Differential Effects of T- and L-Type Calcium Antagonists on Glomerular Dynamics in Spontaneously Hypertensive Rats

Yasuyuki Nakamura, Hidehiko Ono, Edward D. Frohlich

Abstract—To determine whether there is a difference in the effects of T- and L-type calcium antagonists on systemic, renal, and glomerular hemodynamics, the pathological changes of N^δ-nitro-L-arginine methyl ester (L-NAME)–exacerbated nephrosclerosis and clinical alterations were investigated in spontaneously hypertensive rats (SHR). Seven groups of 17-week-old male SHRs were studied: Group 1, control; Group 2, mibefradil, 50 mg · kg⁻¹ · d⁻¹; Group 3, L-NAME in drinking water, 50 mg/L; Group 4, L-NAME (50 mg/L) plus mibefradil (50 mg · kg⁻¹ · d⁻¹); Group 5, L-NAME (50 mg/L) plus amlodipine (10 mg · kg⁻¹ · d⁻¹); Group 6 and 7, L-NAME (50 mg/L) for 3 weeks followed by mibefradil (50 mg · kg⁻¹ · d⁻¹) or amlodipine (10 mg · kg⁻¹ · d⁻¹), respectively, for the subsequent 3 weeks. Both the T- and L-channel calcium antagonists similarly reduced mean arterial pressure and total peripheral resistance index. These changes were associated with significant decreases in afferent and efferent glomerular arteriolar resistances and the ultrafiltration coefficient (P<0.01). Furthermore, the histopathological glomerular and arterial injury scores and urinary protein excretion were also significantly improved (P<0.01), and left ventricular and aortic masses were significantly diminished in all treated groups. Both drugs, mibefradil and amlodipine, had effects of increasing the single-nephron glomerular filtration ratio (SNGFR), and single-nephron plasma flow (SNPF), and of reducing glomerular afferent arteriolar resistance and urinary protein excretion. Thus, the T-type (mibefradil) and L-type (amlodipine) calcium antagonists each prevented and reversed the pathophysiological alterations of L-NAME–exacerbated hypertensive nephrosclerosis in SHR. The T-type calcium antagonist (mibefradil) seemed to have been more effective than the L-type amloidipine antagonist and it produced a greater reduction in afferent arteriolar resistance while preserving SNGFR.

Key Words: L-type receptor calcium antagonist ■ mibefradil ■ amlodipine ■ systemic hemodynamics ■ L-NAME ■ glomerular arteriolar injury ■ proteinuria ■ T-type receptor calcium antagonist ■ renal hemodynamics ■ glomerular dynamics ■ arteriolar injury

The calcium antagonists are widely used for the treatment of hypertension and coronary artery disease.¹⁻³ They have been said to belong to 3 chemical subgroups (dihydropyridines, phenylalkylamines, and benzothiazepines), although at least 5 types of receptors (L, T, N, P/Q, and R), have been identified and cloned that control their respective voltage-gated calcium channels. Moreover, their intracellular effects on controlling free calcium ions are also extremely variable. This class of compounds is highly heterogeneous, and each agent may be distinguished by location and function, structure, pharmacological sensitivities, and electrophysiological and physiological characteristics.⁴ Indeed, in no one organ is their variability more evident than with respect to their renal effects.⁵⁻¹⁴

Recently, the T-type calcium antagonist mibefradil was demonstrated to have developed an ability to inhibit both L- (long lasting) and T- (transient) types of calcium channel receptor sites, with greater selectivity for the T-type channel receptor.¹⁵⁻¹⁸

Little is known on the comparative or differential effects of these 2 receptor antagonists on systemic, renal, and glomerular hemodynamic effects.¹⁹,²⁰ The present study, therefore, was designed to determine whether differences exist between these 2 types of calcium antagonists. To this end, the effects of the L-type calcium antagonist amlodipine and the T-type calcium antagonist mibefradil on systemic and renal hemodynamics and glomerular dynamics were studied in spontaneously hypertensive rats (SHR) with N^δ-nitro-L-arginine methyl ester (L-NAME)–exacerbated nephrosclerosis.²¹

