Renoprotective Effects of Felodipine and/or Enalapril in Spontaneously Hypertensive Rats With and Without L-NAME

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Abstract—To determine the renoprotective effects of a calcium antagonist (felodipine) and an angiotensin-converting enzyme (ACE) inhibitor (enalapril), alone or in combination, 10 groups of 19-week-old spontaneously hypertensive rats (SHR) (with or without N^G-nitro-L-arginine methyl ester [L-NAME]) were studied using renal micropuncture techniques. Group 1 (control), group 2 (felodipine, 30 mg · kg^{-1} · d^{-1}), group 3 (enalapril, 30 mg · kg^{-1} · d^{-1}), and group 4 (felodipine plus enalapril, 15 mg · kg^{-1} · d^{-1} each agent) were studied after 3 weeks of treatment without L-NAME. L-NAME (50 mg/L) cotreatment was administered in drinking water to groups 6 through 10 using the same doses of each agent as in groups 1 through 4: group 5 (only L-NAME), group 6 (felodipine), group 7 (enalapril), and group 8 (felodipine plus enalapril). Groups 9 and 10 received L-NAME initially for 3 weeks followed by felodipine or felodipine plus enalapril, respectively, for the subsequent 3 weeks. All three treatments resulted in reductions in mean arterial pressure and total peripheral vascular resistance ($P<.001$) that were associated with important structural and functional renal microcirculatory improvements. Thus, the pathological nephrosclerosis (subcapsular and juxtamedullary) glomerular and arteriolar injury scores were improved ($P<.05$ at least) in association with normalization of afferent and efferent arteriolar resistances, and single-nephron glomerular filtration rate, plasma flow, and blood flow were significantly improved, as well as the ultrafiltration coefficient (compared with group 5, L-NAME). Thus, the calcium antagonist felodipine, alone or in combination with an ACE inhibitor, not only prevented but also reversed L-NAME–exacerbated hypertensive nephrosclerosis in SHR. **(*Hypertension*. 1998;31:795-801.)**

Key Words: nephrosclerosis ■ nitric oxide synthase ■ renoprotection ■ renal pathological changes ■ glomerular filtration rate ■ arteriolar injury ■ rats, inbred SHR

With widespread use of antihypertensive therapy, morbidity and mortality from major cardiovascular complications of hypertension (ie, strokes, coronary heart disease, cardiac failure, and hypertensive emergencies) have decreased significantly. However, ESRD continues to increase[1] without adequate explanation.[2]

Several experimental models for producing renal failure from hypertension have been developed, usually involving reduction of renal mass, with or without salt loading, steroidal administration, renal infarction, or administration of nephrotoxic drugs. Most of these models elevate glomerular hydrostatic pressure and produce glomerulosclerosis. None of these models, however, involves the natural development of ESRD that occurs with aging in genetically hypertensive animals, as in patients with essential hypertension. We have reported that glomerulosclerosis develops naturally in 73-week-old SHR and that it is associated with increased afferent and efferent glomerular arteriolar resistances and elevated glomerular hydrostatic pressure.[3] We have also demonstrated that the nitric oxide synthase inhibitor L-NAME, when administered to 20- to 23-week-old SHR for 3 weeks, produced systemic, renal, and glomerular hemodynamic changes, proteinuria, and glomerulosclerosis similar to those observed in the 73-week-old SHR.[4] It also appears that antihypertensive drugs may differ in their ability to alter hypertension-induced renal damage, even though they reduced arterial pressure to the same extent. Thus, ACE inhibitor prevented, as well as reversed, the altered renal and glomerular hemodynamics, proteinuria, and associated nephrosclerotic pathological changes.[5] In contrast, hydrochlorothiazide exacerbated the disease.[6] Several others have shown that calcium antagonists and ACE inhibitors afforded renoprotection in other experimental models.[7–13]

The present study therefore was designed to determine whether the calcium antagonist felodipine, alone or in combination with the ACE inhibitor enalapril, would alter the pathophysiological course of nephrosclerosis in 20-week-old SHR with or without L-NAME–exacerbated hypertension.

