Possible Linkage between Renal Injury and Cardiac Remodeling in Dahl Salt-Sensitive Rats Treated with the Calcium Channel Antagonist Benidipine


Interest in cardiovascular protection by calcium channel antagonists has grown over the past decade. We investigated the prevention of cardiac remodeling and renal injury by the long-acting calcium channel antagonist benidipine using 12 week-old Dahl salt-sensitive (Dahl S) rats fed a high-salt (4% NaCl) diet. Six-week benidipine treatment (10 mg/kg chow) decreased systolic blood pressure by 22% in Dahl S rats. This blood pressure reduction was associated with decreases in cardiac mass and weight of the aortic wall. Collagen content in the left ventricle tended to decline with benidipine treatment. In addition, glomerular filtration rate increased by 33% and arterial and glomerular lesions improved morphologically with this treatment. Regression of cardiac mass and collagen content in the left ventricle was due mainly to blood pressure reduction; however, collagen content in the low-pressure right ventricle was not only related to systemic blood pressure but to the severity of renal lesions. These data suggest that the calcium channel antagonist benidipine attenuates cardiac and renal injury in hypertensive Dahl S rats, and that part of the cardiac hypertrophy is due to a non-hemodynamic mechanism that might be responsible for, or a consequence of, the lesions in the kidney. (Hypertens Res 1995; 18: 245-253)

Key Words: calcium channel antagonist, cardiac hypertrophy, renal injury, glomerular sclerosis, Dahl salt-sensitive rats, vascular injury

Mechanical overload is the main determinant of left ventricular hypertrophy in hypertension. Besides this well-documented mechanism, recent studies on the low-pressure right ventricle have highlighted the role of humoral factors in cardiac remodeling. In fact, aldosterone plays a role in increasing collagen in the right ventricle in some forms of experimental hypertension and cardiac type I and type III collagen mRNAs have been demonstrated to be increased in aldosterone-salt hypertension (1, 2). In addition, recent advances in regional renin-angiotensin research have provided evidence for the involvement of the renin-angiotensin system in cardiac hypertrophy and interstitial fibrosis in the pressure overload state (3, 4).

Recent studies have indicated that Dahl salt-sensitive (Dahl S) rats with salt-induced hypertension are characterized by enhanced susceptibility to kidney injury, e.g., glomerulosclerosis, medial necrosis or periarthritis, and tubular dilatation or interstitial expansion, as compared with the rat model for spontaneous hypertension with similar blood pressure levels (3, 6). In this form of hypertension, the collagen content in the injured kidney has been demonstrated to increase. Moreover, in our preliminary study, we found that Dahl S rats with hypertension show cardiac hypertrophy with an increase in interstitial collagen fibers (unpublished data). A series of studies strongly suggested that, besides hemodynamic factors, there must be non-hemodynamic components involved in the progression of kidney injury (5, 6). If non-hemodynamic factors participate in the development of hypertensive injury, they may contribute to the progression of cardiac hypertrophy in Dahl S rats.

In this context, it is of interest to note that certain plasma factors occurring in 5/6 nephrectomized rats or renal dysfunction stimulate collagen synthesis and the progression of sclerosis in the kidney (7, 8, 9). Despite this, to our knowledge there have been few studies addressing whether humoral factors mediate susceptibility to hypertensive organ injury in Dahl S rats. To test the hypothesis that a non-hemodynamic mechanism underlies renal and cardiac injury in hypertension, we investigated whether and how the dihydropyridine calcium chan-
nel antagonist benidipine attenuates renal injury and cardiac hypertrophy in Dahl salt-sensitive rats and, if so, whether there is a mechanistic interrelationship between the progression of kidney injury and cardiac hypertrophy.