Methods

Male SHR (Charles River Laboratories, Wilmington, Mass) aged 17 weeks were housed in plastic cages and maintained at 20°C in a light-controlled room with free access to food, standard rat chow (PMI Feeds Inc, St. Louis, Mo), and tap water. All experimental
studies had been approved previously by our institutional (Alton Ochsner Medical Foundation) animal care and use committee. The rats were divided into 7 groups: Group 1, control (n = 10); Group 2, mibebradil (50 mg · kg⁻¹ · d⁻¹) by gastric gavage for 3 weeks; n = 9); Group 3, L-NAME (Sigma Chemical Co, St. Louis, Mo); (50 mg/L in drinking water for 3 weeks; n = 13); Group 4, L-NAME (50 mg/L) plus mibebradil (50 mg · kg⁻¹ · d⁻¹) for three weeks by gastric gavage; n = 10); Group 5, L-NAME (50 mg/L) plus amlodipine (10 mg · kg⁻¹ · d⁻¹) for 3 weeks by gastric gavage; n = 7); Group 6, (n = 9), L-NAME (50 mg/L) for 3 weeks; and mibebradil (50 mg · kg⁻¹ · d⁻¹) for 3 weeks; and Group 7 (n = 8) L-NAME (50 mg/L) for 3 weeks then amlodipine (10 mg · kg⁻¹ · d⁻¹) for 3 weeks. The average daily dose of L-NAME (7.6 ± 0.7 mg/d in drinking water) was calculated from water intake and determined in our earlier studies. The 24-hour urinary protein (Uₚrotein, Lowry method) and sodium (Uₚrotein, Beckman Astra 8 frame photometer) excretion rates were measured before all renal micropuncture studies as described previously.

**Micropuncture Technique**

Rats were anesthetized with thiobutabarbital (Inactin, 100 mg/kg IP; Byk-Gulden) and then placed on a temperature-regulated table to maintain rectal temperature at 37°C throughout the study.

Inactin was selected for the anesthetic agent because, in our past studies, this agent had less effect on renal function. However, micropuncture studies are extensive and long, and we cannot exclude this factor in altering such renal phenomena such as glomerulotubular feedback. After a tracheostomy (with insertion of polyethylene tubing), an indwelling polyethylene catheter (PE-50) was placed into the right femoral artery to permit blood sampling and measurement of artery pressure and heart rate. Arterial pressure was measured through a transducer (model P23 Dd, Statham Instruments; Oxnard, Calif) that was connected to a multichannel polygraph (Sensor Medics R612, Beckman Instruments; Schiller Park, Ill). The right carotid artery was cannulated with a thermistor microprobe (Type IT-18; Physitemp Instruments, Schiller Park, Ill). The renal surface was illuminated by a fiber-optic catheter for injection of saline at room temperature. The renal surface was dripped around the kidney to form a saline (0.9% NaCl) well at the midpoint of each collection period. After these hemodynamic measurements were obtained, the bladder was cannulated with a polyethylene catheter (PE-100) for collection of urine. The left kidney was then exposed through a flank incision and suspended in a Lucite cup (poured with cotton) while warm agar was dripped around the kidney to form a saline (0.9% NaCl) well at room temperature. The renal surface was illuminated by a fiber-optic lamp. The left ureter was cannulated with PE-10 catheter for timed urine collection. The right jugular vein was cannulated for [³H] methoxy-inulin (850 μCi/mL) infusion at a rate of 0.1 mL/100 g body weight per hour. The right femoral vein was cannulated for 12% albumin infusion during the first 45 minutes of surgery at a rate of 0.4 mL/100 g body weight per hour and, thereafter, with saline containing 1% albumin and 1.5% p-aminohippurate (Merck Sharp and Dohme; West Point, Pa) at a rate of 0.4 mL/100 g body weight per hour. After an appropriate equilibration period, urine was collected over 30-minute periods, with blood samples withdrawn at the midpoint of each collection period. Two or three “star vessels” were punctured directly for collection of efferent glomerular arteriolar blood. To determine single-nephron glomerular filtration rate (SNGFR), precisely timed (90-second) samples of fluid were collected from 4 to 6 superficial proximal tubules. Efferent (Pₑ) and tubular (Pₜ) and stopped-flow (SFP) pressures were measured directly by a servo-null system (Instrumentation for Physiology and Medicine; San Diego, Calif) as reported previously.

