Effects of Lemildipine, a New Calcium Channel Blocker, on Renal Microcirculation in SHR

Masahiko Kawabata, Tetsuya Ogawa, and Toshikazu Takabatake

The effects of lemidipine, a new dihydropyridine calcium channel blocker, on renal hemodynamics and the tubuloglomerular feedback (TGF) mechanism were examined in anesthetized 9- to 10-wk-old spontaneously hypertensive rats (SHR). Lemildipine, 3 µg·kg⁻¹ i.v., did not reduce mean blood pressure (MBP) but tended to increase GFR and increased urinary excretion of sodium (UNaV). Filtration fraction (FF) and fractional excretion of sodium (FENa) remained unaltered. An additional dose of lemidipine, 9 µg·kg⁻¹ i.v., reduced MBP and renal vascular resistance. Renal plasma flow tended to increase, and GFR was unchanged. FF significantly fell. UNaV and FENa remained at the control level. Micropuncture experiments revealed that the maximal reduction in proximal stop-flow pressure (SFP), an index of glomerular capillary hydrostatic pressure (Pgc), induced by perfusion of the loop of Henle was significantly reduced by high-dose treatment (8.8 ± 1.3 vs. 13.7 ± 1.9 mmHg in control). A high dose of lemidipine induced a rightward and slightly upward shift of the TGF curve; the steady-state tubular flow rate (V₁/₂) was increased, the maximal slope of the curve decreased, and SFP at V₁/₂ unaltered. A low dose of lemidipine did not affect TGF response. These results indicate that lemidipine attenuates the TGF response in SHR by dilating afferent arterioles and thereby corrects the left and downward shift of the TGF curve in SHR. In addition, the fall in FF indicates lemidipine-induced efferent arteriolar vasodilatation. Through balanced systemic and glomerular vasodilatation, lemidipine maintains the levels of Pgc and GFR in the face of reduced renal perfusion pressure. (Hypertens Res 1998; 21:121-126)

Key Words: afferent arteriole, efferent arteriole, intraglomerular pressure, renal hemodynamics, tubuloglomerular feedback

Glomerular hemodynamics are controlled by the tubuloglomerular feedback (TGF) mechanism, which alters blood flow and glomerular filtration of a single nephron by changing afferent arteriolar resistance in response to the sodium chloride concentration at the macula densa (1). The TGF mechanism is believed to play an important role in the regulation of water and electrolytes. In spontaneously hypertensive rats (SHR), TGF activity is augmented as compared with normotensive Wistar-Kyoto rats (WKY) (2–6). We have shown that this increased TGF activity is corrected in part by volume replacement (4), renal denervation (5), and calcium channel blocker treatment (6).

Lemildipine, (±)-3-isopropyl 5-methyl 2-carbamoyloxyethyl-4-(2,3-dichlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate, is a newly developed dihydropyridine calcium channel blocker (7, 8). Lemildipine has been reported to have a slow-onset and long-lasting profile of receptor binding (9) and to show significant antihypertensive effects, when given once a day or twice a day, without altering the diurnal variation of blood pressure in hypertensive patients (10).

The aim of this study was to investigate the acute effects of lemidipine on glomerular hemodynamics and on the TGF mechanism in anesthetized SHR. To evaluate glomerular microcirculatory effects, micropuncture experiments were performed in vivo to measure proximal tubular stop-flow pressure (SFP) during perfusion of the loop of Henle at various rates, which enabled us to estimate glomerular capillary hydrostatic pressure (Pgc) and to assess TGF responsiveness in single superficial nephrons during lemidipine treatment.