**Materials and Methods**

**Dahl Salt-Sensitive Rats**
Fifty Dahl-Iwai salt-sensitive (Dahl S) rats were bred at Tukuba Research Laboratories, Eisai Co., Ltd., Tokyo, Japan. This strain was originally from Brookhaven National Laboratories, Upton, New York, USA. The rats were kept on a low-salt (0.3% NaCl) diet after weaning. Forty-two 6-week-old Dahl S rats were fed a high-salt (4% NaCl) diet for 6 weeks and were randomly assigned to 3 groups: 1) 14 Dahl S rats fed a high-salt diet alone (4% control); 2) 14 Dahl S rats fed a high-salt diet containing low-dose benidipine hydrochloride ((+)-(R*)-3-[((R* )-1-benzyl-3-piperidyl)methyl 1,4-dihydro-2,6-dimethyl-4-(m-nitrophenyl)-3,5-pyridinedicarboxylate hydrochloride] (3 mg/kg chow) (low-dose); and 3) 14 Dahl S rats fed a high-salt diet containing high-dose benidipine hydrochloride (10 mg/kg chow) (high-dose) [10, 11]. These rats were maintained for another 6 weeks (therapeutic period). Eight 6-week-old Dahl S rats were fed a low-salt diet for 12 weeks (0.3% control). Water was given ad libitum during the study. Blood pressure was measured every week by the tail cuff method of Friedman et al. [12].

A 24-hour urine specimen was collected on the last day of the therapeutic period. The right kidney was isolated under pentobarbital anesthesia (25 mg/kg body weight) for morphological investigation. Thereafter, blood samples and the organs of interest were removed.

**Assessment of Renal Function**
Creatinine levels were measured by a Beckman Creatinine Analyzer-2 (Beckman Instrument Inc., Brea, California, USA). Sodium and potassium concentrations were measured by an AutoCal Model 643 flame photometer (Instrumental Laboratory, Inc., Lexington, Massachusetts, USA). Urinary protein concentration was measured by the sulfosalicylic acid method. N-acetyl-β-d-glucosaminidase activity (NAG) was determined using sodio-m-cresol-sulfophthaleinyl N-acetyl-β-d-glucosaminide as a substrate (NAG assay kit, Shionion Pharmaceutical Co., Osaka, Japan) [13]. Plasma renin activity (PRA) and plasma aldosterone concentration (PAC) were measured by standard radioimmunoassay methods (RENIN-RIABEAD, Dainabot Co., Ltd., Tokyo, Japan and Aldosterone-kit, Diagnostic Products Corporation, Los Angeles, California, USA).

**Collagen Determination**
Collagen content in the tissue was determined morphologically using a Vanox S model AH-2 microscope (Olympus, Co., Ltd., Tokyo, Japan) and image-analyzer (LUZEX III, Nireco, Tokyo, Japan) according to the method of Achwarz [16, 17]. The
Fig. 1. Chemical structure of benidipine. The left graph (a) shows the chemical structure of the representative dihydropyridine-calcium channel antagonist nifedipine. The right (b) depicts the chemical structure of benidipine. Portions that are different between the two drugs are marked by broken lines. These differences give benidipine long-acting properties and a higher affinity for cellular membranes.

Fig. 2. Cardiac collagen measurement. The graphs show cross-sections of left ventricle with a normal appearance from high-dose benidipine-treated rats (lower graph) and interstitial collagen fibrils (arrows) in the left ventricle from untreated Dahl S rats fed a high-salt diet (upper graph). The collagen fibers stained blue were identified using spectrum analysis of collagen images, and the area was summated by a computer-assisted analysis system according to the method described in the text.
image of collagen fibers stained by azan was analyzed and the specific signals were determined (Fig. 2). Considering spectrum information indicating collagen fibers, the areas of collagen infiltration were determined and summed up to obtain the total square. The analyzer scanned an entire cross-section of the heart, and the measurement was expressed as a ratio of the area of collagen to the total area.

Reagents
All reagents were of analytical grade. Benidipine was kindly donated by Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan.