The Pₑ and Pₜ were obtained from the proximal tubule and the “star vessel”, respectively. Pₑ was calculated from the sum of the Pₑₑ and the plasma πₑ. Arterial plasma protein concentration (Cₐ) was determined, and πₑ and πₜ were calculated using the Landis-Pappenheimer equation. The pressure gradient across the glomerular capillary wall was calculated as ΔP = Pₑₑ – Pₑₜ; the transmembrane colloid osmotic pressure difference (Δπₑ) was calculated according to the equation of Deen et al²³ as modified by Arendshorst and Gottschalk.²⁴ The Pₑₑ, Pₜ, and Pₑₑₑ, and their averages were determined.

[³H] julin radioactivity of all tubular fluid, urine, and plasma samples were counted to determine SNGFR, GFR, and ERPF. These measurements were used to calculate πₑ, πₜ, Rₑ, and Rₜ, and glomerular capillary Kₑ. At the termination of each study, blood was withdrawn for measurement of serum creatinine and uric acid concentrations using a 747-100 Analyzer (Boehringer Mannheim/Hitachi).

**Renal Morphology**

After being fixed in 10% buffered formalin and embedded in paraffin for light microscopy, the kidneys were cut at a thickness of 2 to 3 μm and stained with hematoxylin and eosin, periodic acid-Schiff, and periodic acid-methenamine-silver as also reported previously. Histological examination was conducted in a blinded fashion, and glomerular and arteriolar injury scores were calculated as described previously. Approximately 30 subcapsular and 50 juxtamedullary glomeruli from each specimen were analyzed for glomerular injury as described in previous studies: 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 (GIS): [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 also examined from each specimen in order to determine an arteriolar injury score (AIS) using the serial sections stained with periodic-acid-Schiff. Grading was performed as described previously. Grading was performed as described previously. 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 evidence of luminal encroachment. Each score was then calculated according to the formula for arteriolar injury score: [1 × number of Grade 2 arterioles] + [2 × number of Grade 3 arterioles] + [3 × number of Grade 4 arterioles] × 100/(number of arterioles observed).

**Statistical Analysis**

All data are expressed as mean ± SEM. A 1-way ANOVA, followed by Duncan’s multiple range test, was performed for between-group significance. The confidence level was considered to be statistically significant when the probability value was less than 0.05.

**Results**

**Organ Weights**

Left ventricular and aortic masses were significantly increased by L-NAME and reduced by mibebradil, with respect to the control group (P < 0.01). Moreover, left ventricular and aortic masses were also reduced by both calcium antagonists (with and after L-NAME). In contrast, right ventricular and left kidney masses did not change (Table 1). Body weight was greater in Group 6 rats because they were 3 weeks older.

**Systemic and Cardiac, and Whole Kidney Hemodynamics**

Mibebradil (Group 2) slightly decreased MAP and TPRI as compared with the control group (Table 2). On the other
TABLE 2. Systemic and Whole Kidney Hemodynamics