Methods

Animal Preparation

Male SHR and WKY (8 wk old) were obtained from Charles River Japan (Atsugi, Japan). They were fed a commercial chow diet (Na, 0.1 mEq/g; K, 0.24 mEq/g) and allowed free access to food and water up to the time of surgery. Experiments were carried out as described previously (11), when the rats were 9 to 10 wk of age. Anesthesia was induced with thiopental sodium (Ravonal, Tanabe, Osaka, 110 mg·kg body weight⁻¹ i.p.). A tracheostomy tube was inserted, and a PE-10 polyethylene tube (Clay Adams, Parsippany, USA) was placed in...

the right external jugular vein for infusion of 10% polyfructosan (Inutest, Laevosan, Linz, Austria) in 0.9% saline at a rate of 4.5 ml·kg body weight\(^{-1}\)·h\(^{-1}\). The right femoral artery was cannulated with a PE-10 tube to monitor arterial pressure and collect blood samples. The left kidney was then exposed through a left subcostal flank incision, placed in a plastic cup, and immobilized in agar (30 g·l\(^{-1}\) in 0.9% NaCl). The renal surface was bathed in warmed mineral oil (38°C), and the left renal pelvis was cannulated with a PE-10 tube to collect urine samples. A 25-gauge needle connected to a PE-50 tube was inserted into the left renal vein to collect renal venous blood.

Whole Kidney Clearance Study

Effects of lemildipine on renal hemodynamics were assessed in nine SHRs. One hour after completion of surgery, a 60-min clearance period was started and allowed as a control period. After the control period, lemildipine, 3.0 \(\mu\)g·kg\(^{-1}\)·i.v., was dissolved in 0.3 ml 0.9% saline, was infused over 2 min into the systemic circulation via the jugular venous catheter. Thirty minutes after the bolus infusion, two successive 30-min clearance studies were performed. Then, an additional dose of lemildipine, 9.0 \(\mu\)g·kg\(^{-1}\)·i.v., was administered in the same way. After waiting 30 min, two 30-min clearance studies were done. Arterial and renal venous blood samples were collected at the midpoints for the measurement of polyfructosan and electrolyte concentrations in plasma.

Measurement of Proximal Stop-Flow Pressure (SFP) during Loop Perfusion

Micropuncture studies were started 1 h after completion of surgery in six SHRs. During control, low-dose (3.0 \(\mu\)g·kg\(^{-1}\)·i.v.), and high-dose (3.0 + 9.0 \(\mu\)g·kg\(^{-1}\)·i.v.) lemildipine periods, TGF responses were estimated by measuring the changes in SFP during orthograde perfusion of the loop of Henle with artificial tubular fluid (ATF) as described previously (11). In brief, the course of a nephron was irrigated to estimate \(P_{Gc}\) (1) because SHR does not possess superficial glomeruli that can be punctured directly. ATF, containing (in mM) 136 NaCl, 4 NaHCO\(_3\), 4 KCl, 2 CaCl\(_2\), 7.5 urea and 0.1% FD & C Green (Keystone, Chicago, USA), was prepared and gassed with 5% CO\(_2\)/95% O\(_2\). The feedback response was expressed as the percentage change in the maximal reduction from SFP at zero loop perfusion (%dSFP).

For comparison, TGF response was also assessed in five age-matched WKYs, using the same protocol as in SHR under control condition.

Analytical Techniques

Urine volume was determined by weight. The polyfructosan concentrations in plasma and urine were measured by the anthrone method (12). Plasma and urine concentrations of Na and K were measured by flame photometry (Model 775, Hitachi; Tokyo, Japan). Each measurement was made in duplicate.

Calculations and Statistics

The whole kidney glomerular filtration rate (GFR) was determined from the whole kidney polyfructosan clearance. Renal plasma flow (RPF) was calculated as GFR·AP/(AP-VP), where AP and VP are the arterial and renal venous concentrations of polyfructosan, respectively. Renal blood flow (RBFW) was calculated as RPF/(1-hematocrit) and renal vascular resistance (RVR) as mean arterial pressure/RBF.

TGF was characterized quantitatively by the method of Schermann and Briggs (13). The inverse, sigmoidal relationship between the loop of Henle perfusion rate (Vlp) and SFP can be described by the following equation:

\[
SFP = SFP_0 - \frac{dSFP}{1 + e^{k(V_{1/2} - V_{lp})}}
\]

where SFP is described as a function of Vlp and four variables: SFP\(_0\), SFP at no loop flow; \(k\), an exponential constant; dSFP, the maximal decrease in SFP; and \(V_{1/2}\), the flow rate at which the response is half-maximal. The equation is symmetry around the midpoint; the maximal slope, \(f'(V_{1/2})\) arises at the midpoint and is equal to \(k\cdot dSFP/4\). \(V_{1/2}\) and \(k\) were computed from the five data points for each nephron using a nonlinear least squares procedure. SFP\(_{1/2}\), SFP at half-maximal reduction, was calculated as (SFP\(_0\) - dSFP/2).

