29	62.6*	17.0	45.6*	25.9*	23.5‖	15.0‖	0.027*
LNMMA	±4.0	±.06	±1.4	±10.0	±.02	±2.3	±0.6	±2.3	±3.0	±1.7	±0.7	±.002
Group Ic												
Basal	103.6	1.10	40.6	139.0	0.30	54.4	17.0	37.4	14.5	15.6‖	13.3	0.048
	±5.0	±.08	±1.7	±8.5	±.02	±1.5	±1.0	±1.0	±1.0	±1.4	±1.0	±.005
Glycine +	142.0*‡	1.28*	53.9*§	212.3*‖	0.27	61.6*	16.0	45.0*	22.5*	17.4	10.3*	0.046
LNMMA	±4.0	±.10	±2.5	±18.0	±.03	±1.9	±0.5	±2.0	±3.3	±1.4	±0.6	±.010

Refer to glossary for definition of terms. All values expressed as mean±SEM. * by Student's t test, $P < 0.05$ vs. Basal. ‡ by Tukey test, $P < 0.05$ vs. GLYCINE in group Ia. § by Tukey test, $P < 0.05$ vs. GLYCINE + LNMMA in group Ib. ‖ by Tukey test, $P < 0.05$ vs. other groups, same period.





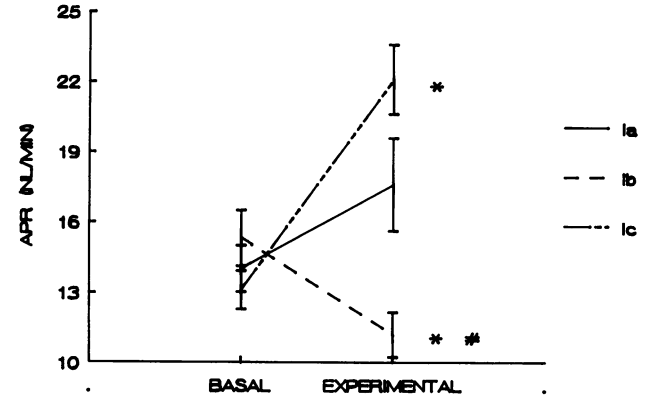


Figure 2. Absolute (*APR*) and fractional (*FR*) proximal tubular reabsorption in basal and experimental period in untreated rats (group *Ia* and *Ib*) and DuP 753–treated rats (group *Ic*). Group Ia received glycine infusion in the experimental period; groups Ib and Ic received infusion of glycine + LNMMA in the experimental period. #$P < 0.05$ vs. other groups, same period; *$P < 0.05$ vs. BASAL.

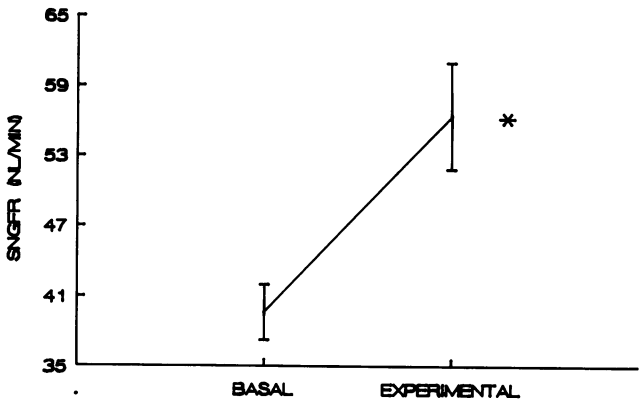


Figure 3. SNGFR and absolute proximal reabsorption (*APR*) in L-arginine–treated rats (group Id) in BASAL period and during infusion of glycine + LNMMA (*EXPERIMENTAL*). *$P < 0.05$ vs. BASAL.