<table>
<thead>
<tr>
<th></th>
<th>Group 1, Control (n=10)</th>
<th>Group 2, Mibefradil (n=9)</th>
<th>Group 3, L-NAME (n=13)</th>
<th>Group 4, L-NAME+Mibefradil (n=10)</th>
<th>Group 5, L-NAME+Amlodipine (n=7)</th>
<th>Group 6, L-NAME then Mibefradil (n=9)</th>
<th>Group 7, L-NAME then Amlodipine (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>184±2</td>
<td>174±4</td>
<td>213±4*</td>
<td>188±5†</td>
<td>187±5‡</td>
<td>181±4†</td>
<td>182±3†</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>335±9</td>
<td>335±10</td>
<td>346±8</td>
<td>344±11</td>
<td>316±3‡</td>
<td>339±9</td>
<td>321±5§</td>
</tr>
<tr>
<td>Cardiac index, mL/min^−1·kg^−1</td>
<td>192±6</td>
<td>208±6</td>
<td>176±9</td>
<td>204±12</td>
<td>184±6</td>
<td>200±15</td>
<td>196±8</td>
</tr>
<tr>
<td>Total peripheral resistance index, U/kg</td>
<td>0.96±0.03</td>
<td>0.84±0.03</td>
<td>1.28±0.09*</td>
<td>0.95±0.06†</td>
<td>1.03±0.06‡</td>
<td>0.96±0.09†</td>
<td>0.94±0.04†</td>
</tr>
<tr>
<td>Stroke Index, mL/min^−1·kg^−1</td>
<td>0.58±0.03</td>
<td>0.63±0.03</td>
<td>0.52±0.03</td>
<td>0.55±0.06</td>
<td>0.58±0.02</td>
<td>0.58±0.05</td>
<td>0.61±0.02</td>
</tr>
<tr>
<td>Effective renal plasma flow, mL/min</td>
<td>2.9±0.1</td>
<td>3.9±0.2*</td>
<td>1.7±0.3*</td>
<td>2.2±0.2**</td>
<td>2.4±0.3</td>
<td>2.3±0.4</td>
<td>2.4±0.3</td>
</tr>
<tr>
<td>Glomerular filtration rate, mL/min^−1·100 g^−1</td>
<td>0.26±0.02</td>
<td>1.35±0.01**</td>
<td>0.16±0.02*</td>
<td>0.25±0.02‡</td>
<td>0.23±0.03†</td>
<td>0.22±0.03‡</td>
<td>0.25±0.03‡</td>
</tr>
</tbody>
</table>

Data is mean±1 SEM.

*P<0.01, **P<0.05 vs Group 1; †P<0.01, ‡P<0.05 vs Group 3.
was no improvement in proteinuria, FF, and GIS. On the other hand, prior reports describing the effects by amlo-
dipine, such as an L-type agent, suggest that these agents dilate
everywhere that most L-type calcium antagonists, including amlo-
dipine, diluted only the afferent glomerular arterioles.

Discussion

The results of this study clearly demonstrate that both mibefradil and amlo-
dipine improved systemic and renal hemodynamics as well as intrarenal glomerular dynamics. These findings are similar to our previous findings concerning the renoprotective effects of felodipine, an L-type channel calcium receptor antagonist. In that study, felodipine not only prevented but also reversed L-NAME-exacerbated hypertensive nephrosclerosis in SHR. On the other hand, prior reports describing the effects by amlo-
dipine, another L-type channel antagonist, have been inconsistent. Dworkin et al found no renoprotective effects of amlo-
dipine in uninephrectomized SHR since there was no improvement in proteinuria, FF, and GIS. On the other hand, Saruta et al demonstrated a protective action in 5/6 nephrectomized SHR. These workers also reported that most L-type calcium antagonists, including amlo-
dipine, diluted only the afferent glomerular arterioles.

In general, most reports on the effects of calcium antagonists, such as an L-type agent, suggest that these agents dilate only the afferent arterioles and have little effect on the efferent vessels (eg, nifedipine in the isolated perfused hydroenphritic model, diltiazem in vasoconstricted isolated perfused kidney, and verapamil in rats in which responses to angiotensin II were determined from perfused juxtedudillary nephrons).

In contrast, L-type receptor antagonists, such as efonidipine and manidipine, have been shown to dilate both afferent and efferent glomerular arterioles. Similarly, Ménard et al reported that mibefradil diminished proteinuria and prevented glomerular lesions in DOCA-salt hypertensive rats. These investigators also suggested that mibefradil may dilate both afferent arterioles and efferent arterioles. Our data support this finding since mibefradil reduced $P_g$, $R_u$, and $P_e$. Our data further suggest that amlo-
dipine also dilated both afferent and efferent arterioles, findings which are consistent with the report by Hayashi et al.

