Contrasting Effects of Selective T- and L-Type Calcium Channel Blockade on Glomerular Damage in DOCA Hypertensive Rats

Habib Karam, Jean-Paul Clozel, Patrick Bruneval, Marie-Françoise Gonzalez, Joël Ménard

Abstract—Mibefradil and amlodipine are calcium antagonists with different channel selectivities. Mibefradil blocks both L- and T-type calcium channels; although in the usual pharmacological doses, it predominantly blocks the T-type channels. In contrast, amlodipine selectively blocks L-type channels. The goal of the present study was to assess whether this differential selectivity would result in different effects on end-organ damage in experimental hypertension. For this purpose, deoxycorticosterone acetate (DOCA)–salt hypertensive rats were treated either with equipotent doses of mibefradil or amlodipine (30 mg · kg⁻¹ · d⁻¹ as food admix). Despite the fact that both drugs decreased systolic arterial pressure to the same extent (140±5 mm Hg in the mibefradil group and 144±3 mm Hg in the amlodipine group versus 225±5 mm Hg in the untreated-DOCA group), only mibefradil decreased proteinuria (35.5±6.5 versus 103.3±14.1 mg/24 h in untreated DOCA-salt animals) and prevented glomerular lesions. Both drugs, however, prevented the occurrence of vascular renal lesions. To elucidate the mechanism responsible for this difference, we evaluated in an additional series of experiments the effects of mibefradil and amlodipine on plasma and renal renin concentrations, as well as the effects of the addition of enalapril, an ACE inhibitor, given on top of both drugs on proteinuria. Amlodipine, in contrast to mibefradil, markedly stimulated the plasma (17.8±2.6 ng Ang I · mL⁻¹ · h⁻¹ in the amlodipine group versus 3.9±0.4 ng Ang I · mL⁻¹ · h⁻¹ in the mibefradil group and 3.2±0.3 ng Ang I · mL⁻¹ · h⁻¹ in the untreated-DOCA group) and renal (2.42±0.37 ng Ang I · mL⁻¹ · h⁻¹ in the amlodipine group versus 0.36±0.04 ng Ang I · mL⁻¹ · h⁻¹ in the mibefradil group and 0.26±0.08 ng Ang I · mL⁻¹ · h⁻¹ in the untreated-DOCA group) renin concentrations. Stimulation of the renin-angiotensin system could explain the absence of a renal protective effect of amlodipine. This was also suggested by the fact that enalapril given in addition to amlodipine could decrease proteinuria. In conclusion, T-type channel blockade by mibefradil decreases blood pressure without stimulation of the renin-angiotensin system and therefore prevents most of the glomerular damage in DOCA hypertensive rats. (Hypertension. 1999;34:673-678.)

Key Words: hypertension, experimental ■ mibefradil ■ amlodipine ■ calcium channels ■ kidney ■ rats

Mibefradil is a selective T-type calcium channel blocker that has been shown to be highly effective as an antihypertensive and antianginal drug in humans. Recently, mibefradil was withdrawn from the market because of the occurrence of metabolic drug interactions independent from its mechanism of action.

Interestingly, mibefradil has been shown to prevent proteinuria in deoxycorticosterone acetate (DOCA)–salt hypertensive rats. This is in contrast to what has been described with amlodipine, a selective L-channel blocker. Therefore, in the present paper, we hypothesized that selective T-channel blockade could have a different effect on renal end-organ damage than selective L-channel blockade. To test this hypothesis, in the first study, we compared the effects of mibefradil with the effects amlodipine. This comparison was performed with equihypotensive doses of both drugs to avoid any interference of blood pressure. This study clearly showed a difference between mibefradil, which could prevent glomerular damage and proteinuria, and amlodipine, which had no beneficial effect on these 2 variables.