Statistical Analysis
Values were expressed as means ± SE. Differences were assessed by Student's t-test, chi-square test, one-way or two-way analysis of variance followed by Student's t-test.

Results
Hemodynamic Effects
Systolic blood pressure was time-dependently increased in Dahl S rats given a high-salt diet. Dahl S rats fed a low-salt diet remained normotensive over the entire experimental period (Fig. 3). With benidipine treatment, the systolic blood pressure fell immediately; this antihypertensive effect was sustained during the therapeutic period. At the end of the experiment, the systolic pressure was 211 ± 5 (SE) mmHg in high-salt control rats, 195 ± 5 mmHg in low-dose treated rats (p < 0.05 vs. high-salt Dahl S rats), 185 ± 3 mmHg in high-dose treated rats (p < 0.001 vs. high-salt Dahl S rats), and 129 ± 1 mmHg in normotensive Dahl S rats fed a low-salt diet (p < 0.0001 vs. high-salt Dahl S rats).

Cardiac Morphometry
Cardiac mass and collagen content are shown in Table 1. There were no differences in body weight among the four groups. The high-salt diet significantly increased aortic weight by 42%, as compared with that in low-salt control rats. This increase in aortic weight was significantly attenuated by benidipine treatment. Similarly, the high-salt diet increased cardiac weight by 35%; however, benidipine treatment significantly attenuated this cardiac hypertrophy in a dose-dependent manner.

Table 1. Alterations in Cardiac Mass by Benidipine Treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>BW (g)</th>
<th>Aorta (mg)</th>
<th>Heart (mg)</th>
<th>LV-collagen (mg)</th>
<th>RV-collagen (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4% control</td>
<td>406±7</td>
<td>18.7±0.6</td>
<td>0.407±0.007</td>
<td>9.58±1.25</td>
<td>15.92±2.24</td>
</tr>
<tr>
<td>Low-dose</td>
<td>410±5</td>
<td>16.9±0.4**</td>
<td>0.383±0.006*</td>
<td>8.58±1.16</td>
<td>ND</td>
</tr>
<tr>
<td>High-dose</td>
<td>415±5</td>
<td>17.1±0.5**</td>
<td>0.375±0.004**</td>
<td>7.95±1.08</td>
<td>14.15±2.20</td>
</tr>
<tr>
<td>0.3% control</td>
<td>429±7</td>
<td>13.2±0.3***</td>
<td>0.301±0.003***</td>
<td>3.04±0.49***</td>
<td>ND</td>
</tr>
</tbody>
</table>

Units are grams for body weight (BW), mg/cm² for aortic weight (Aorta), g/100 g body weight for heart weight (Heart), percentage for left- and right-ventricular collagen content (LV-collagen and RV-collagen). Differences were assessed by one-way analysis of variance (ANOVA) and Student's t-test. *p<0.05, **p<0.01, ***p<0.001 vs. the respective values in the 4% control group. NS indicates statistically not significant. ND = not determined.

In addition, we morphometrically investigated alterations in collagen content in the cardiac tissue. As shown in Table 1, the collagen content in the left ventricle almost tripled in Dahl S rats fed a high-salt diet. Benidipine treatment tended to attenuate this increase in collagen content in the heart. We also determined collagen content in the right ventricle, a low-pressure circulatory system. High-dose benidipine reduced this value by only 11%, and this difference was not significant.

Effects on Renal Function
Plasma variables are shown in Table 2. There were no differences in plasma sodium or potassium levels among the four groups. The high-salt diet significantly increased the plasma creatinine level in Dahl rats.
Calcium Antagonist and Collagen

S rats, as compared with normotensive control rats; however, benidipine treatment reduced plasma creatinine to below the control level in normotensive Dahl S rats. PRA tended to be reduced by benidipine treatment, but the difference with control was not significant. Similarly, the PAC level was significantly lowered by the high-salt diet. The level decreased further in the benidipine-treated groups, but the difference was not significant.