Methods

Male 16-week-old SHR, purchased from Charles River Laboratories (Wilmington, Mass), were housed in plastic cages and maintained at 20°C in a light-controlled room. They were fed standard rat chow (PMI Feeds Inc) and given tap water ad libitum. The experimental protocol was approved by our institutional animal care and use committee.

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Selected Abbreviations and Acronyms

ACE = angiotensin-converting enzyme
ERPF = effective renal plasma flow
ESRD = end-stage renal disease
GFR = glomerular filtration rate
K_F = ultrafiltration coefficient
L-NAME = N^G-nitro-L-arginine methyl ester
MAP = mean arterial pressure
π_A, π_E = afferent or efferent arteriolar osmotic pressure
ΔP = pressure gradient across glomerular capillary wall
P_E = efferent arteriolar pressure
P_G = glomerular capillary hydrostatic pressure
P_T = proximal tubular pressure
R_A, R_E = afferent or efferent glomerular arteriolar resistance
RVW = right ventricular weight
SFP = stop-flow pressure
SHR = spontaneously hypertensive rat(s)
SNBF = single-nephron blood flow
SNFF = single-nephron filtration fraction
SNGFR = single-nephron glomerular filtration rate
SNPF = single-nephron plasma flow
TPR(I) = total peripheral resistance (index)
$U_{Na}V$ = urinary sodium excretion
$U_{Prot}V$ = urinary protein excretion

The rats were divided into 10 experimental groups (Table 1). In brief, the first series of studies involved four treatment groups of 16-week-old SHR not given L-NAME: group 1, control and untreated (n=9); group 2, felodipine (30 mg · kg^{-1} · d^{-1} by gastric gavage for 3 weeks; n=10); group 3, enalapril (30 mg · kg^{-1} · d^{-1} by gastric gavage for 3 weeks; n=8); and group 4, felodipine plus enalapril (15 mg · kg^{-1} · d^{-1} of each agent by gastric gavage; n=9). In the second series, each group (groups 5 through 8) also included 16-week-old male SHR that received L-NAME (50 mg/L) for 3 weeks in drinking water, alone or with their respective agents (the latter were given daily by gastric gavage; Table 1). Thus, each group received its respective treatment (in the same doses used for groups 1 through 4) for 3 weeks: group 5, only L-NAME; group 6, felodipine; group 7, enalapril; and group 8, felodipine plus enalapril. The average daily dose of L-NAME, (7.6±0.7 mg/d in drinking water) was calculated from the water consumed and was determined in previous studies from our laboratory.[4–6] Groups 9 and 10 were used to determine whether felodipine, alone or combined with enalapril, was able to reverse the L-NAME–exacerbated hypertensive renal pathophysiological alterations. These agents were administered in the same doses as used for groups 2 and 8 for 3 weeks after administration of L-NAME for 3 weeks. During the final week of treatment, all rats were placed in metabolic cages for 3 days to measure 24-hour urinary protein (Lowry method)[14] and sodium (Beckman Astra 8 flame photometer) excretion as described previously.[5,6]

Micropuncture Technique

Rats were anesthetized with pentobarbital (40 mg/kg IP) and placed on a temperature-regulated table to maintain rectal temperature at 37°C throughout the study. After a tracheostomy (with insertion of polyethylene tubing), an indwelling polyethylene catheter (PE-50) was placed into the left femoral artery for arterial pressure measurement (Gould-Statham transducer model P23-Db, Statham Instruments) and connected to a multichannel polygraph (Sensor Medics, R612, Beckman Instruments). This same arterial catheter was used to collect blood for measurement (by capillary microcentrifugation) of hematocrit level. The right jugular vein was cannulated with a polyethylene catheter (PE-50), and the right carotid artery was cannulated with a thermistor microprobe Type IT-18 (Physitemp Instruments Inc) connected to a thermodilution device (Cardiotherm 500, Columbus Instruments) for determination of cardiac output. Another polyethylene catheter (PE-50) was inserted into a vein for infusion of solutions. A high-precision syringe (CR-700-200, Hamilton Co) was connected to that venous catheter for injection of saline at room temperature. Cardiac output was displayed on a digital screen and simultaneously recorded; the calculated cardiac output was normalized for body weight and expressed as cardiac index (milliliters per minute per kilogram). TPR was calculated as the quotient of MAP divided by the cardiac index. After these hemodynamic measurements were obtained, the urinary bladder was cannulated with a soft tube for urine collection. The left kidney was exposed through a flank incision and suspended in a Lucite cup packed with cotton and warm agar dripped around the kidney to form a saline (0.9% NaCl) well at room temperature. The renal surface was illuminated by fiber-optic lamp. The left ureter was catheterized with PE-10 catheter for timed urine collection. The right femoral vein was used for [H^3]methoxyinulin (850 μCi/mL) infusion at a rate of 0.1 mL/100 g body wt per hour. The right jugular vein was cannulated for 12% albumin infusion during the first 45 minutes of surgery at a rate of 0.4 mL/100 g body wt per hour and, thereafter, with saline containing 1% albumin and 1.5% *p*-aminohippurate (Merck, Sharp & Dohme) at a rate of 0.4 mL/100 g body wt per hour. After an equilibration period, urine was collected over four 30-minute periods, with blood samples withdrawn at the midpoint of each period.