In a second set of experiments, we attempted to explain these differences. An obvious interfering factor was the renin-angiotensin system (RAS). Mibefradil and amlodipine have been shown to have different effects on the RAS in vivo as well as in vitro. Mibefradil, but not amlodipine, can decrease blood pressure in renal hypertensive rats without stimulating the RAS. In vitro, mibefradil, in contrast to amlodipine, does not stimulate the release of renin by the juxtaglomerular cells. We have, therefore, evaluated the effects of both mibefradil and amlodipine on the plasma and renal renin concentrations. We have also assessed the effects of the blockade of the RAS with an ACE inhibitor given with mibefradil or amlodipine.

Methods

Animal Preparation

Male normotensive Wistar rats (RCC Ltd, Füllinsdorf, Switzerland), 10 weeks of age, were anesthetized with sodium hexobarbital (100

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mg/kg IP). The right kidney was removed through a flank incision, and 1 pellet of DOCA (40 mg) was implanted subcutaneously over the nape of the neck every 2 weeks. These rats were provided with 1% saline to drink. Sham-operated rats were used as controls and received tap water to drink. Four weeks after surgery, systolic arterial blood pressure (SABP) of DOCA rats was measured indirectly by the tail-cuff method, and only rats with blood pressures >200 mm Hg were selected for the study.

The investigation was performed in accordance with the Home Office "Guidance on the Operation of the Animals (Scientific Procedures) Act 1986," published by Her Majesty’s Stationery Office, London, UK.

**Experimental Design**

**Protocol 1**

Four groups of rats were compared. One group contained sham-operated rats and received no treatment (n=16). The selected DOCA hypertensive rats were randomly allocated to 3 different groups and were treated either with oral doses of amlodipine, a calcium antagonist (30 mg·kg⁻¹·d⁻¹ as food admix, n=16), or mibefradil, another new long-acting calcium antagonist (30 mg·kg⁻¹·d⁻¹ as food admix, n=16), the third group was left untreated (DOCA-un-treated group, n=16). Systolic blood pressure was measured in each rat at the middle and the end of the 5-week treatment period. The rats were killed after 5 weeks of treatment, and the blood was withdrawn from the abdominal aorta.

**Protocol 2**

In this protocol, 6 groups of rats were compared. One group contained sham-operated rats and received no treatment (n=30). The selected DOCA hypertensive rats were randomly allocated to 5 different groups and were treated with either oral doses of amlodipine, (30 mg·kg⁻¹·d⁻¹ as food admix, n=30); mibefradil, (30 mg·kg⁻¹·d⁻¹ as food admix, n=30); a combination of amlodipine (30 mg·kg⁻¹·d⁻¹ as food admix, n=30) and enalapril, an ACE inhibitor (30 mg·kg⁻¹·d⁻¹ as food admix, n=30); or a combination of mibefradil (30 mg·kg⁻¹·d⁻¹ as food admix, n=30) and enalapril (30 mg·kg⁻¹·d⁻¹ as food admix, n=30). The sixth group was left untreated (DOCA-un-treated group, n=30). Systolic blood pressure was measured in each rat at the middle and the end of the 5-week treatment period. The rats were killed after 5 weeks of treatment, and the blood was withdrawn from the abdominal aorta.

**Measurements of Maximal Coronary Blood Flow in Isolated Perfused Hearts (Protocol 1)**

Ten rats per group, previously heparinized, were anesthetized with sodium hexobarbital (100 mg/kg IP) to prevent thrombosis of the coronary circulation and thereafter killed by cervical dislocation. The hearts were isolated, cannulated from the aorta, and retrogradely perfused in a Langendorff apparatus with a modified Krebs-Henseleit solution of the following composition (mmol/L): NaCl 114.7, KCl 4.7, MgSO₄ 1.2, KH₂PO₄ 1.5, NaHCO₃ 25, CaCl₂ 2.5, and glucose 11.1. The solution was gassed with 95% O₂ to 5% CO₂, and the pH was adjusted to 7.3. Coronary arterial flow was measured at perfusion pressures of 90, 80, 70, 60, and 50 mm Hg. After perfusion, the hearts were blotted and weighed.