Results are expressed as the means ± SE. Analysis of variance (ANOVA) was performed to study differences among groups. When ANOVA revealed a significant difference, Fisher’s PLSD was applied to identify specific group differences. A probability level of less than 0.05 was considered to indicate statistical significance.

Results

Whole Kidney Clearance

The effects of intravenous lemildipine on whole kidney clearance are summarized in Table 1. Low-dose lemildipine (3.0 \(\mu\)g·kg\(^{-1}\)·i.v.) significantly increased urinary excretion of sodium (\(U_{Na}\)V) and tended to increase GFR and fractional excretion of sodium (FE\(_{Na}\)). MBP and other renal hemodynamic variables remained unchanged. High-dose lemildipine (3.0 + 9.0 \(\mu\)g·kg\(^{-1}\)·i.v.) lowered blood pressure and decreased RVR, indicating renal vascular vasodilatation. RPF tended to increase, and GFR was un-
altered; FF was thus significantly lowered. While urine volume was reduced, other variables of urinary excretion remained unaltered during high-dose administration.

**SFP Response to Loop Perfusion**

SFP responses to varying loop perfusion rates in SHR are depicted in Fig. 1. As the loop perfusion rate was increased, SFP decreased significantly along an inverted sigmoidal curve during the control, low- and high-dose lemildipine periods. In control, loop flow at 20, 30, or 40 nl/min decreased SFP from the unperfused value (SFP₀) of 41.1 ± 2.4 mmHg to 32.4 ± 2.5, 28.4 ± 2.6, and 28.8 ± 2.3 mmHg, respectively. During low-dose lemildipine infusion, SFP was decreased by loop perfusion at 20, 30, or 40 nl/min: from SFP₀ of 44.6 ± 1.7 mmHg to 38.0 ± 2.5, 30.0 ± 2.2, and 30.2 ± 2.7 mmHg, respectively. During high-dose infusion SFP was decreased by loop perfusion at 30 or 40 nl/min: from SFP₀ of 41.4 ± 1.3 mmHg to 33.7 ± 1.6 and 32.7 ± 1.6 mmHg, respectively. SFP at corresponding perfusion rates did not differ among the three groups.

SFP responses in WKY are shown in the lower panel of Fig. 1. In WKY SFP was decreased by loop perfusion at 30 or 40 nl/min: from SFP₀ of 37.7 ± 1.3 mmHg to 30.7 ± 1.4 and 29.4 ± 1.5 mmHg, respectively. SFP at corresponding perfusion rates did not differ among WKY and control SHR.

Percent changes in SFP at each perfusion rate in all four groups are depicted in Fig. 2. SFP changes at 20 and 30 nl/min in WKY were significantly reduced by high-dose lemildipine. The SFP reductions at 20 and 30 nl/min in WKY were significantly smaller than those in control SHR. The corresponding values in SHR treated with high-dose lemildipine did not differ from those in WKY.

