Effect of Calcium Antagonists on Glomerular Arterioles in Spontaneously Hypertensive Rats

Maurizio Sabbatini, Amedeo Leonardi, Rodolfo Testa, Lucia Vitioli, Francesco Amenta

Abstract—Through the use of microanatomic techniques, we investigated the effects of treatment with some dihydropyridine-type calcium antagonists (CAs) (ie, lercanidipine, manidipine, and nicardipine) and with the nondihydropyridine-type vasodilator hydralazine on hypertension-dependent glomerular injury and on the morphology of afferent and efferent arterioles in spontaneously hypertensive rats (SHR). Fourteen-week-old male SHR and age-matched normotensive Wistar-Kyoto rats were left untreated (control groups). Four additional groups of 14-week-old SHR were treated for 12 weeks with daily oral doses of 2.5 mg/kg lercanidipine, 5 mg/kg manidipine, 3 mg/kg nicardipine, or 10 mg/kg hydralazine. These treatments decreased systolic blood pressure values to a similar extent in SHR. Signs of glomerular injury, as characterized by glomerulosclerosis, hypertrophy, and an increased number of mesangial cells, were observed in control SHR. The treatment with CAs improved glomerular morphology and decreased the number of mesangial cells. Lercanidipine and manidipine were more effective than nicardipine in countering glomerular injury. In the SHR, both afferent and efferent arterioles revealed luminal narrowing, accompanied by increased wall thickness in efferent arterioles. The dihydropyridine-type derivatives that were tested decreased the luminal narrowing of afferent arterioles. Lercanidipine and manidipine countered the luminal narrowing of efferent arterioles. Hydralazine had no effect on hypertension-dependent glomerular injury or vascular changes. The present data indicate that lercanidipine and manidipine vasodilate afferent and efferent arterioles in SHR. A vasodilatory activity on efferent arteriole, which is not induced by the majority of CAs, may represent an useful property in the treatment of hypertension complicated by renal disease. (Hypertension. 2000;35:775-779.)

Key Words: calcium antagonists n arterioles n rats, SHR

The kidney is a main target of hypertension. In both essential hypertension and spontaneously hypertensive rats (SHR), the kidneys undergo vascular changes and develop areas of ischemic cortical atrophy that are accompanied by renal fibrosis with glomerular lesions and tubular changes. Hypertension-dependent microanatomic changes in the kidney induce functional impairment with hypoperfusion, elevation of glomerular pressure with increased glomerular filtration rate, proteinuria, and other signs of glomerular injury. Because of the risk that hypertension-dependent injury may cause renal failure, there is considerable interest in the identification of antihypertensive agents able to counter renal hypertensive damage. Calcium antagonists (CAs) represent effective and safe antihypertensive agents that are able to preserve or increase renal blood flow. A limitation of this class of drugs is that the majority of them vasodilate afferent, but not efferent, arterioles. The consequent glomerular hypertension might be associated with progression of the disease, instead of affording renal protection. Recent evidence has shown that unlike the majority of CAs, novel compounds of this class, such as manidipine and efonidipine, vasodilate both afferent and efferent arterioles. However, it has not been established whether this property has an effect on hypertension-dependent glomerular injury.

The present study was designed to assess in SHR the influence of different dihydropyridine-type CAs on the morphology of renal glomerulus and of afferent and efferent arterioles. The second-generation dihydropyridine-type CA nicardipine, the novel CA manidipine, and the recently developed vasoselective dihydropyridine-type derivative lercanidipine were studied. The nondihydropyridine-type vasodilator hydralazine was used as a reference compound.