**Histopathological Analysis (Protocol 1)**

Half of the kidneys from the sham-operated, untreated-DOCA, mibefradil, and amlodipine groups (Protocol 1) were fixed in alcoholic Bouin’s solution and embedded in paraffin. Four-millimeter sections were stained with Masson’s trichrome and examined under light microscopy. Two investigators blinded to the experimental groups assessed the severity of the morphological changes, ie, the presence of infarction, glomerulosclerosis, and tubulointerstitial and vascular lesions. Each type of lesion was graded semiquantitatively as previously described.²

**Biochemical Measurements (Protocol 2)**

The rats were placed in individual metabolic cages, and two 24-hour urine collections were performed consecutively. All the following urinary data are, therefore, the mean values of the 2 measurements for each rat. Urinary volumes were determined gravimetrically. Urinary creatinine and urea nitrogen were measured by a centrifugal analyzer (Roche-Cobas Fara II). Urinary protein concentrations were determined after precipitation with trichloroacetic acid (0.2 mol/L). Turbidity was then determined by measuring absorbance at a wavelength of 450 nm with the centrifugal analyzer. Plasma urea and creatinine were measured by the centrifugal analyzer (Roche-Cobas Fara II).

Plasma renin concentration (PRC) was measured by radioimmunoassay of the angiotensin I that was generated by the incubation of plasma, with an excess of angiotensinogen provided by renin-free plasma obtained from rats that were nephrectomized 24 hours previously.³

**Statistical Analysis**

All results are expressed as mean±SEM. All variables were compared by a 1-way ANOVA. Data in which a significant F value was obtained were further analyzed with Fischer’s protected least significant difference test. P<0.05 was considered significant.

**Results**

**Hemodynamic Variables**

At the beginning of the treatment period, 5 weeks after nephrectomy, SABP values were identical in all DOCA groups of both protocols and were significantly higher than the SABP in the sham-operated groups (Figure 1).
**TABLE 1.** Body Weights, Organ Weights, and Hemodynamic Parameters in the Rat Groups of Protocol 1 (A) and 2 (B)

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No.</th>
<th>BW, g</th>
<th>LVW/BW, *1000</th>
<th>KW/BW, *1000</th>
<th>SABP, mm Hg</th>
<th>HR, bpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Sham operated</td>
<td>Untreated</td>
<td>16</td>
<td>408±9</td>
<td>1.90±0.01</td>
<td>2.9±0.1</td>
<td>128±3</td>
<td>339±6</td>
</tr>
<tr>
<td>DOCA</td>
<td>Untreated</td>
<td>16</td>
<td>322±9*</td>
<td>3.36±0.17*</td>
<td>7.6±0.3*</td>
<td>225±5*</td>
<td>376±11*</td>
</tr>
<tr>
<td>DOCA</td>
<td>Mibefradil</td>
<td>16</td>
<td>356±7*</td>
<td>2.38±0.07*</td>
<td>6.4±0.3*</td>
<td>140±5*</td>
<td>291±7*</td>
</tr>
<tr>
<td>DOCA</td>
<td>Amlodipine</td>
<td>16</td>
<td>361±11†</td>
<td>2.57±0.06†</td>
<td>7.6±0.5</td>
<td>144±3*</td>
<td>364±10</td>
</tr>
<tr>
<td>B. Sham operated</td>
<td>Untreated</td>
<td>30</td>
<td>393±6</td>
<td>1.65±0.03</td>
<td>3.1±0.1</td>
<td>136±2</td>
<td>344±7</td>
</tr>
<tr>
<td>DOCA</td>
<td>Untreated</td>
<td>25</td>
<td>317±8*</td>
<td>2.63±0.08*</td>
<td>7.6±0.4*</td>
<td>218±6*</td>
<td>370±95</td>
</tr>
<tr>
<td>DOCA</td>
<td>Mibefradil</td>
<td>26</td>
<td>330±6</td>
<td>2.07±0.06†</td>
<td>7.3±0.5</td>
<td>146±3*</td>
<td>319±9*</td>
</tr>
<tr>
<td>DOCA</td>
<td>Amlodipine</td>
<td>29</td>
<td>320±6</td>
<td>2.20±0.03‡</td>
<td>8.1±0.4</td>
<td>143±3*</td>
<td>359±9</td>
</tr>
<tr>
<td>DOCA</td>
<td>Mibefradil+Enalapril</td>
<td>29</td>
<td>306±8</td>
<td>2.27±0.07‡</td>
<td>7.5±0.2</td>
<td>148±3*</td>
<td>312±8*</td>
</tr>
<tr>
<td>DOCA</td>
<td>Amlodipine+Enalapril</td>
<td>30</td>
<td>333±7</td>
<td>2.12±0.05‡</td>
<td>7.8±0.4</td>
<td>129±2*</td>
<td>361±7*</td>
</tr>
</tbody>
</table>