The values of APR and FR in the basal period and during glycine or glycine + LNMMA infusion are reported in Fig. 2. Administration of DuP 753 in group Ic resulted in a significant decrease in the basal value of FR; FR was 0.33±0.02 in DuP 753 rats and 0.39±0.02 in both groups of untreated rats ($P < 0.05$). Interestingly, glycine infusion did not change either APR or FR in control rats (group Ia) whereas simultaneous infusion of LNMMA and glycine (group Ib) produced a significant reduction in both APR and FR; APR decreased from 15.3±1 to 11.2±1 nl/min and FR from 0.39±0.02 to 0.27±0.02 ($P < 0.05$ vs. Basal). In contrast, DuP 753 administration (group Ic) prevented the decrease in proximal reabsorption observed in untreated rats (group Ib); indeed, in group Ic both APR and FR increased significantly during infusion of LNMMA + glycine (from 13.1±0.8 to 22.1±1 nl/min and from 0.33±0.02 to 0.42±0.02, respectively, $P < 0.05$ vs. basal period).

Pretreating the rats with L-arginine (group Id) blunted the increase in blood pressure observed in group Ib (Table I); in arginine-treated rats no significant change in MAP was detected during LNMMA + glycine infusion (MAP was 102±3 mmHg in basal period and 110±2 mmHg during glycine + LNMMA), whereas untreated rats (group Ib) responded to the same infusion with a 23% increase in MAP. Fig. 3 shows the effect of L-arginine on SNGFR and APR in basal period and during infusion of glycine + LNMMA. L-Arginine completely restored the normal glomerular and tubular response to glycine; as opposed to group Ib, SNGFR increased 42% and APR increased to maintain a constant FR during simultaneous infusion of LNMMA and glycine in the experimental period.

Protocol II

Glomerular and tubular interaction between NO and AII. In this study we evaluated the effects of inhibition of NO synthesis on unstimulated glomerular and tubular dynamics. No difference was detected in body weight between control rats (234±6 g) and DuP 753 rats (225±9 g). The results of the glomerular hemodynamic study in untreated rats (group IIa) and DuP 753–treated rats (group IIb) are reported in Table II. As observed in protocol I, basal MAP in DuP 753–treated rats (group IIb) was 8 mmHg lower than in untreated rats ($P < 0.05$) and no major difference was detected between the two groups with regard to basal glomerular hemodynamics.

LNMMA administration in control untreated rats (group IIa) induced a slight but not significant decrease in whole-kidney GFR. Both SNGFR and SNPF were reduced to the same extent (−17%, $P < 0.05$ vs. BASAL) by LNMMA, therefore no change in SNFF was observed. The vasoconstrictor response to LNMMA was characterized by increases in MAP associated with an elevation in glomerular resistances. The parallel and

Table II. Glomerular Hemodynamics in Control Rats (Group IIa) and DuP 753–treated Rats (Group IIb) in Basal Period and During LNMMA Infusion

	MAP	GFR	SNGFR	SNPF	SNFF	P_G	P_{US}	ΔP	EFP	AR	ER	LpA
	mmHg	*ml/min*	*nl/min*			*mmHg*				*×10⁹ dyne·s·cm⁻⁵*		*nl/s/mmHg*
Group IIa												
Basal	103.0	0.89	37.8	132.0	0.30	52.6	16.5	36.1	13.0	16.1	13.8	0.065
	±1.2	±.03	±1.5	±7.0	±.03	±1.6	±0.4	±1.5	±3.4	±0.9	±1.3	±.015
LNMMA	120.0*	0.79	32.2*	108.0*	0.30	50.8	11.5*	39.3	17.1	25.9*	17.6*	0.044*
	±3.0	±.06	±1.6	±5.0	±.01	±2.0	±0.4	±2.0	±3.4	±1.3	±0.9	±.012
Group IIb												
Basal	94.5‡	1.02	33.0	112.0	0.30	49.2	15.0	34.2	16.7	18.5	15.7	0.035
	±2.2	±.08	±1.7	±7.5	±.02	±1.0	±0.3	±0.8	±2.5	±1.1	±1.1	±.004
LNMMA	119.6*	0.98	34.8	117.0	0.29	51.4	14.2‡	37.2	13.7	28.2*	17.5	0.051
	±3.6	±.13	±3.0	±9.0	±.03	±1.9	±0.4	±1.9	±2.3	±2.3	±1.7	±.016

Refer to glossary for definition of terms. All values expressed as mean±SEM. By Student's *t* test: * $P < 0.05$ vs. Basal; ‡ $P < 0.05$ vs. group IIa, same period.

important increase in both AR and ER resulted in no changes in glomerular pressure and ΔP. A significant reduction in LpA was also detected in control rats during LNMMA infusion.