Two or three "star vessels" were punctured for collection of efferent glomerular arteriolar blood and fluid from four proximal tubules during a 2- to 5-minute period, with particular care to keep a column of oil at the micropipette tip. The data thus obtained permitted calculation of SNGFR, P_E, and P_T. The SFP was measured directly by a servo-null system (Instrumentation for Physiology and Medicine). The P_E and P_T were obtained from the "star vessel" and

TABLE 1. Outline of Experimental Groups of 20- to 23-Week-Old Male SHR

Groups	Rats, n	Treatment (Dose)
No L-NAME		
1	9	Control
2	10	Felodipine (30 mg · kg^{-1} · d^{-1})
3	8	Enalapril (30 mg · kg^{-1} · d^{-1})
4	9	Felodipine+enalapril (15 mg · kg^{-1} · d^{-1}, each agent)
With L-NAME treatment for 3 wk		
5	7	L-NAME (50 mg/L)
6	8	L-NAME (50 mg/L)+felodipine (30 mg · kg^{-1} · d^{-1})
7	7	L-NAME (50 mg/L)+enalapril (30 mg · kg^{-1} · d^{-1})
8	8	L-NAME (50 mg/L)+felodipine+enalapril (15 mg · kg^{-1} · d^{-1}, each agent)
9	9	L-NAME then felodipine (in doses described above)
10	7	L-NAME then felodipine+enalapril (in doses described above)

proximal tubule, respectively. P_G was calculated from the sum of P_{SFP} and plasma π_A. Concentration of protein was determined refractometrically, and π_A was calculated by the Landis-Pappenheimer equation.[15] Transglomerular hydrostatic pressure across the glomerular capillary was calculated as $\Delta P = P_G - P_T$, and transmembrane colloid osmotic pressure difference ($D\pi$) was calculated according to the equation of Deen et al[16] as modified by Arendshorst and Gottschalk.[17] The tubular fluid, urine, and plasma samples were measured for [^{3}H]inulin radioactivity by placement in 10-mL scintillation vials (Bio-Safe II) for counting in a β-scintillation counter, which allowed calculation of SNGFR, GFR, and ERPF. These measurements permit calculation of π_A and π_E, R_A and R_E, and the glomerular capillary K_F. At the termination of each study, blood was drawn for measurement of serum creatinine and uric acid concentrations by a 747-100 Analyzer (Boehringer Mannheim/Hitachi).

Renal Morphology

The kidneys, after being fixed in 10% buffered formalin and embedded in paraffin for light microscopy, were cut at thicknesses of 2 to 3 μm and stained with hematoxylin and eosin, periodic acid-Schiff, and periodic acid-methenamine-silver as reported previously.[3–6] Histological examination was conducted in a blinded fashion, and glomerular and arteriolar injury scores were calculated as described previously.[3–6] Approximately 50 subcapsular and 50 juxtamedullary glomeruli of each specimen were analyzed for glomerular injury, as described in previous studies[3,4,13] (grade 1, normal glomerulus by light microscopy; grade 2, involvement of up to one third of the glomerular area; grade 3, involvement of one to two thirds of the glomerulus; and grade 4, two thirds to global sclerosis). Each scoring permitted calculation of a glomerular injury score: [(1×number of grade 2 glomeruli)+(2×number of grade 3 glomeruli)+(3×number of grade 4 glomeruli)]×100/(number of glomeruli studied).

