Nephroprotective Effect of Treatment with Calcium Channel Blockers in Spontaneously Hypertensive Rats

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ABSTRACT

The influence of hypertension and of treatment with some dihydropyridine-type Ca\(^{2+}\) channel blockers and with the non- dihydropyridine-type vasodilator hydralazine on the morphology of kidney was investigated in 26-week-old spontaneously hypertensive rats (SHR) and in age-matched Wistar-Kyoto rats. Fourteen-week-old SHR were treated for 12 weeks with a non-hypotensive dose of lercanidipine or with equihypotensive doses of lercanidipine, manidipine, nicardipine, and hydralazine. In control SHR, systolic pressure values were significantly higher in comparison with Wistar-Kyoto rats. Treatment with the low dose of lercanidipine did not reduce systolic blood pressure in SHR, whereas the higher dose of lercanidipine or other compounds tested significantly decreased systolic pressure values. Glomerular hypertrophy accompanied by signs of glomerulosclerosis, increase of mesangial cells, and convoluted tubules degeneration were observed in control SHR. Hypotensive doses of Ca\(^{2+}\) antagonists countered glomerular injury, the increase of mesangial cells, the reduction of capsular space, and tubular degeneration. Hydralazine, in spite of its hypotensive activity, displayed a slight nephroprotective action. The nonhypotensive dose of lercanidipine countered in part glomerular injury, narrowing of capsular space, and tubular degeneration, and decreased mesangial cell augmentation in SHR. These results suggest that treatment with dihydropyridine-type Ca\(^{2+}\) antagonists counters hypertensive glomerular and tubular changes occurring in SHR. The demonstration of nephroprotection by the nonhypotensive dose of lercanidipine suggests that the renal effects of the compound may be in part unrelated to its hemodynamic activity.
and efferent arterioles, such as lercanidipine and manidipine. The nondihydropyridine-type vasodilator hydralazine was also investigated as a reference vasodilator.

Materials and Methods

Animals and Pharmacological Treatment. Male SHR and normotensive Wistar-Kyoto (WKY) rats (Charles River, Calco, Italy) of 12 weeks of age were used. They were handled according to internationally accepted principles for care of laboratory animals (European Community Council Directive 86/609, O.J. no. L358, Dec. 15, 1986). One group of SHR (n = 10) and one group of WKY rats (n = 10) were treated with vehicle and used as control groups. SHR were randomly allotted to the following five groups: 1) lercanidipine-treated with a nonhypotensive dose (0.5 mg/kg/day, n = 7); 2) lercanidipine-treated with a hypertensive dose (2.5 mg/kg/day, n = 8); 3) manidipine-treated (5 mg/kg/day, n = 7); 4) nicardipine-treated (3 mg/kg/day, n = 8); and 5) hydralazine-treated (10 mg/kg/day, n = 8). Drugs were added daily to rat drinking water for 12 weeks starting from the 14th week of age. Drug-containing water was put in lightproof containers. Drug concentration was adjusted every 3 days to ensure exposure to the established doses. Ca\(^2\) antagonist or hydralazine consumption was within 99 to 101% of the target doses (mean values 99–101%).

Body weight of animals was determined every 2 weeks. Systolic blood pressure and heart rate values were measured every week by an indirect tail-cuff method in conscious rats after prewarming at 37°C for 20 min. To minimize inaccuracies in blood pressure measurement, rats were conditioned in the 2 weeks preceding the beginning of experiments. At the 10th week of treatment, animals were accommodated in metabolic cages for 2 days. In the first day they became familiar with the environment of the metabolic cage. On the second day, 24-h urine production, Na\(^+\) and K\(^+\) concentrations, and albumin excretion were measured.