Of significance, pretreating the rats with DuP 753 did not prevent the rise in blood pressure induced by LNMMA. In the DuP 753 group (group IIb) MAP increased 26% but the glomerular vasoconstriction observed in untreated rats was completely abolished by DuP 753. No significant change was detected in GFR as well as SNGFR and SNPF; afferent glomerular resistance rose during LNMMA infusion, probably in response to the increased MAP, demonstrating appropriate renal autoregulation in this group. No difference was detected among the two groups of rats with regard to the Hct and πA values in basal state; moreover, LNMMA infusion did not affect either Hct or πA in the second period.

Remarkable results were obtained from the examination of tubular reabsorption in these groups of rats; results are depicted in Fig. 4. As observed in protocol I, DuP 753 administration was associated with decreased proximal reabsorption. In DuP 753–treated rats (group IIb), basal values of APR and FR were 11.5±1.3 nl/min and 0.34±0.02, respectively; whereas in untreated rats, APR was 15.7±1.2 nl/min and FR was 0.40±0.02 ($P < 0.05$). The most striking result was the impressive decrease in proximal reabsorption detected during LNMMA infusion in untreated control rats. In this group, LNMMA infusion decreased APR 47% and FR 35% ($P < 0.05$ vs. control period). As observed in protocol I, DuP 753 treatment before LNMMA infusion (group IIb) prevented the reduction in APR and FR as well the glomerular vasoconstriction, suggesting an interaction between AII and NO in the control of both basal and stimulated glomerular and tubular function.

As observed in DuP 753–treated rats, saralasin administration (group IIc) did not prevent the increase in MAP induced by LNMMA (99.8±4.7 mmHg, first period; 128.7±5.6 mmHg during LNMMA, $P < 0.05$). Saralasin also resulted in prevention of the decrease in both SNGFR and proximal reabsorption during LNMMA (Fig. 5). In saralasin-treated rats, SNGFR was 39.7±1.9 nl/min in the first period and 40.8±2.1 nl/min during LNMMA; similarly both APR and FR did not change significantly between the two periods, APR was 17.1±1 and 18.6±1 nl/min, respectively, FR was 0.43±0.02 before and 0.47±0.02 after LNMMA. These data clearly demonstrate that DuP 753 prevents the LNMMA effects on both glomerular and tubular function through AII antagonism rather than other AII-independent mechanisms.