Forty to 50 afferent arterioles were examined from each specimen to determine an arteriolar injury score using the serial sections stained with periodic acid-Schiff. Grading was performed as described previously[3–6]: grade 1, no arteriolar changes; grade 2, arteriolar wall hyalinosis up to 50% of circumference; grade 3, 50% to 100% hyalinosis of the wall circumference but without luminal narrowing; and grade 4, complete hyalinosis of the wall with luminal encroachment. Each score was then calculated according to the formula for arteriolar injury score: [(1×number of grade 2 arterioles)+(3×number of grade 4 arterioles)]×100(number of arterioles observed).

Statistical Analysis

Results were expressed as mean±1 SEM. An ANOVA analysis followed by Bonferroni's correction for multiple comparisons (also termed Dunn's multiple comparison procedure) was used for statistical analysis.[18] Scheffé's comparison was used for statistical analysis of nephron glomerulosclerosis score.[19] Finally, the 5% confidence level ($P<.05$) was considered to be statistically significant.

Results

Effects Without L-NAME

Organ Weights

There was no difference in body weight among the groups. Left ventricular weight was significantly reduced by enalapril and by felodipine plus enalapril with respect to the control group ($P<.001$; Table 2). Right ventricular, aortic, and renal masses did not change.

Systemic and Renal Hemodynamics

MAP and TPR were markedly reduced by each treatment ($P<.001$; Table 2). Renal plasma flow and blood flow were increased and renal vascular resistance was reduced by each treatment ($P<.001$). GFR was increased by felodipine ($P<.001$) but remained unchanged by enalapril and the combination treatment. Filtration fraction was reduced by enalapril ($P<.001$) and the combination therapy ($P<.005$; Table 2).

Glomerular Dynamics

SNPF and SNGFR were increased significantly by felodipine, and although R_A was significantly diminished ($P<.001$), R_E did not change. In contrast, enalapril and the combination of the two agents significantly increased SNPF and SNGFR and reduced SNFF, R_A, R_E, and P_G ($P<.001$; Table 2).

Effects With L-NAME

Organ Weights

Body weight was reduced in L-NAME rats as reported in earlier studies.[4,13] Left ventricular mass was increased by L-NAME, but enalapril and the combined therapy prevented that increase in left ventricular mass; after L-NAME, the combined therapy reversed the increase in mass ($P<.005$). Moreover, all treatments (with and after L-NAME) reversed the L-NAME–induced increase in aortic mass (Table 3).

Systemic and Renal Hemodynamics

MAP and TPR were markedly increased by L-NAME and were associated with a significant reduction in cardiac index ($P<.001$; Table 3). Cotreatment of L-NAME with each of the three treatments prevented these alterations ($P<.005$; Table 3). L-NAME significantly reduced renal plasma flow and GFR, while renal vascular resistance and filtration fraction increased markedly ($P<.005$; Table 3). Both felodipine and enalapril prevented these L-NAME–induced alterations in whole-kidney hemodynamics ($P<.005$; Table 3), although the filtration fraction remained unaltered by any of these treatments.

Glomerular Dynamics

L-NAME drastically reduced SNPF, SNBF, SNGFR, and K_F, whereas R_A and R_E rose ($P<.005$; Table 3). The P_G and SFP only increased slightly, presumably because the SNBF was diminished so markedly due to the intense R_A constriction. All three treatments prevented these adverse alterations in glomerular dynamics. Twenty-four-hour proteinuria and serum uric acid concentration were significantly increased by L-NAME; these changes were prevented by felodipine, enalapril, and the combined treatment ($P<.005$), although the serum creatinine concentration reduction was not significant.

Effects of Treatment After L-NAME

Organ Weights

The reduced body weight induced by L-NAME was significantly reversed by felodipine and the combined therapy ($P<.05$), and the L-NAME–predicted increases in left ventricular and aorta mass were significantly prevented by both treatments ($P<.001$; Table 3).