Urinary parameters are shown in Table 3. Urine volume and urinary excretion of sodium increased with the high-salt diet; however, there were no differences in these parameters between untreated and benidipine-treated Dahl S rats. The high-salt diet significantly increased urine protein and NAG excretion in Dahl S rats, as compared with low-salt Dahl S rats. GFR was greater in normotensive control rats than in untreated Dahl S rats, but the difference was not statistically significant. Benidipine did not reduce urine protein or NAG excretion, as compared with that in untreated control rats; however, in benidipine-treated Dahl S rats, the creatinine clearance rate (GFR) was significantly increased in a dose-dependent manner.

Renal Morphology

We examined the effects of benidipine on glomerular sclerosis (Fig. 4). In untreated Dahl S rats, the severity of the glomerular lesions was evenly distributed between GS-1 and GS-2. Benidipine markedly reduced the percentage of glomeruli exhibiting a

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**Table 2. Plasma Parameters in Dahl S rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>Na</th>
<th>K</th>
<th>Cr</th>
<th>PRA</th>
<th>PAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>4% control</td>
<td>(13)</td>
<td>153.6±0.6</td>
<td>4.7±0.1</td>
<td>0.49±0.02</td>
<td>2.52±1.16</td>
<td>83.92±13.15</td>
</tr>
<tr>
<td>Low-dose</td>
<td>(13)</td>
<td>155.8±1.3</td>
<td>4.1±0.1</td>
<td>0.35±0.01*</td>
<td>1.22±0.25</td>
<td>73.23±4.29</td>
</tr>
<tr>
<td>High-dose</td>
<td>(14)</td>
<td>158.4±0.2</td>
<td>4.7±0.0</td>
<td>0.31±0.02*</td>
<td>1.04±0.22</td>
<td>65.71±3.59</td>
</tr>
<tr>
<td>0.3% control</td>
<td>(8)</td>
<td>156.8±1.2</td>
<td>4.7±0.0</td>
<td>0.37±0.02*</td>
<td>3.47±1.23</td>
<td>152.12±18.45**</td>
</tr>
</tbody>
</table>

*p values (ANOVA) NS NS <0.05 NS <0.001

Units are mEq/l for plasma sodium (Na) and potassium (K) concentration, mg/dl for plasma creatinine (Cr) concentration, ng/ml/h for plasma renin activity (PRA) and pg/ml for plasma aldosterone concentration (PAC). Differences were assessed by one-way analysis of variance (ANOVA) and Student’s t-test. *p<0.05. **p<0.001 vs. the respective values in the 4% control group.

**Table 3. Urinary Parameters in Dahl S rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>UV</th>
<th>U_{Na}V</th>
<th>U_{K}V</th>
<th>U_protein</th>
<th>U_{NAG}</th>
<th>GFR</th>
</tr>
</thead>
<tbody>
<tr>
<td>4% control</td>
<td>(13)</td>
<td>8.96±1.25</td>
<td>1.49±0.22</td>
<td>0.56±0.06</td>
<td>179.6±29.5</td>
<td>2.05±0.25</td>
<td>793.3±34.3</td>
</tr>
<tr>
<td>Low-dose</td>
<td>(13)</td>
<td>8.96±1.30</td>
<td>1.93±0.25</td>
<td>0.59±0.06</td>
<td>194.1±24.9</td>
<td>2.57±0.13</td>
<td>1,122.6±156.9</td>
</tr>
<tr>
<td>High-dose</td>
<td>(14)</td>
<td>8.83±0.62</td>
<td>1.98±0.22</td>
<td>0.66±0.06</td>
<td>183.3±17.4</td>
<td>1.96±0.20</td>
<td>1,233.6±109.4*</td>
</tr>
<tr>
<td>0.3% control</td>
<td>(8)</td>
<td>3.93±0.44**</td>
<td>0.14±0.03**</td>
<td>0.44±0.05*</td>
<td>49.7±3.9**</td>
<td>1.28±0.16**</td>
<td>1,066.6±109.2</td>
</tr>
</tbody>
</table>