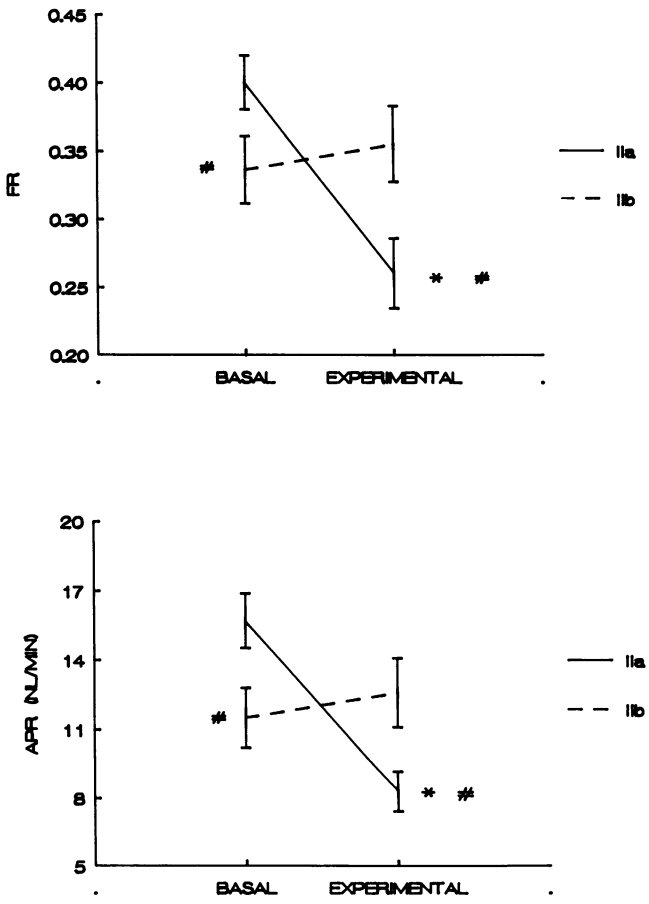
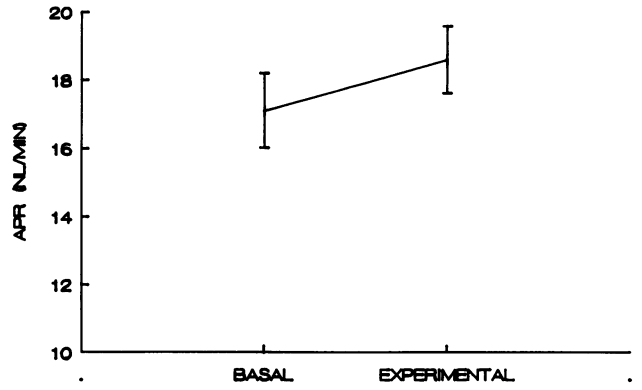
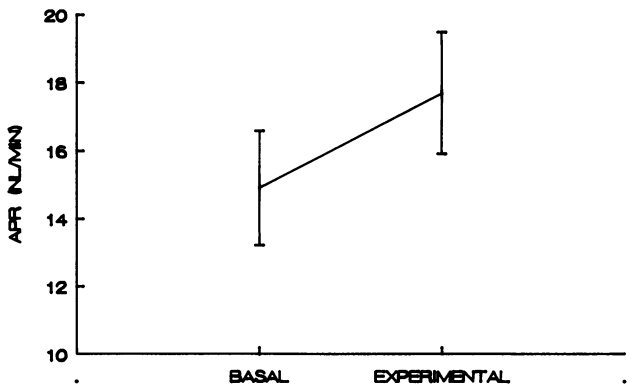


Figure 4. Absolute (*APR*) and fractional (*FR*) proximal tubular reabsorption in untreated rats (group IIa) and rats treated with DuP 753 (group IIb) in basal period and during LNMMA infusion (*EXPERIMENTAL*). *$P < 0.05$ vs. other groups, same period; *$P < 0.05$ vs. BASAL.





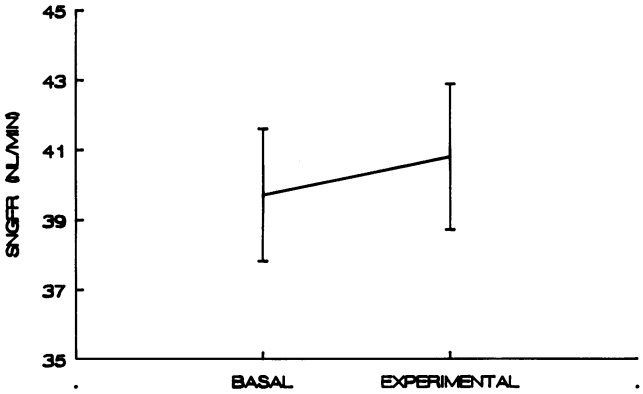


Figure 5. SNGFR and absolute proximal tubular reabsorption (*APR*) in saralasin-treated rats (Group IIc) in basal period and during LNMMA infusion (*EXPERIMENTAL*).

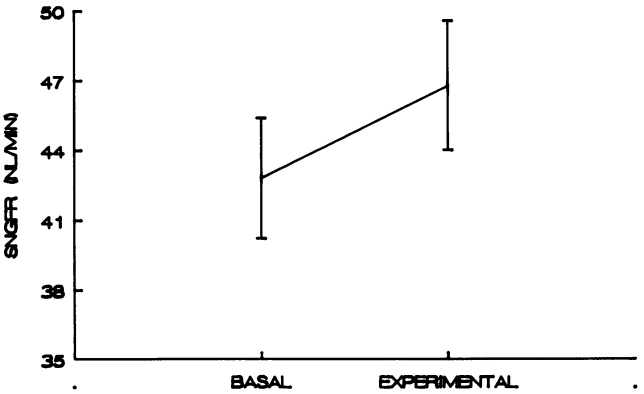


Figure 6. SNGFR and absolute proximal tubular reabsorption (*APR*) in group IId in basal period and during infusion of L-arginine + LNMMA (*EXPERIMENTAL*).