BW indicates body weight; LVW, left ventricular weight; KW, kidney weight; SABP, systolic arterial blood pressure; and HR, heart rate.

*P<0.05, †P<0.01, $P<0.001 vs untreated-DOCA rats.

§P<0.05, ‡P<0.01, ¶P<0.001 vs sham-operated rats.

**Protocol 1**
Both amlodipine and mibefradil significantly decreased the SABP to 36% and 38%, respectively, throughout the entire treatment period (Table 1A). Mibefradil, but not amlodipine, decreased significantly the heart rate by 23% compared with the DOCA-untreated group (Table 1A).

**Protocol 2**
During the treatment period, the SABP of the sham-operated rats remained unchanged. In contrast with that of the untreated-DOCA rats in which the SABP remained high, SABP decreased dramatically in the 4 treated groups and almost reached the normal value of the sham-operated group of rats. Amlodipine and mibefradil+enalapril had no effects on heart rate. In contrast, mibefradil and mibefradil+enalapril decreased heart rate significantly (Figure 1, Table 1B).

**Body and Organ Weights**
Before treatment, all the DOCA groups in the 2 protocols had equivalent baseline body weights.

**Protocol 1**
At the end of the treatment period, body weights increased significantly in the amlodipine (by 12%) and mibefradil (by 11%) groups without, however, reaching the weight of the sham-operated rats (Table 1A).

Untreated-DOCA rats showed marked left ventricular and kidney hypertrophy, with significant increases of 77% and 145% in the ratio of left ventricular weight over body weight and kidney weight over body weight, respectively. Both mibefradil and amlodipine significantly improved the left ventricular hypertrophy, but only mibefradil was effective in the reduction of kidney hypertrophy (Table 1A).

**Protocol 2**
At the end of the treatment period, body weights were unchanged in the 5 groups, although a tendency toward an increase in body weight was observed in the mibefradil, amlodipine, and amlodipine+enalapril groups (Table 1B). Untreated-DOCA rats showed marked left ventricular and kidney hypertrophy with significant increases of 59% and 145% in the ratio of left ventricular weight over body weight and kidney weight over body weight, respectively. All treatments significantly improved the left ventricular hypertrophy but were ineffective in the reduction of kidney hypertrophy (Table 1B).

**Coronary Reserve**
During adenosine infusion, coronary autoregulation was abolished and coronary blood flow was linearly related to perfusion pressure (Figure 2). Maximal coronary blood flow (MCBF) measured at a pressure of 90 mm Hg decreased by 39% in untreated-DOCA rats. At this pressure, mibefradil was the only drug that significantly improved MCBF (P<0.01) by 43% compared with the untreated-DOCA group. At the remaining perfusion pressures both mibefradil (P<0.01) and amlodipine (P<0.05) significantly improved the MCBF (Figure 2).

**Renal Function and Proteinuria**

**Protocol 1**
Urinary volume increased significantly in all DOCA rats compared with sham-operated rats (Table 2A). Mibefradil.
decreased urinary volume by 32% without normalizing it. Proteinuria increased by 330% in the DOCA group. Only mibefradil decreased it significantly at 66%, reaching almost normal values (Table 2A). Creatinine clearance decreased by 35% in the untreated-DOCA group. However, mibefradil normalized it. Nonsignificant differences were observed, however, for urea clearance (Table 2A).