*p values (ANOVA) <0.05 <0.0001 <0.05 <0.005 NS <0.05

Units are ml/100 g body weight/d for urinary volume (UV), mmol/100 g body weight/d for sodium excretion (U_{Na}V) and urinary potassium excretion (U_{K}V), mg/d for urinary protein excretion (U_{protein}), units/g creatinine for urinary NAG excretion (U_{NAG}) and ml/100 g body weight/d for creatinine clearance rate (GFR). Numbers in parentheses represent the numbers of rats. Differences were assessed by one-way analysis of variance (ANOVA) and Student’s t-test. NS indicates statistically not significant. *p<0.05. **p<0.001 vs. the respective values in high-salt control Dahl S rats (4% control).

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![Fig. 4. Glomerular sclerotic lesions in Dahl S rats. Distribution pattern of glomeruli with different severity of glomerulosclerosis is shown for each group. Values on the ordinate axis represent the percentage of glomeruli that were assigned each glomerulosclerosis score. 4% control, untreated rats fed a high-salt diet; low-dose, low-dose benidipine-treated rats; high-dose, high-dose benidipine-treated rats; and 0.3% control, normotensive rats fed a low-salt diet. The difference was assessed using values in the three high-salt groups by the chi-square test on lxm contingency table; $\chi^2$=16.8 (p<0.05).](image)
severe degree of sclerotic lesions, i.e., GS-2, GS-3 and GS-4, and increased the number of glomeruli with a normal appearance (GS-0). The attenuation of glomerular lesions was also reflected in an improvement in overall GS score, as shown in Table 4.

Morphological alterations in the intrarenal (radiating and arcuate) arteries are shown in Fig. 5. In untreated Dahl S rats, the arterial lesions were evenly distributed among various degrees of severity. In contrast, benidipine strikingly decreased the number of arteries showing severe lesions, corresponding to AI-2 and AI-3. Similarly, benidipine treatment significantly attenuated the overall arterial injury score (Table 3). Despite such vascular protection by benidipine treatment, renal tubular injuries were not improved by the calcium channel antagonist treatment.

**Correlation between Cardiometry and Various Indices**

Next, we investigated whether the indices of cardiac hypertrophy were correlated with those of kidney damage. As shown in Table 5, alterations in cardiac weight were closely related to the level of systolic blood pressure and the indices of renal injuries, such as urinary protein excretion, AI- and GS-scores. On the other hand, collagen content in the

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**Table 4. Alterations in Overall Injury Score by Benidipine Treatment**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>AI score</th>
<th>GS score</th>
<th>TI score</th>
</tr>
</thead>
<tbody>
<tr>
<td>4% control</td>
<td>(13)</td>
<td>125.6±27.2</td>
<td>158.6±12.8</td>
<td>2.69±0.15</td>
</tr>
<tr>
<td>Low-dose</td>
<td>(13)</td>
<td>76.3±21.3</td>
<td>138.3±14.6</td>
<td>2.69±0.15</td>
</tr>
<tr>
<td>High-dose</td>
<td>(14)</td>
<td>69.4±21.9*</td>
<td>110.8±13.6**</td>
<td>2.36±0.20</td>
</tr>
<tr>
<td>0.3% control</td>
<td>(8)</td>
<td>7.0±2.2***</td>
<td>75.8±3.3***</td>
<td>1.00±0.00</td>
</tr>
<tr>
<td>$p$ values (ANOVA)</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

The scores were determined according to the equations described in text. AI score, arterial injury score; GS score, glomerulosclerosis score; TI score, tubular injury score. 4% control, untreated rats fed a high-salt diet; Low-dose, low-dose benidipine-treated rats; High-dose, high-dose benidipine-treated rats; 0.3% control, control Dahl S rats fed a low-salt diet. Differences were assessed by one-way analysis of variance and Student’s t-test. * $p<0.05$, ** $p<0.01$, *** $p<0.005$ vs. the respective values in the 4% control group.