When L-arginine was infused concurrently with LNMMA (10-fold excess of L-arginine to LNMMA) in group IId rats, no change was detected in blood pressure, SNGFR, or tubular reabsorption. L-Arginine prevented the increase in MAP observed in untreated rats during LNMMA infusion (group IIa); in group IId, MAP was 112±4 mmHg in the basal period and 115±7 mmHg during infusion of L-arginine + LNMMA. As depicted in Fig. 6, L-arginine prevented the decrease in both SNGFR and APR associated with LNMMA. The findings in the latter group as well as in group Id demonstrate that the changes in systemic blood pressure and in both stimulated and unstimulated renal dynamics induced by LNMMA are completely reversed by infusion of L-arginine.

Discussion

Glomerular and tubular function are clearly influenced by NO, both under basal conditions and during the infusion of glycine, as clearly demonstrated by the results of the current study. Inhibition of AII does not modify the increase in blood pressure associated with LNMMA but prevents the glomerular hemodynamic changes associated with NO inhibition. Inhibition of NO production with LNMMA has also revealed an important interaction with AII in affecting proximal tubular reabsorption, an effect that can be prevented by the administration of specific AII receptor antagonists. The effect of LNMMA must have resulted from an enhanced inhibitory action of AII on proximal reabsorption, an influence brought out by elimination of NO effects.

Our results demonstrate that administration of LNMMA produced significant increases in systemic blood pressure with simultaneous changes in renal resistances (afferent and efferent) and decreases in the glomerular ultrafiltration coefficient (Table II). Similar findings have recently been demonstrated by Zatz and De Nucci (28) using normal rats and significantly higher doses of LNMMA. As pointed out by Zatz and De Nucci (28), the changes in resistances, LpA, and ΔP are similar to those previously described in this laboratory by Blantz and co-workers (29) during the systemic infusion of AII. Our glomerular hemodynamic data obtained in both DuP 753 and saralasin-treated rats further support the concept that inhibition of NO generation somehow increases this AII influence. Both DuP 753 and saralasin prevented the decrease in SNGFR during LNMMA due to the prevention of changes in SNPF, ER, and LpA, as specifically observed in DuP 753–treated rats. A similar picture was observed in the first protocol, in which administration of DuP 753 (group Ic) permitted a normal increase in GFR, SNGFR, and SNPF during glycine despite the presence of LNMMA. These data suggest that under both normal and glycine-stimulated conditions, NO functions as a natural AII antagonist. Similar findings suggesting the presence of

NO–AII interactions have been demonstrated in vivo by Tolins and Raij (11), using clearance techniques, and in vitro by Schultz et al. (19), using mesangial cells.

A very important finding of the current study was the effect of LNMMA on proximal tubular reabsorption. Both under normal and stimulated conditions, administration of LNMMA produced a significant decrease in proximal tubular reabsorption (Figs. 2 and 4). The fact that a 10-fold excess of L-arginine prevented the decrease in APR clearly demonstrates that the effect of LNMMA on APR depends specifically on the ability of this agent to inhibit NO synthase and presumably reduce NO generation. A role for NO in the control of proximal tubular reabsorption has not been previously demonstrated. Previous studies have reported increased urinary volume and sodium excretion after LNMMA and have suggested a possible inhibitory effect at the level of the distal or collecting tubule (28, 30), but no direct evidence has been provided relating to an inhibitory effect on proximal tubular reabsorption. Interestingly, Ishii et al. (31) have recently provided evidence that porcine tubular epithelial cells (LLC-PK_1 cells) are capable of producing NO (31). However, the in vitro techniques used in Ishii's study precluded any conclusion of a role for NO in affecting tubular transport. Other studies have shown that proximal convoluted tubules are capable of the synthesis of L-arginine, the substrate for this reaction (32).