Systemic and Renal Hemodynamics

The increased MAP and TPR produced by L-NAME were markedly reduced (ie, reversed) by both felodipine and felodipine plus enalapril ($P<.001$; Table 3). Furthermore, the increased renal vascular resistance was reduced by felodipine and by felodipine plus enalapril ($P<.001$), which were associated with improved ERPF ($P<.005$) and GFR ($P<.05$; Table 3).

TABLE 2. Systemic Renal and Glomerular Hemodynamics in SHR Without L-NAME

Treatment	Group 1 Control (n=9)	Group 2 Felodipine (n=10)	Group 3 Enalapril (n=8)	Group 4 Enalapril+Felodipine (n=9)
Weight, mg/g				
Body	346±5.8	340±6.1	353±3.9	339±4.8
LVW	2.5±0.05	2.5±0.05	2.2±0.06†	0.52±0.14*
RVW	0.58±0.032	0.67±0.021	0.55±0.026	0.61±0.035
LKW	3.4±0.1	3.4±0.05	3.2±0.04	3.2±0.07
RKW	3.2±0.06	3.4±0.05	3.2±0.05	3.2±0.02
Systemic hemodynamics				
MAP, mm Hg	170±5.8	142±3.9*	140±2.4*	124±3.5*§
HR, bpm	357±3.0	361±5.0	353±3.9	344±8.0
CI, mL · min^{-1} · kg^{-1}	223±10	246±6.0	247±9.7	236±4.2
TPRI, U/kg	0.77±0.24	0.58±0.32*	0.57±0.21*	0.32±0.07*
Renal hemodynamics				
ERPF, mL · min^{-1} · g^{-1}	2.8±0.07	4.2±0.1*	4.2±0.33*	3.7±0.1‡
RBF, mL · min^{-1} · g^{-1}	6.4±0.17	9.6±0.3*	9.3±0.72*	8.2±0.3*
RVR, U	26±1.1	15±0.6*	16±1.6*	15±0.6*
GFR, mL · min^{-1} · g^{-1}	0.86±0.09	1.3±0.08*	0.64±0.05	0.87±0.09
FF, %	29±3.0	31±2.1	17±3.1*	23±1.1†
Hct, %	55±0.005	55±0.004	55±0.006	55±0.007
Glomerular dynamics				
SNPF, nL/min	124±2.8	142±3.5‡	145±4.7‡	152±7.3*
SNBF, nL/min	279±7.6	320±7.0‡	323±9.0‡	336±14*
SNGFR, nL/min	34±0.6	40±0.84‡	33±1.8	34±1.6
SNFF, %	27±0.7	28±0.9	23±1.3†	22±0.36*
P_T, mm Hg	12±0.2	13±0.8	13±0.4	14±0.7
SFP, mm Hg	35±0.9	39±0.9†	30±0.6*	30±0.6†
P_G, mm Hg	56±0.73	59.5±0.82	51±0.89*	52±0.64*
R_A, U	3.2±0.24	2.0±0.1*	2.2±0.08*	1.7±0.12*
R_E, U	1.3±0.03	1.3±0.7	0.92±0.02*	0.90±0.04*
P_E, mm Hg	16±0.5	16±0.54	17±0.5	16±0.4
ΔP, mm Hg	44±0.7	45±1.1	37±0.7*	38±0.6*
Coloid A, mm Hg	21±0.47	20±0.24	20±0.55	22±0.62
Coloid B, mm Hg	35±1.0	34±0.84	30±1.5	32±1.2
K_F, nL · s^{-1} · mm Hg^{-1}	0.038±0.02	0.041±0.07	0.048±0.05*	0.052±0.06*
Renal function				
Creatinine, mg/dL	0.53±0.05	0.60±0.05	0.55±0.09	0.50±0.06
Uric acid, mg/dL	1.9±0.24	1.2±0.14	1.6±0.24	1.3±0.15
$U_{Prot}V$, mg/24 h	26±2.7	18±2.2	23±1.6	23±1.4
$U_{Na}V$, mg/24 h	1.4±0.42	1.7±0.66	1.4±0.41	1.6±0.47

LVW indicates left ventricular weight; RVW, right ventricular weight; LKW, left kidney weight; RKW, right kidney weight; HR, heart rate; CI, cardiac index; RBF, renal blood flow; RVR, renal vascular resistance; and FF, filtration fraction. Other abbreviations are defined in "Selected Abbreviations and Acronyms." Values are mean±1 SEM.