Methods

Animals and Pharmacological Treatment
Male SHR and normotensive Wistar-Kyoto (WKY) rats (12 weeks old; Charles River) were used in the study and were handled according to internationally accepted principles for the care of laboratory animals (European Community Council Directive 86/609, OJ no L358, December 18, 1986). Starting from 2 weeks before the experiments, animals were kept under a constant light-dark cycle at

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an ambient temperature of 22±1°C, with free access to water and laboratory chow. One group of SHR (n=10) and 1 group of WKY rats (n=10) were treated with vehicle and used as control groups. Four groups of SHR were treated with lercanidipine (2.5 mg · kg⁻¹ · d⁻¹; n=8), manidipine (5 mg · kg⁻¹ · d⁻¹; n=7), nicardipine (3 mg · kg⁻¹ · d⁻¹; n=8), or hydralazine (10 mg · kg⁻¹ · d⁻¹; n=8). Drugs were added to the drinking water, which was put into lightproof containers. The body weight of the animals was determined every 2 weeks, and systolic blood pressure and heart rate were measured every week with the use of an indirect tail-cuff method in conscious rats. At the 10th week of treatment, animals were put in metabolic cages for the determination of 24-hour urine production, Na⁺ and K⁺ concentrations and ratio, and albumin excretion.

**Tissue Preparation**

After 12 weeks of treatment, at the age of 26 weeks, systolic blood pressure was measured in the animals, which were then weighed, anesthetized with diethyl ether, and perfused through the left ventricle with a 0.9% NaCl solution containing 0.5% polyvinylpyrrolidone, 20 IU heparin, and 25 mg/mL EDTA to produce maximal arteriolar dilatation. This solution was maintained at 37°C, and perfusion lasted for 10 to 15 minutes. The first solution was then replaced by a second solution of 10% formalin in 0.1 mol/L phosphate buffer (pH 7.4) at 25°C. Perfusion pressure was adjusted to a constant rate of 1 mL · min⁻¹ · 100 g body wt⁻¹ with the use of a catheter connected to a pressure transducer inserted in the abdominal aorta. After 30 minutes of perfusion, the kidneys were removed, weighed, fixed in the same perfusion fixative for 1 week, and then processed for paraffin embedding. Alternate consecutive parasagittal sections (6 μm thick, 50 μm apart) were stained with hematoxylin and eosin or Masson’s trichrome stain to allow investigation of the morphology of the glomerulus and vascular components. After staining, sections were viewed under a light microscope connected with an image analyzer.

For glomerular morphometry, 6 consecutive sections stained with Masson’s trichrome stain were viewed under a microscope connected via a TV camera to an IAS 2000 image analyzer (Delta Sistemi). Ten renal corpuscles were identified per slide; this allowed analysis of 60 glomeruli per rat. With the use of an overlap option, glomeruli (including glomerular capillaries and mesangium) and renal corpuscles (glomerulus plus glomerular capsule) were delineated. The area occupied by glomeruli (rG) and renal corpuscles (rC) was calculated with use of a program of the image analyzer. The rG-to-rC ratio was then determined. An increase in this value indicates the occurrence of glomerular hypertrophy. Mesangial nuclei were also counted in a 10×10³ μm² area of the same glomeruli selected for morphometric analysis.

**Vascular Morphometry**

Glomerular afferent and efferent arterioles were measured on 10 consecutive sections per rat according to the described image analysis system. On each section, 12 afferent and efferent arterioles were examined. This sampling protocol included examination of the vascular pole of 120 glomeruli per animal. Sections were viewed at a final magnification of ×400. The luminal and wall areas were then independently measured for afferent and efferent glomerular arterioles. Further details on morphometric analysis are reported elsewhere.¹³

**Data Analysis**

Mean values of the different parameters that were investigated were calculated from single animal data. Group mean±SEM values were derived from single animal data. The significance of differences between mean values was analyzed with the use of ANOVA, followed by the Newman-Keuls multiple range test. For rG-to-rC and wall-to-lumen ratios, normal distributions of theoretic frequencies and frequencies observed in single measurements were assessed through the use of χ² analysis.