The mechanism by which NO inhibition decreases proximal reabsorption has yet to be defined. The increase in blood pressure observed after LNMMA cannot explain this finding; indeed, DuP 753 and saralasin did not prevent the rise in MAP but did prevent the reduction in APR during LNMMA infusion. Since DuP 753 and saralasin reversed this phenomenon it is logical to suggest a role for AII as either a mediator or a permissive factor. In vitro studies by Harris and Young (33) demonstrated that low AII concentrations (10^{-11}–10^{-12} M) increase proximal tubular reabsorption whereas higher concentrations (10^{-6}–10^{-8} M) inhibit proximal tubular sodium reabsorption. It is difficult to imagine that administration of LNMMA could produce such increases in AII concentration (10^{-6}–10^{-8} M), but recent data by Seikaly et al. (34) measured tubular AII concentrations and provided another possible explanation. Seikaly et al. recently demonstrated that under normal euvolemic conditions, tubular AII concentrations are in the range of 10^{-8}–10^{-9} M (6–8 nM). Under such circumstances, we can speculate that NO may counteract the inhibitory effect of AII on proximal tubular reabsorption, allowing the expression of the AII stimulatory effect on proximal reabsorption with which most of us are familiar. Administration of DuP 753 under normal circumstances should block the stimulatory effect of AII on proximal tubular reabsorption leading to decreases in APR as demonstrated in this study and previously by Xie et al. (35). Blockade with DuP 753 of both the inhibitory as well as the stimulatory effects of AII on proximal tubular reabsorption can explain the absence of changes in APR in the presence of both DuP and LNMMA (group IIb). One could postulate that under both AII and NO blockade, glomerulotubular balance may be the overriding factor that determines proximal tubular reabsorption, such that increases in SNGFR are associated with increases in APR as seen in our group Ic. The experimental technique used in this study precludes any further analysis of the interaction between AII and NO in the control of proximal tubular reabsorption but provides important observations for future research.

NO appears to mediate the renal hyperemia and hyperfiltration after acute amino acid infusion. Two different studies have demonstrated that LNMMA inhibits the increase in GFR and renal plasma flow observed during amino acid or glycine infusion in normal rats, however, none of these studies has provided insights regarding the mechanism underlying this phenomenon (11, 12). The present study confirms the importance of NO release in the response to glycine. Infusion of LNMMA (group Ib, Table I) induced systemic vasoconstriction, an increase in blood pressure, absence of vasodilation during glycine, and a significant reduction in LpA. The changes in glomerular hemodynamics were coincident with significant decreases in both APR and FR (Fig. 2). Although there is a debate on the role of the TGF on the protein-induced increase in GFR (36–38), on the basis of our findings, we can speculate that the absence of a response to glycine during LNMMA is most likely the result of activation of TGF due to increases in distal tubular delivery. We have previously demonstrated in three different experimental models of renal injury in rats, two-kidney one-clip Goldblatt hypertension (17), diabetes mellitus (18), and experimental glomerulonephritis (unpublished observations), the presence of a correlation between lack of normal response to glycine, and reduction in proximal tubular reabsorption. In these conditions, as in the current study, an activation of the TGF system may be responsible for limiting the response. However, under pathophysiological conditions (experimental diabetes and Goldblatt hypertension) we have recently obtained evidence that TGF activation may not be the only factor that limits the response to glycine, since restoring tubular reabsorption to normal values is not sufficient to restore the response to glycine (reference 18; unpublished observations).

In conclusion, the present study demonstrates for the first time that NO is a physiological antagonist of AII at both the glomerular and tubular level. Such an interaction may be a critical factor in the control of the basal renal function and, moreover, in the tubular and glomerular response to glycine.

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References

1. Pitts, R. F. 1944. The effects of infusing glycine and of varying the dietary protein intake on renal hemodynamics in the dog. *Am. J. Physiol.* 142:355–365.

2. Meyer, T. W., I. Ichikawa, R. Zatz, and B. M. Brenner. 1983. The renal hemodynamic response to amino acid infusion in the rat. *Trans. Assoc. Am. Phys.* 96:76–83.