*$P<.001$, †$P<.005$, ‡$P<.05$ vs group 1; §$P<.05$ vs group 2.

Glomerular Dynamics

Improvements in SNPF and SNGFR were associated with marked reductions in both R_A and R_E ($P<.005$; Table 3). Moreover, the K_F rose significantly with the combined therapy. Twenty-four-hour proteinuria was markedly decreased by both felodipine and felodipine plus enalapril ($P<.005$); the serum uric acid concentration was reduced by felodipine ($P<.001$; Table 3).

Glomerular and Arteriolar Injury Scores

The glomerular injury to the subcapsular and juxtamedullary lesions produced by the interaction of L-NAME and hypertensive disease was equally severe ($P<.001$). Each of the two

TABLE 3. Systemic, Renal, and Glomerular Hemodynamics of SHR With L-NAME

Treatment	Group 5 L-NAME (n=7)	Group 6 Felodipine+ L-NAME (n=8)	Group 7 Enalapril+ L-NAME (n=8)	Group 8 Felodipine+ Enalapril+ L-NAME (n=7)	Group 9 L-NAME Then Felodipine (n=9)	Group 10 L-NAME Then Felodipine+Enalapril (n=7)
Weights						
Body weight, g	226±12‖	291±7.8‖	293±5.8‖	283±11‖	355±6.4	351±7.2
LVW, mg/g	2.9±0.06§	2.6±0.09*	2.5±0.09*	2.3±0.06†	2.5±0.06‡	2.2±0.04†
RVW, mg/g	0.53±0.023	0.53±0.02	0.58±0.03	0.58±0.024	0.54±0.02	0.51±0.011
LKW, mg/g	3.5±0.07	36±0.04	3.6±0.06	3.4±0.06	3.2±0.04	3.5±0.09
Aorta weight, mg/cm^2	0.068±0.008	0.054±0.005	0.049±0.003*	0.046±0.02†	0.046±0.004†	0.048±0.003*
Systemic hemodynamics						
MAP, mm Hg	241±6.3‖	193±3.8†	183±7.1†	180±5.4†	169±5.7‡	159±5.0‡
HR, bpm	346±7.4	347±12	364±7.1	367±6.1	362±4.9	355±7.3
CI, mL · min^{-1} · kg^{-1}	138±17§	206±8.4†	205±10†	218±6.1	197±4.2†	202±6.2†
TPRI, U/kg	1.95±0.29§	0.96±0.05†	0.91±0.068†	0.83±0.05†	0.86±0.036‡	0.78±0.029‡
Renal hemodynamics						
ERPF, mL · min^{-1} · g^{-1}	0.88±0.14‖	2.3±0.25†	2.2±0.2†	2.5±0.23†	2.6±0.19‡	2.3±0.28‡
RVR, U	139±21‖	40±4.0†	38±3.0†	34±3.5†	32±2.1‡	39±4.0‡
GFR, mL · min^{-1} · g^{-1}	0.34±0.07	1.0±0.13†	0.8±0.07	1.0±0.15*	0.8±0.07†	0.9±0.14*
FF, %	43±6.1	45±4.1	36±3.5	39±3.1	31±3.3	37±6.0
Hct_A, %	56±0.008	55±0.01	55±0.007	55±0.005	51±0.007†	52±0.007*
Glomerular dynamics						
SNPF, nL/min	41±5.8‖	120±8.7†	107±6†	107±5.3*	115±8.3‡	104±6.5‡
SNBF, nL/min	94±14.4‖	268±21†	239±12.6†	239±13†	234±17‡	217±12‡
SNGFR, nL/min	15±21	37±2.3†	31±2.7†	31±2.0†	33±2.8	34±3.8
SNFF, %	37±1.6‖	31±1.4	29±2.0	29±1.2*	30±3.0	32±3.0
P_T, mm Hg	14±0.95	14±0.65	13±0.56	13±0.37	14±0.6	12±0.6
SFP, mm Hg	39±1.2	37±0.92	35±1.2	35±1.2	36±1.4	32±1.3*
P_G, mm Hg	59±1.0	58±1.3	56±1.4	56±1.4	57±1.6	54±1.1
R_A, U	18±3.0‖	4.2±0.4†	4.3±0.21†	4.3±0.4†	4.0±0.41‡	4.0±0.42‡
R_E, U	5.0±0.63‖	1.5±0.12†	1.6±0.11†	1.6±0.11†	1.7±0.13‡	1.7±0.11‡
P_E, mm Hg	16±0.56	16±0.61	15±0.47	16±0.29	17±0.64	15±0.86
ΔP, mm Hg	44±1.8	44±1.4	42±1.4	42±1.4	43±1.6	40±1.3*
Coloid A, mm Hg	20±0.6	21±0.4	21±0.3	20±0.3	21±0.46	21±0.31
Coloid B, mm Hg	42±2.2	39±1.3	36±2.0	33±2.5‖	38±2.4	40±1.3
K_F, nL · s^{-1} · mm Hg^{-1}	0.021±0.003	0.049±0.007	0.043±0.008	0.039±0.003	0.048±0.012	0.077±0.019†
Renal functions						
Creatinine, mg/dL	0.94±0.15	0.67±0.02‡	0.55±0.07‡	0.66±0.10‡	0.61±0.035‡	0.80±0.10*
Uric acid, mg/dL	2.5±0.23‖	1.3±0.26†	1.4±0.17†	0.9±0.26†	1.1±0.15‡	1.6±0.20
$U_{Prot}V$, mg/24 h	44±4.4‖	28±3.0*	21±2.7†	23±2.5†	29±2.4*	24±4.0†
$U_{Na}V$, mg/24 h	1.7±0.28	1.6±0.36	1.1±0.063	0.95±0.073	1.3±0.10	1.3±0.26