Tissue Preparation. At the final age of 26 weeks, animals underwent the last pressure measurements. They were then weighed, anesthetized with 50 mg/kg sodium pentobarbital, and perfused through the left ventricle with a 0.9% NaCl solution containing 0.5% polyvinylpyrrolidone, heparin (20 U I.U.), and EDTA (25 mg/ml) to produce maximal vasodilation. This solution was kept at 37°C and the perfusion lasted 10 to 15 min. The first solution was then replaced by a second one of 10% formalin in 0.1 M phosphate buffer (pH 7.4). The second solution was kept at room temperature. Perfusion pressure was adjusted at a constant rate of 1 ml/min/100 g b.wt. with a catheter connected to a pressure transducer inserted into the aorta.

After 30 min of perfusion, kidneys were dissected out and weighed. Right kidneys were cut perpendicular to the hilum, and fixed in the same perfusion fixative for 1 week. Left kidneys were divided in two halves parallel to major axis. Both kidneys were then washed and processed for paraffin embedding. Consecutive sections (3–4 μm thick) of right kidney were stained with 1) Masson’s trichromic staining to investigate the morphology of different components of nephron, with particular reference to accumulation of connective tissue and to the development of areas of tissue degeneration; 2) H&E to verify microanatomical details; and 3) periodic acid-Schiff (PAS) staining to detail development of areas of tissue degeneration; 2) H&E to verify microanatomical details; and 3) periodic acid-Schiff (PAS) staining to detail development of areas of tissue degeneration.

Data Analysis. Means of values of different parameters investigated were calculated. Group means were derived from single-animal values. Data are expressed as mean ± S.E. for body weight values. The significance of differences between means was assessed by ANOVA followed by Newman-Keuls multiple range test for parametric data. Nonparametric data (injury scores) were analyzed with the Mann-Whitney-Wilcoxon test, followed by Kruskal-Wallis test.

Results

Data of systolic arterial pressure values of WKY rats and of both control and pharmacologically treated SHR during the...
course of the experiment are shown in Fig. 1. In control SHR arterial pressure was significantly higher in comparison with normotensive WKY rats (Fig. 1). Pharmacological treatments reduced to a similar extent systolic pressure starting from the 6th week except for the dose of 0.5 mg/kg/day lercanidipine that did not affect systolic pressure of SHR (Fig. 1). Heart rate values averaged 300 ± 10 beats/min and were similar in the different animal groups investigated (data not shown). Data of body weight values at the beginning and at the end of experiment are summarized in Table 1. Body weight showed a tendency to increase at the end of the experiment with the exception of animals treated with lercanidipine or nicardipine (Table 1). Kidney weight values were similar in WKY rats or SHR either control or pharmacologically treated (data not shown).

Sections of kidney revealed in control SHR compared with normotensive WKY rats the occurrence of vascular changes consisting of increased thickness of tunica media accompanied by luminal narrowing (Fig. 2, A and B). The occurrence of connective tissue accumulation also was observed in renal cortex and medulla (data not shown). The nonhypotensive dose of lercanidipine or hydralazine (Fig. 2F) did not change vascular morphology. The hypotensive dose of lercanidipine, manidipine, or nicardipine countered luminal narrowing of renal artery branches (Fig. 2, C–E) and decreased connective tissue accumulation.

**Glomerular Morphometry.** Renal glomeruli in SHR developed hypertrophy, capillary sclerosis, and decreased capsular lumen (Fig. 3, A and B). A reduction of total cortical volume was found in SHR compared with WKY rats, indicating that renal cortex is smaller in SHR (Table 2).

In control SHR, the number of glomeruli was decreased and the volume of glomerular capsule was unchanged (data not shown), whereas volume of glomerular tufts (Fig. 3, A and B), total glomerular volume, and number of mesangial cells were increased compared with normotensive WKY rats (Table 2). In control SHR injury score was also higher in cortical and juxtamedullary glomeruli and in proximal and distal tubules but not in other portions of nephron (loop of Henle and collecting tubules; Table 3).