**Protocol 2**

As observed in protocol 1, urinary volume increased significantly in all DOCA rats compared with sham-operated rats (Table 2B). Proteinuria also increased significantly in the untreated-DOCA group of rats. Amlodipine had no effect on it. Mibefradil, mibefradil+enalapril, and amlodipine+enalapril, however, decreased proteinuria significantly (Table 2B). Creatinine clearance decreased in all the DOCA groups compared with the sham-operated group (Table 2B). A nonsignificant tendency toward a decrease in urea clearance was observed in the untreated-DOCA group compared with the sham-operated group. Only mibefradil and amlodipine+enalapril increased urea clearance significantly (Table 2B).

**Renal Histology**

Histological lesions were evident in the untreated-DOCA rats. These consisted of moderate to severe nephroangiosclerosis: glomerulosclerosis (score of 44.2±9.1), tubulointerstitial atrophy and inflammation (score of 10.9±1.4), and arterial wall thickening (score of 6.2±1.3). Mibefradil significantly decreased glomerulosclerosis and tubulointerstitial lesions (scores of 6.4±2.5 and 2.9±0.9, respectively) and normalized vascular wall lesions (score 0). Amlodipine had no effect on glomerular and tubular lesions but decreased vascular lesions significantly (0.2±0.1), nearly to the same level as mibefradil (Figure 3).

**Plasma and Renal Renin Concentrations**

Plasma renin concentration was decreased dramatically throughout the experiment in the untreated, mibefradil, and mibefradil+enalapril groups, with respect to the sham-operated group. At the beginning of the treatment period, the PRC in the amlodipine and amlodipine+enalapril groups was significantly lower than in the sham-operated group (10.6±1.1 and 12.2±2.2 ng Ang I·mL⁻¹·h⁻¹ versus 20.6±2.0 ng Ang I·mL⁻¹·h⁻¹, respectively), but it was significantly higher than in the untreated-DOCA, mibefradil, and mibefradil+enalapril groups (2.9±0.3, 3.1±0.3, and 4.3±0.6 ng Ang I·mL⁻¹·h⁻¹, respectively). Throughout the experiment, the PRC remained elevated in the amlodipine and amlodipine+enalapril groups (Figure 4). However, the PRC increased between weeks 3 and 5 in the amlodipine+enalapril group. Renal renin content was significantly lower in untreated-DOCA, mibefradil, and mibefradil+enalapril groups with respect to the sham-operated group (0.26±0.08, 0.36±0.04, 0.42±0.08 ng Ang·mg protein⁻¹·h⁻¹ versus 3.38±0.26 ng Ang·mg protein⁻¹·h⁻¹, respectively). As for PRC, renal renin content was significantly higher in the amlodipine and amlodipine+enalapril groups (2.42±0.37 and 2.15±0.30 ng Ang·mg protein⁻¹·h⁻¹) compared with the untreated-DOCA group, but it did not reach the value of the sham-operated rats (Figure 4).

**Discussion**

The present study showed that selective T-channel blockade with mibefradil⁶–⁸ prevents glomerular damage in DOCA-salt hypertensive rats and that selective L-channel blockade with amlodipine has no such beneficial effect. A likely hypothesis to explain this difference is that mibefradil, in contrast to amlodipine, does not stimulate the RAS in the kidney.