3. Bosch, J. P., A. Saccaggi, A. Lauer, C. Ronco, M. Belledonne, and S. Labman. 1983. Renal functional reserve in humans. Effect of protein intake on glomerular rate. *Am. J. Med.* 75:943–950.

4. Castellino, P., B. Coda, and R. A. DeFronzo. 1986. Effect of amino acid infusion on renal hemodynamics in humans. *Am. J. Physiol.* 251:F132–F140.

5. Hirschberg, R. R., R. D. Zipser, L. A. Slomowitz, and J. D. Kopple. 1988. Glucagon and prostaglandin are mediators of amino acid-induced rise in renal hemodynamics. *Kidney Int.* 33:1147–1155.

6. Krishna, G. G., G. Newell, E. Miller, P. Heeger, R. Smith, M. Polansky, S. Kapoor, and R. Hoeldtke. 1988. Protein-induced glomerular hyperfiltration: role of hormone factors. *Kidney Int.* 33:578–583.

7. Hirschberg, R. R., and J. D. Kopple. 1987. Role of growth hormone in the amino acid-induced acute rise in renal function in man. *Kidney Int.* 32:382–387.

8. Alverstrand, A., L. Zimmerman, and J. Bergstrom. 1988. Potential role of a liver-derived factor in mediating renal response to protein. *Blood Purif.* 6:276–284.

9. Woods, L. L., H. L. Mizelle, J. Montani, and J. Hall. 1986. Mechanisms controlling renal hemodynamics and electrolyte excretion during amino acids. *Am. J. Physiol.* 252:F303–F312.

10. Claris Appiani, A., B. M. Assael, A. S. Tirelli, G. Cavanna, C. Corbetta, and G. Marra. 1988. Proximal tubular function and hyperfiltration during amino acid infusion in man. *Am. J. Nephrol.* 8:96–101.

11. Tolins, J. P., and L. Raij. 1991. Effects of amino acid infusion on renal hemodynamics. Role of endothelial-derived relaxing factor. *Hypertension (Dallas)* 17:1045–1051.

12. King, A. J., J. L. Troy, S. Anderson, J. R. Neuringer, M. Gunning, and B. M. Brenner. 1991. Nitric oxide: a potential mediator of amino acid-induced renal hyperemia and hyperfiltration. *J. Am. Soc. Nephrol.* 1:1271–1277.

13. Hostetter, T. H., J. L. Olson, H. G. Renke, M. A. Venkatachalam, and B. M. Brenner. 1981. Hyperfiltration in remnant nephrons: a potentially adverse reaction to renal ablation. *Am. J. Physiol.* 241:F85–F93.

14. Anderson, S., T. W. Meyer, H. G. Renke, and B. M. Brenner. 1985. Control of glomerular hypertension limits glomerular injury in rats with reduced renal mass. *J. Clin. Invest.* 76:612–619.

15. Dworkin, L. D., T. H. Hostetter, H. G. Renke, and B. M. Brenner. 1984. Hemodynamic basis for glomerular injury in rats with desoxycorticosterone-salt hypertension. *J. Clin. Invest.* 73:1448–1461.

16. Zatz, R., T. W. Meyer, H. G. Renke, and B. M. Brenner. 1985. Predominance of hemodynamic rather than metabolic factors in the pathogenesis of diabetic glomerulopathy. *Proc. Natl. Acad. Sci. USA.* 82:5963–5967.

17. De Nicola, L., R. C. Blantz, and F. B. Gabbai. 1991. Renal functional reserve in treated and untreated hypertensive rats. *Kidney Int.* 40:406–412.

18. De Nicola, L., R. C. Blantz, and F. B. Gabbai. Renal functional reserve in the early stage of experimental diabetes. *Diabetes.* In press.

19. Shultz, P. J., A. E. Schorer, and L. Raij. 1990. Effects of endothelium-derived relaxing factor and nitric oxide on rat mesangial cells. *Am. J. Physiol.* 258:F162–F167.