**Results**

Systolic blood pressure values of control WKY rats and of different groups of SHR along the course of the study are shown in Figure 1. In control SHR, a significant increase in systolic blood pressure was observed in comparison with age-matched WKY rats. The 4 antihypertensive compounds that were tested significantly reduced the systolic blood pressure of SHR. Starting from the 6th week of treatment, the drugs decreased systolic blood pressure to similar extents. Heart rate values were similar in the groups investigated (data not shown). A tendency to increase body weight was observed from the beginning through the end of treatment, with the exception of lercanidipine-treated SHR (data not shown). Neither the presence of hypertension nor different pharmacological treatments changed kidney weight values (data not shown).

In control SHR, an increase in urine volume was noticeable. This effect was countered with lercanidipine, hydralazine, and manidipine but not nicardipine (data not shown). Urinary albumin concentrations were increased in control SHR (278±12 mg/d) in comparison with normotensive WKY rats (80±4 mg/d, p<0.01 versus SHR). This phenomenon was countered by treatment with lercanidip-
lens (200±7 mg/d, \(P<0.01\) versus control SHR), manidipine (190±9 mg/d, \(P<0.01\) versus control SHR), and nicardipine (230±13 mg/d, \(P<0.01\) versus control SHR) but not hydralazine (273±10 mg/d). In control SHR, urinary Na\(^+\) and K\(^+\) concentrations were decreased in comparison with normotensive WKY rats (data not shown). Lercanidipine, manidipine, nicardipine, and hydralazine countered Na\(^+\) retention of SHR (data not shown). Treatment with manidipine and nicardipine enhanced urinary K\(^+\) decrease compared with lercanidipine and hydralazine and increased the Na\(^+\)-to-K\(^+\) ratio (data not shown).

**Glomerular Morphology**
Analysis of the morphology of renal glomeruli in control SHR revealed the occurrence of hypertrophy and sclerosis of glomerular capillaries and an increase in mesangial cells compared with age-matched normotensive WKY rats (Figure

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**Figure 2.** Photomicrographs of renal corpuscle and glomerular afferent (A) and efferent (E) arterioles in a normotensive WKY rat (A), control SHR (B), lercanidipine-treated SHR (C), manidipine-treated SHR (D), nicardipine-treated SHR (E), and hydralazine-treated SHR (F) (Masson’s trichrome stain). Note in control SHR occurrence of glomerular hypertrophy, reduction in capsular lumen (*), and increase of mesangial cell nuclei (arrowheads). A decreased luminal area of afferent and efferent arterioles accompanied by increased thickness of efferent arterioles. Treatment with lercanidipine, manidipine, or nicardipine reduced degree of glomerular hypertrophy, decreased number of mesangial cell nuclei, and increased luminal area of afferent arterioles. Lercanidipine and manidipine were also able to increase luminal area of efferent arterioles. (Calibration bar 25 \(\mu\)m.)
2). Decreased space between glomerular capsule and glomeruli (capsular lumen) was also noticeable in SHR as a consequence of glomerular hypertrophy (Figure 2). The ratio of the area occupied by renal glomeruli and capsule (rG-to-rC ratio) was also increased in SHR (Table 1). The studied drugs countered these phenomena (Table 1), with lercanidipine and manidipine being the most effective in reversal of the rG-to-rC ratio (Table 1). Moreover, pharmacological treatment with CAs normalized the number of nuclei of mesangial cells (Table 1 and Figure 2).

Afferent and Efferent Glomerular Arterioles

Data on the influence of hypertension and of pharmacological treatment on the morphology of afferent and efferent glomerular arterioles are summarized in Table 2. A significant vasoconstriction (decreased luminal area) was observed in afferent and efferent arterioles of SHR compared with those of WKY rats (Figure 2A). In control SHR, afferent arterioles showed luminal narrowing, whereas luminal narrowing accompanied by an increase in wall thickness was observed in efferent arterioles (Table 2 and Figure 2B). This phenomenon was not accompanied by changes in the number of nuclei of smooth muscle cells (data not shown). Among the compounds that were investigated, only lercanidipine and manidipine countered hypertension-dependent changes in both afferent and efferent glomerular arterioles (Figures 2C and 2D and Table 2). Treatment with nicardipine dilated the afferent, but not the efferent, arterioles (Figure 2E and Table 2). Hydralazine had no effect on afferent or efferent arterioles (Figure 2F and Table 2). The antihypertensive drugs that were tested did not counter the increased wall thickness of efferent arterioles expressed as wall area (Table 2).