Pharmacological treatment did not affect renal cortex volume, glomerular capsule volume, or the number of glomeruli (Table 2). Volume of glomerular tuft was decreased in pharmacologically treated SHR compared with control SHR (Fig. 3, B–F). The nonhypotensive dose of lercanidipine, hydralazine, and nicardipine exerted a similar effect. The hypotensive dose of lercanidipine and manidipine had a more pronounced effect on volume of glomerular tufts. Treatment with dihydropyridines at hypotensive or nonhypotensive doses, but not with hydralazine, reduced the number of mesangial cell nuclei in SHR (Table 2).

Data on the influence of pharmacological treatment on GIS are summarized in Table 3. Kidneys of WKY rats did not show damage. Treatment with 2.5 mg/kg/day lercanidipine or with manidipine reduced significantly GIS, both in cortical and juxtamedullary glomeruli. Nicardipine had an intermediate effect, followed by the nonhypotensive dose of lercanidipine and hydralazine (Table 3).

**Tubular Morphometry.** Analysis of renal cortex convoluted tubules revealed in control SHR dark cell profiles corresponding to degenerating epithelial cells (Fig. 4). These cells were not observed in normotensive WKY rats (Fig. 4A). Pharmacological treatment improved the morphology of convoluted tubules (Fig. 4, C–F). Data of TIS are summarized in Table 3. As observed for glomeruli, no signs of injury were noticeable in normotensive WKY rats, whereas the highest score was attributed to control SHR (Table 3). Among the drugs investigated, the best score was obtained with the hypotensive dose of lercanidipine, followed by manidipine, nicardipine, the nonhypotensive dose of lercanidipine, and hydralazine (Table 3).

**Urine Analysis.** In control SHR compared with WKY rats an increase of urine volume was found (Table 4). This effect was countered by the drugs tested except for nicardipine (Table 4). Treatment with the hypotensive dose of lercanidipine or hydralazine normalized urine volume values in SHR (Table 4). A significant increase of urinary albumin concentration was found in control SHR compared with normotensive WKY rats (Table 4). Albuminuria was remarkably decreased by treatment with the different drugs investigated with the exception of hydralazine. The most powerful drugs in reducing albuminuria were the hypotensive dose of lercanidipine and manidipine (Table 4). In control SHR urinary sodium and potassium concentrations were decreased in comparison with normotensive WKY rats (Table 4). Pharma-

**Fig. 1.** Systolic pressure values of control WKY rats and of different groups of SHR during the course of the experiment. Treatment started when rats were 14 weeks old. Data are the mean ± S.E. ■, control SHR; ▲, lercanidipine (0.5 mg/kg/day)-treated SHR; ●, nicardipine-treated SHR; □, manidipine-treated SHR; ▲, lercanidipine (2.5 mg/kg/day)-treated SHR; □, hydralazine-treated SHR; ●, normotensive WKY rats. Systolic pressure values were significantly different (P < .01) from control SHR and WKY rats in the 12 weeks of experiments. In treated SHR, systolic pressure values were significantly lower than in control SHR after 1 week (2nd week) in hydralazine-treated SHR (P < .01) and after 2 weeks (3rd week) in nicardipine-treated SHR (P < .05) or in manidipine-treated and lercanidipine-treated SHR (P < .01).
cological treatments partially countered this phenomenon, with the exception of the lower dose of lercanidipine for sodium and potassium. Manidipine and nicardipine enhanced urinary potassium decrease (Table 4).