Previous studies concluded that the low renin levels, caused by the suppression of the RAS observed in the DOCA model,⁹ allow us to distinguish the relative importance of hemodynamic factors from circulating hormones in the mediation of these responses.¹⁰,¹¹ The DOCA model is also characterized by increased plasma volume, which is most marked during the initial 6 weeks of DOCA-salt treatment,¹² and severe renal lesions that consist of segmental sclerosis and mesangial expansion in the glomeruli, vascular wall thickening, typical onion skin pattern and ultimately fibrinoid

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**TABLE 2. Urinary Excretion, Proteinuria, Urea, and Creatinine Clearances in the Rat Groups of Protocol 1 (A) and 2 (B)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No.</th>
<th>Urinary Volume, mL/24 h</th>
<th>Urinary Proteinuria, mg/24 h</th>
<th>C&lt;sub&gt;cr&lt;/sub&gt; (mL/min)</th>
<th>C&lt;sub&gt;urea&lt;/sub&gt; (mL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Sham operated</td>
<td>Untreated</td>
<td>16</td>
<td>17±2</td>
<td>24.0±4.4</td>
<td>2.3±0.1</td>
<td>1.04±0.03</td>
</tr>
<tr>
<td></td>
<td>DOCA</td>
<td>16</td>
<td>40±5*</td>
<td>103.3±14.1§</td>
<td>1.5±0.1§</td>
<td>0.83±0.05</td>
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<tr>
<td></td>
<td>Mibefradil</td>
<td>16</td>
<td>27±5*</td>
<td>35.5±6.5†</td>
<td>2.0±0.1†</td>
<td>1.11±0.13</td>
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<tr>
<td></td>
<td>Amlodipine</td>
<td>16</td>
<td>36±4</td>
<td>109.9±11.4</td>
<td>1.7±0.1</td>
<td>0.97±0.08</td>
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<td>B. Sham operated</td>
<td>Untreated</td>
<td>29</td>
<td>17±1</td>
<td>13.8±0.9</td>
<td>1.9±0.1</td>
<td>0.77±0.04</td>
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<td></td>
<td>DOCA</td>
<td>21</td>
<td>38±5§</td>
<td>48.4±9.9§</td>
<td>1.4±0.1§</td>
<td>0.67±0.03</td>
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<td></td>
<td>Mibefradil</td>
<td>26</td>
<td>39±5</td>
<td>25.1±3.9†</td>
<td>1.5±0.1</td>
<td>0.86±0.06*</td>
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<td>28±3</td>
<td>35.1±6.8</td>
<td>1.3±0.1</td>
<td>0.67±0.04</td>
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<td></td>
<td>Mibefradil+Enalapril</td>
<td>28</td>
<td>44±5</td>
<td>19.1±2.9‡</td>
<td>1.3±0.1</td>
<td>0.66±0.03</td>
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<td></td>
<td>Amlodipine+Enalapril</td>
<td>30</td>
<td>29±3</td>
<td>31.6±5.9*</td>
<td>1.5±0.1</td>
<td>0.84±0.08*</td>
</tr>
</tbody>
</table>

C<sub>cr</sub> indicates creatinine clearance; C<sub>urea</sub> indicates urea clearance.

*P<0.05, †P<0.01, ‡P<0.001 vs untreated-DOCA rats.

§P<0.001 vs sham-op rats.
necrosis with tubular atrophy and casts, and interstitial inflammation.\textsuperscript{2,13}

In the present study, we confirmed that elevated blood pressure is the major determinant of the decrease of coronary vascular reserve in the DOCA rats.\textsuperscript{14} Indeed, amlodipine and mibefradil reduced systemic blood pressure to the same extent. They also reduced left ventricular hypertrophy and improved MCBF. This was also the case for the vascular lesions in the kidney. However, this was different for proteinuria and the glomerular lesions. Indeed, although amlodipine reduced blood pressure to the same extent as mibefradil, amlodipine failed to reduce proteinuria, kidney weight, and glomerular morphological lesions unlike mibebradil. This is in accordance with studies published by Dworkin et al\textsuperscript{3} and Ménard et al.\textsuperscript{2} The differential effect of these 2 drugs on proteinuria is parallel to that observed on glomerular lesions but is independent from the vascular lesions that seem determined by blood pressure.

Figure 3. Semiquantitative evaluation of glomerular, tubulointerstitial, and vascular lesions in untreated and amlodipine- and mibefradil-treated groups of protocol 1.