**Table 5. Correlation Coefficients**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cardiac weight</th>
<th>LV-collagen</th>
<th>RV-collagen</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP</td>
<td>0.912***</td>
<td>0.518**</td>
<td>0.411*</td>
</tr>
<tr>
<td>Aortic weight</td>
<td>0.750***</td>
<td>0.313*</td>
<td>0.098</td>
</tr>
<tr>
<td>Uprotein</td>
<td>0.612***</td>
<td>0.322*</td>
<td>0.238</td>
</tr>
<tr>
<td>NAG</td>
<td>0.237</td>
<td>0.222</td>
<td>0.067</td>
</tr>
<tr>
<td>GFR</td>
<td>−0.078</td>
<td>−0.032</td>
<td>−0.090</td>
</tr>
<tr>
<td>AI-score</td>
<td>0.726***</td>
<td>0.203</td>
<td>0.486*</td>
</tr>
<tr>
<td>GS-score</td>
<td>0.760***</td>
<td>0.242</td>
<td>0.419*</td>
</tr>
<tr>
<td>PRA</td>
<td>0.263</td>
<td>−0.136</td>
<td>−0.169</td>
</tr>
<tr>
<td>PAC</td>
<td>0.431**</td>
<td>0.079</td>
<td>0.248</td>
</tr>
<tr>
<td>Cardiac weight</td>
<td>ND</td>
<td>0.425*</td>
<td>0.441*</td>
</tr>
<tr>
<td>LV-collagen</td>
<td>ND</td>
<td>ND</td>
<td>0.038</td>
</tr>
</tbody>
</table>

Coefficients were determined using rats fed a high-salt diet for cardiac weight ($n=40$) and left-ventricle collagen ($n=40$) and using high-salt control rats and high-dose benidipine treated Dahl rats for right-ventricle collagen ($n=27$). * $p<0.05$, ** $p<0.01$, *** $p<0.001$. ND = not determined.
Discussion

Food consumption was estimated in the last week of the therapeutic period. Food intake averaged 28 ± 3 (SE) g/d for untreated Dahl S rats, 31 ± 2 g/d for low-dose benidipine-treated rats, 30 ± 3 g/d for high-dose benidipine-treated rats, and 33 ± 4 g/d for rats fed a low-salt diet. There were no differences in food intake among the four experimental groups. The dosage of benidipine, calculated from the food consumption in high-dose benidipine-treated Dahl S rats, was 1.12 ± 0.02 mg/kg body weight/d. It has been reported that with oral administration at 3 mg/kg body weight or more in spontaneously hypertensive rats (SHR), this drug exhibits an antihypertensive effect lasting 24 hours (18). The dosage used in the present study was in the range of usual doses for the treatment of SHR, but approximately 20- to 40-fold greater than those used in human subjects (19, 20).

There are an increasing number of studies suggesting that Dahl S rats are more susceptible to organ injury due to hypertension than a genetic rat model for spontaneous hypertension (SHR) (5, 6). In the present study, we demonstrated that the calcium channel antagonist benidipine attenuated the development of hypertension. This blood pressure reduction was associated with improvement of the renal injury, including morphological regression of glomerular and arterial lesions; however, it should be noted that the organ protection was apparently greater than expected judging from the blood pressure reduction alone. In fact, there was a marked difference between low- and high-dose benidipine in the improvement in renal injury, even though the blood pressure values were quite similar. These data suggested a role for non-hemodynamic components in the attenuation of renal injury (21–23). This histologic improvement was reflected in an increase in creatinine clearance rate and a decrease in plasma creatinine levels. Such renal protection by benidipine was in agreement with that observed in several calcium channel antagonists recently introduced to clinical usage (24).