20. Tolins, J. P., M. J. Palmer, S. Moncada, and L. Raij. 1990. Role of endothelium-derived relaxing factor in regulation of renal hemodynamic responses. *Am. J. Physiol.* 258:H655–H662.

21. Wong, P. C., W. A. Price, A. T. Chiu, D. J. Duncia Carini, R. R. Wexler, A. L. Johnson, and P. B. W. M. Timmermans. 1990. Nonpeptide angiotensin II receptor antagonists. IX. Antihypertensive activity in rats of DuP 753, an orally active antihypertensive agent. *J. Pharmacol. Exp. Ther.* 252:726–732.

22. Blantz, R. C., and B. J. Tucker. 1978. Measurements of glomerular dynamics. *In* Methods in Pharmacology, Renal Pharmacology. Vol. 4B. M. Martinez Maldonado, editor. Plenum Press, New York, 141–163.

23. Tucker, B. J., and R. C. Blantz. 1978. Determinants of proximal tubular reabsorption as mechanisms of glomerulotubular balance. *Am. J. Physiol.* 235:F142–F150.

24. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265–275.

25. Landis, E. M., and R. J. Pappenheimer. 1963. Exchanges of substances through capillary walls. *In* Handbook of Physiology, Circulation (Sect. 2, Vol. II). American Physiological Society, Washington, DC. 931–1034.

26. Blantz, R. C. 1974. Effect of mannitol upon glomerular ultrafiltration in the hydropenic rat. *J. Clin. Invest.* 54:1135–1143.

27. Bliss, C. I. 1970. Statistics in Biology. McGraw-Hill, New York. 186–205.

28. Zatz, R., and G. De Nucci. 1991. Effects of acute nitric oxide inhibition on rat glomerular microcirculation. *Am. J. Physiol.* 261:F360–F363.

29. Blantz, R. C., K. S. Konnen, and B. J. Tucker. 1976. Angiotensin II effects upon the glomerular microcirculation and ultrafiltration coefficient. *J. Clin. Invest.* 57:419–434.

30. Baylis, C., P. Harton, and K. Engels. 1990. Endothelium-derived relaxing factor controls renal hemodynamics in the normal rat kidney. *J. Am. Soc. Nephrol.* 1:875–881.

31. Ishii, K., B. Chang, J. F. Kerwin, F. L. Wagenaar, Z. J. Huang, and F. Murad. 1991. Formation of endothelium-derived relaxing factor in porcine kidney epithelial LLC-PK_1 cells: an intra- and intercellular messenger for activation of soluble guanylate cyclase. *J. Pharmacol. Exp. Ther.* 256:38–43.

32. Levillain, O., A. Hus-Citharel, F. Morel, and L. Bankir. 1990. Localization of arginine synthesis along rat nephron. *Am. J. Physiol.* 259:F916–F923.

33. Harris, P. F., and J. A. Young. 1977. Dose dependent stimulation and inhibition of proximal tubular sodium reabsorption by angiotensin II in the rat kidney. *Pfluegers Arch. Eur. J. Physiol.* 367:295–297.

34. Seikaly, M. G., B. S. Arant, Jr., and F. D. Seney, Jr. 1990. Endogenous angiotensin concentrations in specific intrarenal fluid compartments of the rat. *J. Clin. Invest.* 86:1352–1357.

35. Xie, M. H., F. Y. Liu, P. C. Wong, P. B. M. W. M. Timmermans, and M. C. Cogan. 1990. Proximal nephron and renal effects of DuP 753, a nonpeptide angiotensin II receptor antagonist. *Kidney Int.* 38:473–479.

36. Brown, S. A., and L. G. Navar. 1990. Single-nephron responses to systemic administration of amino acids in dogs. *Am. J. Physiol.* 259:F739–F746.

37. Woods, L. L., and E. W. Young. 1991. Impaired renal hemodynamic response to protein feeding in dogs with experimental Fanconi syndrome. *Am. J. Physiol.* 261:F14–F21.

38. Woods, L. L., D. R. DeYoung, and B. E. Smith. 1990. Furosemide abolishes the increase in glomerular filtration rate (GFR) after a meat meal. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 4:A437. (Abstr.)