Figure 4. A, PRC during the treatment period of protocol 2; and B, renal renin measured at the end of the treatment period of protocol 2. ***P<0.001 vs untreated-DOCA rats.

The different effects of both drugs on the function and structure of the kidney could be explained by their different action on the RAS. Indeed, it was shown that L- and T-type calcium channels blockers exert opposite effects on the RAS.\textsuperscript{4} T-type calcium channels blockers inhibit renin secretion and renin gene expression in vivo, whereas L-type channel blockers act as stimulators of the renin system. In our study, both the systemic and local RAS were evaluated, and amlodipine has a stimulatory effect in both systems. DOCA treatment suppressed the RAS. This was shown by the marked decrease of plasma renin activity in the untreated-DOCA rats. Interestingly, despite the very low basal levels of PRC and renal renin, amlodipine was able to induce a significant rise of the RAS. The inhibition of the RAS in the DOCA model can, therefore, be bypassed. The stimulation of the RAS in the kidney by amlodipine is maximal because enalapril added in addition to amlodipine was unable to potentiate it. The effect of amlodipine on RAS is not unexpected, because it has been shown that a major side effect of L-type calcium antagonists in the treatment of hypertension is the activation of the RAS.\textsuperscript{15–18} However, in the plasma, it seems that enalapril could further stimulate the release of renin by amlodipine as shown by the increase of PRC between 3 and 5 weeks. We have no explanation for this dissociation. Thus, it is likely that the reactivation of the RAS plays a role in the proteinuria and glomerular lesions observed in the amlodipine group. However, another mechanism could be involved because enalapril added to amlodipine decreased proteinuria more than with amlodipine alone, although it did not reach the level observed in the mibebradil group.

The deleterious effects of the activation of the RAS could be due to an increased level of angiotensin II. Indeed, several
in vitro\textsuperscript{19,20} and in vivo\textsuperscript{21} studies have shown the potential role of angiotensin II in inducing fibrosis. Another possible mechanism could be a direct action of amlodipine on L-type calcium channels present in the renal preglomerular afferent arterioles,\textsuperscript{22} leading to an increased intraglomerular pressure and to proteinuria. Enalapril and other blockers of the RAS\textsuperscript{23} may have a predominant postglomerular effect and therefore decreased intraglomerular pressure and proteinuria by dilating the efferent arteriole. Recently, a low voltage–activated calcium channel block selected by mibebradil was cloned. This T-type channel is highly expressed in the kidney.\textsuperscript{24} Mibebradil, acting on these channels possibly present on both afferent and efferent arterioles, could lead to a hemodynamic equilibrium without an increase in intraglomerular pressure and proteinuria. Another possible mechanism of action of amlodipine could be a direct effect on renin release at the level of renal juxtaglomerular cells, which are the main site of renin gene expression and secretion. It has been shown that exocytosis of renin from these cells is inhibited by a rise in the cytosolic calcium concentration.\textsuperscript{25}

In conclusion, our study shows that in DOCA-salt hypertension, pure L-type calcium channel blockers, such as amlodipine, and selective T-type calcium channel blockers, such as mibebradil, decreases blood pressure to the same extent, but do not have similar effects on renal structure and function. The L-channel blocker amlodipine stimulates the RAS and therefore fails to decrease proteinuria and glomerular lesions. This was not the case with the T-channel blocker mibebradil. Mibebradil inhibits the \( \text{P}450 \) cytochrome system, which is involved in the metabolism of several drugs such as simvastatin. This interaction has led to clinically relevant drug interactions, such as rhabdomyolysis, because of an increase in plasma levels of statin. As a result of these interactions, and independently of its mechanism of action, mibebradil was recently withdrawn from the market. However, T-channel blockers without such drug interactions might represent a new therapeutic approach in hypertension, especially for the prevention of renal damage.

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**References**

Contrasting Effects of Selective T- and L-Type Calcium Channel Blockade on Glomerular Damage in DOCA Hypertensive Rats
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