LVW indicates left ventricular weight; RVW, right ventricular weight; LKW, left kidney weight; RKW, right kidney weight; HR, heart rate; CI, cardiac index; RBF, renal blood flow; RVR, renal vascular resistance; and FF, filtration fraction. Other abbreviations are defined in "Selected Abbreviations and Acronyms." Values are mean±1 SEM.

*$P<.05$; †$P<.005$; ‡$P<.001$ vs group 5; §$P<.05$; ‖$P<.005$ vs group 1. The multiple comparison analyses between groups 1, 5, 6, 7, and 8 were done separately; groups 1, 5, 8, and 9 were followed with the same statistical analysis as the previous groups.

pharmacological interventions (felodipine or enalapril) significantly reduced the glomerular injury score of both the cortical and juxtamedullar glomeruli ($P<.001$; Table 4), although the probability level was $P<.01$ with the combined therapy. Furthermore, the arteriolar injury scores were normalized by all three treatments ($P<.01$; Table 4).

Discussion

The results of this study demonstrate that felodipine, enalapril, and the combination of each of these agents (in half-doses) improved systemic and renal hemodynamics and the intrarenal glomerular dynamics. Felodipine produced afferent glomerular

TABLE 4. Glomerular and Arteriolar Injury Score in SHR With L-NAME

Score	Control	L-NAME	L-NAME+Felodipine	L-NAME+Enalapril	L-NAME+Felodipine+Enalapril
Glomerular injury					
Subcapsular	13±3.9	59±1.8¶	10.6±1.8†	5.7±0.63†	14±3.9*
Juxtamedullary	11±1.6	56±12.6¶	14.0±5.6†	12.7±3.7†	19.6±5*
Arteriolar injury	42.6±22	92.3±17.4‖	11±2.6§	15±2.6§	7.6±2.3§

Values are mean±1 SEM.
*$P<.05$, †$P<.01$, §$P<.001$ in comparison with L-NAME; ‖$P<.05$, ¶$P<.01$ in comparison with control rats.