### Table 1. Effects of Intravenous Lemildipine on Whole Kidney Function in SHR

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Lemildipine 3.0 µg/kg i.v.</th>
<th>Lemildipine 3.0+9.0 µg/kg i.v.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBP (mmHg)</td>
<td>137 ± 4</td>
<td>134 ± 4</td>
<td>117 ± 4*</td>
</tr>
<tr>
<td>RVR (mmHg·ml⁻¹·min⁻¹·gKW⁻¹)</td>
<td>23.5 ± 2.6</td>
<td>21.2 ± 2.0</td>
<td>19.0 ± 2.4*</td>
</tr>
<tr>
<td>RBF (ml·min⁻¹·gKW⁻¹)</td>
<td>6.24 ± 0.62</td>
<td>6.76 ± 0.68</td>
<td>6.83 ± 0.63</td>
</tr>
<tr>
<td>RPF (ml·min⁻¹·gKW⁻¹)</td>
<td>2.61 ± 0.23</td>
<td>2.88 ± 0.25</td>
<td>3.11 ± 0.25</td>
</tr>
<tr>
<td>GFR (ml·min⁻¹·gKW⁻¹)</td>
<td>0.78 ± 0.04</td>
<td>0.85 ± 0.04</td>
<td>0.80 ± 0.07</td>
</tr>
<tr>
<td>FF</td>
<td>0.31 ± 0.03</td>
<td>0.31 ± 0.02</td>
<td>0.26 ± 0.02*</td>
</tr>
<tr>
<td>UV (µl·min⁻¹·gKW⁻¹)</td>
<td>4.9 ± 0.4</td>
<td>5.2 ± 0.4</td>
<td>4.0 ± 0.4*</td>
</tr>
<tr>
<td>U₄NaV (nEq·min⁻¹·gKW⁻¹)</td>
<td>76 ± 19</td>
<td>102 ± 28*</td>
<td>62 ± 11</td>
</tr>
<tr>
<td>FENa (%)</td>
<td>0.69 ± 0.17</td>
<td>0.83 ± 0.21</td>
<td>0.58 ± 0.12</td>
</tr>
<tr>
<td>U₄K (nEq·min⁻¹·gKW⁻¹)</td>
<td>959 ± 161</td>
<td>1,134 ± 75</td>
<td>783 ± 99</td>
</tr>
<tr>
<td>FEN (%)</td>
<td>261 ± 42</td>
<td>301 ± 12</td>
<td>222 ± 18</td>
</tr>
<tr>
<td>Ht (%)</td>
<td>58 ± 1</td>
<td>57 ± 1*</td>
<td>55 ± 1*</td>
</tr>
</tbody>
</table>

Values are means±SE; n=9 rats. Control, control period; Lemildipine, experimental period of lemildipine infusion. MBP, mean blood pressure; RVR, renal vascular resistance; RBF, renal blood flow; RPF, renal plasma flow; GFR, glomerular filtration rate; FF, filtration fraction; UV, urine volume; U₄NaV, urinary excretion of sodium; FENa, fractional excretion of sodium; U₄K, urinary excretion of potassium; FENK, fractional excretion of potassium; Hct, hematocrit; KW, kidney weight. *p<0.05 vs. control.

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**Fig. 1.** Proximal tubular stop-flow pressure (SFP) at different rates of loop of Henle perfusion with artificial tubular fluid in control SHR (upper and lower, 10 nephrons/4 rats), lemildipine-treated SHR (upper, low-dose lemildipine infusion, 11 nephrons/6 rats; upper, high-dose lemildipine infusion, 12 nephrons/6 rats), and control WKY (lower, 9 nephrons/5 rats). Symbols, means; bars, SE. *p<0.05 vs. zero perfusion.
The variables of the feedback curves are shown in Table 2. Both dSFP and %dSFP were smaller with high-dose lemildipine than in control SHR. High-dose lemildipine increased V1/2 and decreased f'(V1/2). Low-dose lemildipine, however, did not affect any TGF variable. When TGF variables were compared between WKY and control SHR, both dSFP and %dSFP were significantly larger in SHR than in WKY, although SFPo and SFPmin, SFP at maximal reduction were similar. V1/2 was smaller and f'(V1/2) larger in SHR than in WKY. SFP1/2 did not differ between WKY and SHR.

### Relationship between SFP and MBP

During the control period in SHR, SFPo and SFPmin in individual nephrons positively correlated with simultaneously measured MBP ($r = 0.738$, and 0.746, respectively; both $p < 0.05$). During high-dose lemildipine, however, a similar relationship was observed with SFPo ($r = 0.687$; $p < 0.05$) but not with SFPmin. Lemildipine reduced both the linear slope and probability level of the relationship between MBP and SFP1/2 in a dose-dependent manner (Fig. 3); the slope was $0.554 \pm 0.148$ in control, $0.299 \pm 0.157$ in low dose, and $0.138 \pm 0.091$ in high dose. SFP1/2 averaged in each group was unaltered (Fig. 3 and Table 2).