Discussion

Complicated or inadequately treated hypertension causes renal damage, which may progress to renal failure. It is generally accepted that renal changes that occur during the first phases of hypertension are adaptations to counter the partial loss of function.14,15 Increased glomerular capillary pressure and compensatory kidney growth are the most common renal compensatory adaptations present in the long-term damaged kidney.5,14,15 The counter of increased glomerular capillary pressure may represent a therapeutic principle by which to counter hypertensive renal damage.14,15 ACE inhibitors reduce glomerular capillary pressure and hypertensive glomerular injury under different experimental conditions in SHR.16 Other antihypertensive drugs, including CAs, had less constant or no effects on glomerular pressure.17

Glomerular pressure normalization and the subsequent increase in glomerular filtration rate that is impaired in hypertension may represent important properties of antihypertensive drugs.18 First- and second-generation CAs were able to vasodilate afferent arterioles but not efferent arterioles.19 This does not normalize glomerular pressure and could contribute to the occurrence of time-dependent glomerular damage.20 In the present study, we analyzed the influence

### Table 1. Morphometry of Renal Glomeruli in the Different Animal Groups Investigated

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WKY (n = 10)</th>
<th>SHR (n = 10)</th>
<th>SHR + LERC (n = 8)</th>
<th>SHR + MANI (n = 7)</th>
<th>SHR + NICA (n = 8)</th>
<th>SHR + HYDR (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rG-to-rC ratio</td>
<td>0.71 ± 0.03</td>
<td>0.91 ± 0.04*</td>
<td>0.74 ± 0.03</td>
<td>0.73 ± 0.04</td>
<td>0.78 ± 0.04*†§</td>
<td>0.83 ± 0.02*†§</td>
</tr>
<tr>
<td>Mesangial cell nuclei</td>
<td>62 ± 5.3</td>
<td>92.4 ± 7.6*</td>
<td>61.4 ± 4.3†</td>
<td>58.5 ± 3.3†</td>
<td>60.2 ± 4.1†</td>
<td>90.2 ± 6.1*†§</td>
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LERC indicates lercanidipine; MANI, manidipine; NICA, nicardipine; HYDR, hydralazine; rG and rC, area occupied by glomeruli and renal corpuscle, respectively. Values are mean ± SE.

### Table 2. Quantitative Image Analysis of Afferent and Efferent Glomerular Arterioles in the Different Animal Groups Investigated