**Discussion**

As mentioned in the Introduction, hypertension is accompanied by renal damage characterized by glomerular hypertrophy, nephrosclerosis, and albuminuria (Feld et al., 1977; Dworkin et al., 1987; Martinez-Maldonado et al., 1987; Epstein and Sowers, 1992). Hypertension is not only a cause of renal disease but also may represent the result of disease-induced glomerular damage as in diabetes (Epstein and Sowers, 1992; Mogensen, 1994). Both in hypertension and in concurrent disease such as diabetes mellitus, excessive glomerular protein excretion contributes to the progression of renal injury leading, if not appropriately treated, to chronic nephropathy. Thus, attention of investigators was focused on how to inhibit or to reduce proteinuria and glomerulosclerosis (Epstein and Sowers, 1992; Mogensen, 1994). Animal and human studies have documented that ACE inhibitors more than other antihypertensives limit proteinuria and glomerulosclerosis (Ruggenenti, 1997). A vasodilating activity more marked for efferent than for afferent glomerular arterioles is the most probable reason of nephroprotective effects of these compounds (Anderson et al., 1986). Another important class of antihypertensive drugs, Ca\(^{2+}\) antagonists, although safe and effective in the majority of cases do not vasodilate efferent arteriole, with consequent increased glomerular pressure and the risk of increasing glomerular injury (Loutzenhiser and Epstein, 1985; Dworkin and Feiner, 1986; Hayashi et al., 1996). The inadequate glomerular protection and the lack of influence on proteinuria may be the cause of adverse effects on renal tubular function reported after treatment with nifedipine (Holdaas et al., 1991).

It has been shown that the last-generation dihydropyridine-type Ca\(^{2+}\) antagonists, in contrast to older compounds of the same class, induce vasodilatation of glomerular efferent arterioles (Tojo et al., 1992; Hayashi et al., 1996; Sabbatini et al., 2000). The consequent decrease of glomerular pressure may reduce glomerular injury affording nephroprotection. To verify this hypothesis, this study has investigated the influence of long-term treatment with new-generation Ca\(^{2+}\) an-

**TABLE 1**

<table>
<thead>
<tr>
<th></th>
<th>WKY (n = 10)</th>
<th>SHR (n = 10)</th>
<th>SHR + LER 0.5 (n = 7)</th>
<th>SHR + LER 2.5 (n = 8)</th>
<th>SHR + MAN (n = 7)</th>
<th>SHR + NIC (n = 8)</th>
<th>SHR + HYD (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beginning of treatment (14 weeks of age)</td>
<td>359 ± 5</td>
<td>341 ± 5</td>
<td>382 ± 8</td>
<td>404 ± 4</td>
<td>309 ± 8</td>
<td>385 ± 7</td>
<td>350 ± 5</td>
</tr>
<tr>
<td>End of experiment (26 weeks of age)</td>
<td>391 ± 7(^a)</td>
<td>380 ± 8(^a)</td>
<td>393 ± 6</td>
<td>405 ± 5</td>
<td>352 ± 7(^a)</td>
<td>395 ± 5</td>
<td>372 ± 7(^a)</td>
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</tbody>
</table>

LER, lercanidipine; MAN, manidipine; NIC, nicardipine; HYD, hydralazine.
\(^a\) P < .05 in comparison with the value found at the beginning of treatment.

Fig. 2. Sections of small-sized renal artery branches stained with Masson’s trichromic technique. A, normotensive WKY rat. B, control SHR. C, SHR treated with 2.5 mg/kg/day lercanidipine. D, SHR treated with manidipine. E, SHR treated with nicardipine. F, SHR treated with hydralazine. Note in control SHR, luminal narrowing and an increased thickness of the tunica media. Treatment with lercanidipine and to a lesser extent with the other drugs investigated countered luminal narrowing and thickening of the tunica media. Arrowheads, tunica adventitia; \(*\), tunica media; L, lumen. Scale bar, 10 \(\mu m\).
tagonists such as manidipine and lercanidipine (Testa et al., 1997) on nephroprotection in SHR. The effects of these compounds were compared with those of the second-generation Ca\textsuperscript{2+} antagonist nicardipine and of the nondihydropyridine-type vasodilator hydralazine. To make results of morphometric analysis comparable, we have chosen doses of compounds reducing systolic arterial pressure to a similar extent. Moreover, to evaluate whether nephroprotective effects of Ca\textsuperscript{2+} antagonists might be independent by blood pressure lowering, lercanidipine also was used at a nonhypotensive dose. Consistent with previous findings, a decreased volume of renal cortex and a reduced number of glomeruli were found in control SHR (Skov et al., 1994). This phenomenon was not affected by pharmacological treatment, suggesting that it was already established at the beginning of experiment (Skov et al., 1994) or it was not sensitive to the drugs investigated. Other glomerular parameters were influenced positively by treatment with antihypertensive compounds examined as well as, to a lesser extent, by the nonhypotensive dose of lercanidipine. Consistent with data of another group, glomerular injury was more pronounced in juxtamedullary than in cortical glomeruli (Kimura et al., 1991). Effects of antihypertensives on glomerular injury probably have functional relevance because dihydropyridine-type Ca\textsuperscript{2+} antagonists reduced albuminuria in SHR. Hypotensive dosages of compounds investigated caused natriuresis. The occurrence of marked na-