### Discussion

In clearance experiments, a low dose of lemildipine, which did not show a hypotensive effect, tended to

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**Table 2. Variables of Feedback Curves**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Lemildipine 3.0 µg/kg i.v.</th>
<th>Lemildipine 3.0+9.0 µg/kg i.v.</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFPo (mmHg)</td>
<td>41.1±2.4</td>
<td>44.6±1.7</td>
<td>41.4±1.3</td>
<td>37.7±1.3</td>
</tr>
<tr>
<td>SFPmin (mmHg)</td>
<td>27.7±2.5</td>
<td>29.6±2.2</td>
<td>32.6±1.5</td>
<td>29.3±1.6</td>
</tr>
<tr>
<td>dSFP (mmHg)</td>
<td>13.7±1.9</td>
<td>15.0±1.6</td>
<td>8.8±1.3*</td>
<td>8.3±0.7*</td>
</tr>
<tr>
<td>%dSFP (%)</td>
<td>32.8±4.5</td>
<td>33.9±3.9</td>
<td>21.0±3.0*</td>
<td>22.3±2.2*</td>
</tr>
<tr>
<td>MBP (mmHg)</td>
<td>139±3</td>
<td>137±3</td>
<td>113±4*</td>
<td>91±2*</td>
</tr>
<tr>
<td>f'(V1/2)</td>
<td>0.86±0.16</td>
<td>1.04±0.20</td>
<td>0.44±0.09*</td>
<td>0.35±0.05*</td>
</tr>
<tr>
<td>V1/2 (nl/min)</td>
<td>18.6±1.5</td>
<td>20.6±1.1</td>
<td>23.7±1.3*</td>
<td>24.0±1.0*</td>
</tr>
<tr>
<td>SFP1/2 (mmHg)</td>
<td>34.4±2.3</td>
<td>37.1±1.8</td>
<td>37.0±1.2</td>
<td>33.5±1.4</td>
</tr>
<tr>
<td>n (nephron/rat)</td>
<td>10 / 4</td>
<td>11 / 6</td>
<td>12 / 6</td>
<td>9 / 5</td>
</tr>
</tbody>
</table>

Values are means±SE; Control, control period; Lemildipine, experimental period of lemildipine infusion. SFPo, stop-flow pressure (SFP) measured in the absence of loop flow; SFPmin, SFP at maximal reduction; dSFP, maximal decrease in SFP; %dSFP, percent change in maximal decrease of SFP; f'(V1/2), maximal slope; V1/2, flow rate at which feedback response is half-maximal; SFP1/2, SFP at half-maximal reduction; n, number of nephrons/no. of animals. *$p<0.05$ vs. control SHR.

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**Fig. 2.** Percentage changes in SFP to loop perfusion at different rates in control SHR, lemildipine-treated SHR, and control WKY. Symbols, means; bars, SE. *$p<0.05$ vs. zero perfusion. †$p<0.05$ vs. corresponding value in control SHR.

**Fig. 3.** Relationship between SFP at half-maximal reduction (SFP1/2) and MBP in individual tubules in control and lemildipine-treated SHR. Bars represent means±SE.
increase both GFR and RPF, with no change in FF. This may indicate a vasodilatory effect of lemilidine on the preglomerular vessels, as has been shown with other calcium channel blockers (14-16). A high dose of lemilidine, which substantially reduced systemic blood pressure, tended to increase RPF with no change in GFR, which clearly decreased FF, indicating that depressor doses of lemilidine predominantly dilate efferent arterioles. A vasodilatory effect on postglomerular vessels has not been previously reported with calcium channel blockers, except for fendiline (6, 16) and efonidipine (16-18), both dihydropyridine derivatives. Preglomerular vasodilatation increases glomerular-capillary hydrostatic pressure, which could be mitigated by the concomitant dilation of efferent arterioles. Because the protective effects of anti-hypertensive drugs on the kidney may be related to reductions in glomerular hypertension as well as systemic blood pressure, the effect of lemilidine on efferent arterioles may play a role in nephroprotection.

Calcium channel blockers have been reported to induce mild diuresis and natriuresis (19, 20). The mechanisms include an increase in GFR and inhibition of sodium reabsorption in the proximal tubules (19, 20). Our present findings are in accordance with this. Urinary sodium excretion significantly increased during low-dose lemilidine but returned to the control level with high-dose lemilidine, parallel to the changes in GFR and FENa. Thus, both mechanisms seem to participate in mild natriuresis during low-dose lemilidine treatment.