### TABLE 4. Serum Creatinine and Uric Acid Concentrations

<table>
<thead>
<tr>
<th>Group 1, Control</th>
<th>Group 2, Mibefradil</th>
<th>Group 3, L-NAME</th>
<th>Group 4, L-NAME+Mibefradil</th>
<th>Group 5, L-NAME+Amlo dipine</th>
<th>Group 6, L-NAME then Mibefradil</th>
<th>Group 7, L-NAME then Amlo dipine</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n=10$</td>
<td>$n=9$</td>
<td>$n=13$</td>
<td>$n=10$</td>
<td>$n=7$</td>
<td>$n=9$</td>
<td>$n=8$</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>0.55±0.05</td>
<td>0.60±0.05</td>
<td>0.74±0.05</td>
<td>0.74±0.06</td>
<td>0.71±0.07</td>
<td>0.71±0.06</td>
</tr>
<tr>
<td>Uric acid, mg/dL</td>
<td>1.6±0.2</td>
<td>1.2±0.2</td>
<td>2.1±0.4</td>
<td>1.6±0.2</td>
<td>2.1±0.3</td>
<td>1.5±0.2</td>
</tr>
<tr>
<td>$U_{\text{crea}}, V, mg/24 h$</td>
<td>15±1.1</td>
<td>14±1.1</td>
<td>55±10*</td>
<td>13±1.9†</td>
<td>21±1.6†</td>
<td>17±1.6†</td>
</tr>
<tr>
<td>$U_{\text{crea}}, V, mg/24 h$</td>
<td>14±0.1</td>
<td>1.3±0.1</td>
<td>1.5±0.1</td>
<td>1.6±0.1</td>
<td>1.8±0.1*</td>
<td>1.5±0.1</td>
</tr>
</tbody>
</table>

Data is mean±SEM.

*$P<0.01$, **$P<0.05$ vs Group 1, †$P<0.01$ vs Group 3, §$P<0.01$, ||$P<0.05$ vs Group 4.
Our study does not define the mechanism by which either mibefradil or amlopidine dilate afferent and efferent arteries although these 2 agents clearly reduce the arteriolar smooth muscle tone. Some studies have suggested that angiotensin II-induced afferent arteriolar vasconstriction may be mediated by activation of the voltage-gated calcium channels.5,34,35 Both voltage-operated T- and L-type calcium channels prevail in afferent arterioles, although similar actions may be lacking in the efferent arterioles. Saruta et al,5 however, demonstrated that efondipine and manidipine not only inhibited voltage-operated calcium channels but also seem to affect other mechanisms involved in arteriolar smooth muscle contraction, because these agents inhibited angiotensin II-induced vasoconstriction of efferent arterioles. In a recent report,13 we have summarized the results of those studies that examined the nephroprotective effects of calcium antagonists in hypertension. In that review we emphasized that the nephroprotective effect of all calcium antagonists were inconsistent and appeared to vary with the experimental model, the type and dose of the calcium antagonist used, and their possible differences in their renal microcirculatory effects. Bidani and Griffin36 suggested that glomeruloprotection with the type and dose of the calcium antagonist used, and their possible differences in their renal microcirculatory effects. Bi- 

<table>
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<tr>
<th>TABLE 5. Glomerular and Tabular Injury Scores</th>
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<tr>
<td>Group 1, Control (n=10)</td>
</tr>
<tr>
<td>Glomerular injury score</td>
</tr>
<tr>
<td>Subcapsular glomeruli</td>
</tr>
<tr>
<td>Juxtamedullary glomeruli</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>Arterial injury score</td>
</tr>
</tbody>
</table>

Data is mean±SEM.

Our study does not define the mechanism by which either mibefradil or amlopidine dilate afferent and efferent arterioles. Some studies have suggested that angiotensin II-induced afferent arteriolar vasconstriction may be mediated by activation of the voltage-gated calcium channels.5,34,35 Both voltage-operated T- and L-type calcium channels prevail in afferent arterioles, although similar actions may be lacking in the efferent arterioles. Saruta et al,5 however, demonstrated that efondipine and manidipine not only inhibited voltage-operated calcium channels but also seem to affect other mechanisms involved in arteriolar smooth muscle contraction, because these agents inhibited angiotensin II-induced vasoconstriction of efferent arterioles. In a recent report,13 we have summarized the results of those studies that examined the nephroprotective effects of calcium antagonists in hypertension. In that review we emphasized that the nephroprotective effect of all calcium antagonists were inconsistent and appeared to vary with the experimental model, the type and dose of the calcium antagonist used, and their possible differences in their renal microcirculatory effects. Bi- 

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