| TABLE 2 |

Quantitative image analysis glomerular morphometry parameters in different animal groups investigated

Data are the mean ± S.E. Details on morphometric analysis protocol are reported under Materials and Methods. For the significance of abbreviations see Table 1.

<table>
<thead>
<tr>
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<th>SHR + NIC (n = 8)</th>
<th>SHR + HYD (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortex volume (mm\textsuperscript{3})</td>
<td>538 ± 31</td>
<td>420 ± 23\textsuperscript{a}</td>
<td>423 ± 30\textsuperscript{a}</td>
<td>428 ± 33\textsuperscript{a}</td>
<td>426 ± 28\textsuperscript{a}</td>
<td>425 ± 35\textsuperscript{a}</td>
<td>422 ± 31\textsuperscript{a}</td>
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<tr>
<td>Number of glomeruli (× 10\textsuperscript{3})</td>
<td>30.1 ± 2.1</td>
<td>25.3 ± 1.9\textsuperscript{a}</td>
<td>25.6 ± 1.3\textsuperscript{a}</td>
<td>25.6 ± 1.5\textsuperscript{a}</td>
<td>25.8 ± 2.1\textsuperscript{a}</td>
<td>25.4 ± 1.7\textsuperscript{a}</td>
<td>25.4 ± 2.2\textsuperscript{a}</td>
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<tr>
<td>Total glomerular volume (mm\textsuperscript{3})</td>
<td>21.4 ± 0.2</td>
<td>23.8 ± 0.3\textsuperscript{a}</td>
<td>22.6 ± 0.2\textsuperscript{a,b}</td>
<td>19.4 ± 0.3\textsuperscript{a,b,c}</td>
<td>19.2 ± 0.2\textsuperscript{a,b,c}</td>
<td>20.3 ± 0.2\textsuperscript{a,b,c,d,e}</td>
<td>22.5 ± 0.3\textsuperscript{a,b,c,d,e}</td>
</tr>
<tr>
<td>Volume of glomerular tuft (10\textsuperscript{3} μm\textsuperscript{3})</td>
<td>711.7 ± 16.1</td>
<td>915.5 ± 23.2\textsuperscript{a}</td>
<td>843.6 ± 16.6\textsuperscript{a,b}</td>
<td>730.1 ± 22.0\textsuperscript{a,b,c}</td>
<td>736.1 ± 30.1\textsuperscript{a,b,c}</td>
<td>798.3 ± 33.8\textsuperscript{a,b,c,d,e}</td>
<td>846.8 ± 15.8\textsuperscript{a,b,c,d,e}</td>
</tr>
<tr>
<td>Mesangial cell nuclei/volume glomerular tuft</td>
<td>85.0 ± 6.3</td>
<td>128.4 ± 9.6\textsuperscript{a}</td>
<td>104.3 ± 8.5\textsuperscript{a,b}</td>
<td>86.4 ± 6.2\textsuperscript{a,b,c}</td>
<td>86.9 ± 7.3\textsuperscript{a,b,c}</td>
<td>87.2 ± 6.1\textsuperscript{b,c,d}</td>
<td>98.2 ± 6.1\textsuperscript{a,b,c,d,e,f}</td>
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\textsuperscript{a} P < .05 versus WKY.
\textsuperscript{b} P < .05 versus SHR.
\textsuperscript{c} P < .05 versus SHR + LER 0.5.
\textsuperscript{d} P < .05 versus SHR + LER 2.5.
\textsuperscript{e} P < .05 versus SHR + MAN.
\textsuperscript{f} P < .05 versus SHR + NIC.