Micropuncture experiments demonstrated that SFP decreases in response to an increase in distal tubular flow in both control and lemilidine-treated animals, supporting the existence of feedback regulation of glomerular hemodynamics (I). However, the reduction in SFP induced by loop perfusion, dSFP and %dSFP, was clearly smaller during high-dose lemilidine than control. Because the TGF response involves the vasomotor response of the afferent arteriole, the bluntened maximal TGF response suggests dilation of this arteriolar segment by high-dose lemilidine. Since the fall in FF shown in the clearance study indicates a reduction in efferent arteriolar resistance, lemilidine at the depressor dose seems to dilate both afferent and efferent arterioles in the glomerular microcirculation.

In young SHR, the TGF response curve is shifted to the left and downward, indicating augmented TGF sensitivity, as compared with age-matched WKY (2-6). Such alterations in the TGF have been suggested as one of the reasons for the elevated preglomerular vascular resistance in SHR, which maintains an intraglomerular pressure comparable to that in WKY (3). Consistent with these previous reports, the maximal TGF response, dSFP or %dSFP, was greater and V1/2 was smaller in SHR than in WKY in the present experiments, indicating a shift of the TGF curve in SHR. Furthermore, SFP1/2 in our study, 34.4 mmHg in SHR and 33.5 mmHg in WKY, support comparable Pgc in both strains.

The high dose of lemilidine shifted the TGF curve rightward and slightly upward and diminished TGF sensitivity in SHR to the level seen in WKY; dSFP and %dSFP decreased, V1/2 increased, and the maximal slope which arises at the midpoint of the curve [f(V1/2)] decreased. Such correction of TGF hyperactivity, however, is not specific to lemilidine; similar effects have been observed in SHR treated with volume replacement (4), renal denervation (5), and also with a renal vasodilator (6). Because the predominant effect of the TGF mechanism is the efferent arteriole, a resistance change at this site by renal vasodilatory maneuvers or drugs could modulate TGF sensitivity.

V1/2 has been shown to represent the steady-state tubular flow rate at a late proximal tubular site (21). Increments in V1/2 by high-dose lemilidine suggest an increased load of tubular fluid into the loop of Henle due to inhibition of proximal tubular reabsorption because the clearance studies indicate unaltered glomerular filtration. The vasodilatory effect of lemilidine on efferent arterioles would increase the hydrostatic pressure in peritubular capillaries of postglomerular vessels, which diminishes proximal tubular reabsorption. One of the reasons why lemilidine at this dose did not induce diuresis and natriuresis in the whole kidney may be the compensatory reabsorption of water and sodium in the more distal parts of nephrons (19), which could have been augmented by volume decrease due to unreplaced urinary loss.

Lemildipine reduced both the slope and the probability level of the relationship between MBP and SFP1/2 in a dose-dependent manner, but did not change mean SFP1/2 value. These findings most likely reflect constant Pgc due to balanced vasodilating effects of lemilidine on systemic and glomerular resistance arterioles, including efferent arterioles, and are in accordance with results from whole kidney studies where GFR was unaltered despite reduced renal perfusion pressure.

The augmented TGF response in SHR is considered an intrarenal regulatory abnormality, accompanied by increased renal vascular resistance and reduced RPF and RBF and contributing to renal retention of sodium and water (5). Although the precise mechanism responsible for the augmentation is not known, this renal abnormality may play a role in the initiation or the maintenance of systemic hypertension. The present study demonstrated that lemilidine produces a right and upward shift of the TGF curve and diminishes the augmented TGF response in SHR to the level in WKY. Lemildipine induces preglomerular and postglomerular vasodilatation and maintains the levels of Pgc and GFR, which may prevent glomerular hypertension and thereby play a role in nephroprotection.

Acknowledgements

Lemildipine was a generous gift from Kowa Company Ltd. (Tokyo, Japan). We are deeply grateful to Yoshiko Tanaka for her excellent technical assistance.
References