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WKY (n = 10)</th>
<th>SHR (n = 10)</th>
<th>SHR + LERC (n = 8)</th>
<th>SHR + MANI (n = 7)</th>
<th>SHR + NICA (n = 8)</th>
<th>SHR + HYDR (n = 8)</th>
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</thead>
<tbody>
<tr>
<td>Afferent arteriole</td>
<td></td>
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<tr>
<td>Lumen area, μm²</td>
<td>79.2 ± 5.7</td>
<td>63.6 ± 2.3*</td>
<td>79.1 ± 3.4†</td>
<td>78.8 ± 2.4†</td>
<td>79.2 ± 5.7†</td>
<td>64.7 ± 2.5*§</td>
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<tr>
<td>Wall area, μm²</td>
<td>91.6 ± 4.5</td>
<td>96.6 ± 5.1</td>
<td>91.1 ± 5.2</td>
<td>91.6 ± 4.8</td>
<td>91.9 ± 7.6</td>
<td>95.7 ± 4.1</td>
</tr>
<tr>
<td>Wall-to-lumen ratio</td>
<td>1.18 ± 0.04</td>
<td>1.51 ± 0.03*</td>
<td>1.16 ± 0.07†</td>
<td>1.16 ± 0.03†</td>
<td>1.18 ± 0.10</td>
<td>1.48 ± 0.03*§</td>
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<tr>
<td>Efferent arteriole</td>
<td></td>
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<tr>
<td>Lumen area, μm²</td>
<td>60.2 ± 2.8</td>
<td>50.5 ± 3.1*</td>
<td>60.0 ± 3.3†</td>
<td>59.7 ± 1.1†</td>
<td>50.2 ± 2.0*§</td>
<td>49.7 ± 1.7*§</td>
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<tr>
<td>Wall area, μm²</td>
<td>121.4 ± 5.1</td>
<td>146.2 ± 6.7*</td>
<td>140.2 ± 5.3*</td>
<td>141.4 ± 2.0*</td>
<td>146.1 ± 10.8*</td>
<td>146.7 ± 4.3*</td>
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<tr>
<td>Wall-to-lumen ratio</td>
<td>2.03 ± 0.06</td>
<td>2.93 ± 0.08*</td>
<td>2.37 ± 0.12†</td>
<td>2.37 ± 0.02†</td>
<td>2.90 ± 0.15*§</td>
<td>2.99 ± 0.18*§</td>
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Values mean ± SE. Details regarding the morphometric analysis protocol are reported in Methods. Abbreviations and P values are as in Table 1.
of hypertension on the morphology of glomerular arterioles and the effect of pharmacological treatment on these changes. Treatment included 3 dihydropyridine-type CAs (lercanidipine, manidipine, and nicardipine) and the nondihydropyridine-type vasodilator hydralazine. Manidipine was chosen in view of its favorable renal profile, which includes vasodilatation of afferent and efferent arterioles, as demonstrated in functional studies. Nicardipine, which to some extent counters hypertension-related renal microanatomic changes, was used as a reference CA. Lercanidipine is a newly developed long-lasting and vasoselective CA that displays the same vasodilatory potency as manidipine on the renal vascular tree.

Our morphometric analysis demonstrated luminal narrowing of both afferent and efferent arterioles in SHR and an increased wall thickness of only efferent arterioles. This latter change probably is the result of hypertrophy of efferent arteriole smooth muscle, as documented by no change in the number of smooth muscle nuclei for SHR and normotensive WKY rats. The occurrence of afferent arteriole vasoconstriction parallel to the increase in blood pressure was documented in both SHR and deoxycorticosterone-salt hypertensive rats. The occurrence of afferent arteriole vasoconstriction parallel to the increase in blood pressure was documented in both SHR and deoxycorticosterone-salt hypertensive rats. Our data that show in SHR a decreased afferent arteriole luminal area that is not accompanied by increased thickness of the arteriolar wall suggest the occurrence of remodeling in afferent arterioles. Remodeling is a phenomenon that is characterized by a reduction in the external arterial diameter and luminal encroachment independent of hypertrophy of the arterial wall. In this situation, the arterial smooth muscle may become shorter and unable to extend to its original length during relaxation, resulting in the impairment of maximal dilatation capability. This phenomenon, which affects the afferent arterioles of SHR, is sensitive to pharmacological treatment with CAs. Both lercanidipine and manidipine induced vasodilatation of both afferent and efferent arterioles in SHR. Functional studies have shown that the treatment of SHR with manidipine reduced afferent and efferent arteriolar resistance. This decreased resistance probably is the result of vasodilatation of the 2 arterioles documented in the present study. The observation that both lercanidipine and manidipine caused a similar degree of vasodilatation to afferent and efferent arterioles suggests that the compounds may improve glomerular capillary pressure. In the present work, the vasodilatory activity of lercanidipine on glomerular efferent arteriole was documented for the first time. This suggests that the compound may represent a beneficial antihypertensive agent for patients affected by renal disorders. In addition to the vascular effect, treatment with lercanidipine and manidipine countered glomerular injury occurring in SHR. These findings, together with the observation of decreased albuminuria in manidipine- or lercanidipine-treated SHR, suggest that the glomerular effects of these drugs may have functional relevance.

References