![Fig. 3. Sections of rat renal cortex stained with PAS. Glomeruli. A, normotensive WKY rat. B, control SHR. C, SHR treated with 0.5 mg/kg/day lercanidipine. D, SHR treated with 2.5 mg/kg/day lercanidipine. E, SHR treated with manidipine. F, SHR treated with nicardipine. Note in control SHR hypertrophy of glomerular tuft and glomerulosclerosis (shown by arrows). Treatment with lercanidipine, manidipine, and nicardipine improved glomerular morphology. ⋆, capsular lumen; G, glomerulus. Scale bar, 75 μm.](image-url)
triuresis after administration of Ca\(^{2+}\) antagonists to hypertensives is well documented and is probably mediated through the interaction of these drugs with renal tubules (Romero et al., 1988). Data on the influence of Ca\(^{2+}\) antagonists on urinary potassium are sparse and indicative of no change in electrolyte excretion in SHR (Nagaoka and Shibota, 1989). Based on our data we are unable to hypothesize on the significance of the different effect of lercanidipine, manidipine, and nicardipine on urinary potassium elimination in SHR. More information on this topic can contribute to further differentiation of the renal profile of these drugs.

Comparative analysis of reversal hypertensive microanatomical changes by the drugs investigated suggests that Ca\(^{2+}\) antagonists vasodilating efferent arterioles such as lercanidipine and manidipine (Tojo et al., 1992; Hayashi et al., 1996; Sabbatini et al., 2000) exerted a more pronounced effect on glomerular injury than nicardipine. This supports the assumption that efferent arteriole vasodilatation may represent a valuable property of some Ca\(^{2+}\) antagonists (Tojo et al., 1992; Hayashi et al., 1996; Sabbatini et al., 2000), conferring them a nephroprotective effect.

The findings that the nonhypotensive dose of lercanidipine improved glomerular morphology and reduced proteinuria suggest that part of the nephroprotective effects of the compound are independent of blood pressure-lowering activity, similarly as reported for ACE inhibitors (Ruggenenti, 1997). This property, if of clinical relevance, may offer new perspectives in the treatment of hypertension with concurrent nephropathy or renal diseases in which nephroprotection without lowering of blood pressure is desirable.

Our study also has shown the occurrence of tubular damage in SHR and that this phenomenon is countered by the
drugs investigated. Both in human pathology and experimental models of hypertension, natriuresis is abnormally increased by elevated pressure (Hall et al., 1996). In renal tubules L-type channels blocked by dihydropyridine agents are one of the main gates of cellular Ca\(^{2+}\) entry (van Zwieten and Pfaffendorf, 1993). Our data of degenerating proximal and distal tubule epithelium, recovered by treatment with Ca\(^{2+}\) antagonists, support evidence for a tubular effect of dihydropyridine derivatives. It cannot be excluded, as reported in other cell populations (Fleckenstein et al., 1989; Nicotera et al., 1992), that a derangement in Ca\(^{2+}\) balance in proximal and distal tubule epithelium may induce degeneration. Effects of the drugs tested on degenerating tubules, including the nonhypotensive dose of lercanidipine, may be useful in diseases associated with impaired renal function.

**References**