arteriolar dilation but not efferent arteriolar dilation in rats not given L-NAME. However, the changes were all the more striking when the hypertensive systemic and renal hemodynamic involvement were exacerbated pathophysiologically with nitric oxide synthase inhibition (using L-NAME). The changes induced by L-NAME are in accordance with those changes seen with hypertensive nephrosclerosis and with aging. These L-NAME–induced renal pathophysiological alterations were both prevented and reversed by the respective treatments. Thus, MAP, TPR, total renal vascular resistance, R_A, and R_E were reduced; whole-kidney and single-nephron blood flows and GFR were increased; and the glomerular and arteriolar histopathological alterations were dramatically improved after only 3 weeks of treatment either concurrently with L-NAME (ie, prevention) or after L-NAME (ie, reversed). The more intense increase in R_A than R_E produced by L-NAME may be explained by the marked reductions in effective whole-kidney and single-nephron blood flows. These physiological alterations were related to the marked proteinuria and severe histopathological changes involving the glomeruli and renal arterioles.

The respective treatment interventions used either with or after L-NAME demonstrated that regardless of whether the calcium antagonist was used alone or with the ACE inhibitor, it was able to either prevent or reverse the L-NAME–induced exacerbation of the hypertensive nephrosclerosis in the SHR/L-NAME model. These remarkable improvements were very similar to those we reported earlier with an ACE inhibitor[3,5,20] but not hydrochlorothiazide.[6] However, unlike the ACE inhibitor that reduced both R_A and R_E, felodipine only reduced R_A despite significant improvements in single-nephron blood flows and filtration. Nevertheless, when both agents were used together (in one-half doses), they reduced R_E and similarly prevented and reversed the proteinuria and histopathological lesions.

The precise mechanism(s) whereby a calcium antagonist prevents or reverses the renal effects of L-NAME is still highly speculative.[21,22] When felodipine was administered to normotensive rats given L-NAME (also for 3 weeks), it was associated with prevention of the reduced renal flow, vascular resistance, GFR, and filtration coefficient, which was related to inhibition of mesangial proliferation.[22] Moreover, although the filtration fraction had slightly increased, the proteinuria diminished. It is possible that the failure of felodipine to reduce R_E reflected stimulation of renin and the effects of intrarenally generated angiotensin II on the efferent arteriole.[23] Indeed, other calcium antagonists have also been shown to increase R_E,[8,24] and a rise in P_G has been reported to follow amlodipine administration.[25] In contrast to these findings with the above-cited dihydropyridine agents, we reported reductions in R_A, R_E, and P_G associated with an increased blood flow with two nondihydropyridine compounds, diltiazem[26,27] and clentiazem.[28,29] However, another nondihydropyridine compound, verapamil, did not reverse the renal vasoconstriction when given after L-NAME.[30,31] Still another calcium antagonist (a dihydropyridine) blunted the vasoconstrictor effects of L-NAME when administered acutely.[32] However, when nifedipine was given chronically to L-NAME–induced hypertensive (but not SHR) Wistar rats, no renal circulatory improvements were observed, even though nifedipine attenuated the systolic pressure rise, normalized plasma renin activity, and improved the glomerulosclerosis and the responses of fibroblasts and mesangial and smooth muscle cellular elements.[33] Moreover, when nifedipine was administered to patients, it blunted the vasoconstrictor effect of acutely infused L-NAME.[34]

It is important to note that most of the aforementioned studies were conducted in originally normotensive rats, in whom renal vascular resistance was normal before L-NAME administration. On the other hand, in our study, we observed an exacerbation of the increased renal and systemic resistances associated with L-NAME, which induced pathophysiological changes that are very similar to those we reported earlier in the hypertensive nephrosclerosis of aged SHR.[4] We now report that felodipine, as well as enalapril (and the combination of both of these agents), not only prevented but reversed these severe pathophysiological alterations involving systemic and renal hemodynamics, glomerular dynamics, proteinuria, and glomerular and arteriolar histopathological changes. Furthermore, these physiological and pathological effects were improved by cotreatment with the ACE inhibitor enalapril. Thus, L-NAME treatment exacerbated the glomerulosclerosis of both cortical and juxtamedullar nephrons. In contrast, the juxtamedullar nephrons were affected primarily in old SHR with naturally occurring nephrosclerosis. Nevertheless, felodipine and the combined therapy promoted the prevention and reversal of the glomerular disease as well as other arterioles. Thus, our findings in SHR/L-NAME nephrosclerosis are in accordance with the reversal of glomerulosclerosis with felodipine in old SHR.[35]

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