The most important finding in the present study was that the calcium channel antagonist benidipine decreased cardiac hypertrophy following a high-salt diet. The regression of cardiac hypertrophy was associated with a trend toward a decrease in collagen content in the left ventricle. Analysis of the correlation coefficients showed that the regression of cardiac hypertrophy was closely related to the reduction in systolic blood pressure, thereby suggesting that the alterations of hemodynamics may be fundamental to the progression of left ventricular hypertrophy.

Similar types of cardiovascular protection by long-acting dihydropyridine calcium channel antagonists, introduced very recently into clinical usage, have been reported in the same rat model (24, 25). In addition, it is also reported that a calcium channel antagonist reduces collagen synthesis in the kidney (25). Despite this, there are few studies that meticulously investigate the effects of the prototype calcium channel antagonist nifedipine on cardiovascular lesions. Thus, it seems a little bit difficult to state whether the cardiovascular protection is common to all forms of calcium channel antagonists or if it is unique to the newly developed antagonists. In fact, there are quite a few different properties between nifedipine and the long-acting dihydropyridine analogues (10, 24–26). The analogues have a high affinity for the cardiovascular system and bind more directly to cellular membranes. This property lets the compounds intrude into intracellular spaces and may provide more actions than those expected from blocking calcium channels.

More interestingly, the collagen content in the right ventricle was shown to be regulated by mechanisms different from those in the left ventricle; the content in the right ventricle was more closely correlated to the severity of the renal injury. In addition, the collagen content in the left ventricle was not correlated to the collagen content in the right ventricle. These data strongly suggest that the low-pressure right ventricle is more likely to be influenced in part by a mechanism that is also responsible for renal injury.

The common mechanism for the linkage of cardiac remodeling and renal injury is unclear. Improved renal function may attenuate volume overload, thereby decreasing wall stress in the right ventricle. Such a mechanism might explain the linkage between the heart and kidney injuries; however, in the present study, the renin profile did not support participation of volume-reduction in the regression of organ damage by the calcium channel antagonist. More interestingly, recent studies have shown that some of the long-acting dihydropyridine analogues, introduced very recently into clinical usage, directly inhibit protein synthesis at the ribosomal level, and that this mechanism may lead to a decrease in collagen synthesis in vascular smooth muscle cells (26, 27). Protein synthesis inhibition is also responsible for the inhibition of vascular smooth muscle cell proliferation, an integral component of cardiovascular injury in hypertension. Although we have not investigated whether benidipine directly inhibits protein synthesis in vitro, such a mechanism may underlie the decrease in collagen content and the improvement in renal injury.

Further, there is much evidence that enhancement of the regional renin-angiotensin system is responsible for vascular hypertrophy or renal injury in various forms of hypertension, including models of volume-dependent hypertension such as Dahl S rats (28–32). We did not investigate directly the regional renin-angiotensin system; however, neither PRA nor PAC was related to cardiac collagen contents.

Another important point presented in this study was that the calcium channel antagonist benidipine attenuates glomerular sclerotic lesions in Dahl S rats.
rats. The effects of calcium channel antagonists on glomerular sclerotic lesions have been debated for several years (33-36). The reason for the discrepancy between our data and those obtained in other studies, reporting negative effects with renal ablation or renovascular hypertension models, is not yet understood. However, benidipine may decrease not only afferent arteriole resistance, but also postcapillary resistance, thereby resulting in a reduction in glomerular pressure. In fact, some investigators have reported that calcium channel antagonists decrease glomerular capillary pressure in a reduced renal-mass model (37). In addition, in the malignant hypertensive stage in salt-induced hypertension, the renin-angiotensin system is enhanced, which presumably results in the progression of glomerular sclerotic changes. The resolution of arterial injury by benidipine leads to lowering of the renin-angiotensin system and thereby has a beneficial effect on glomerular sclerotic changes.

Benidipine increased the creatinine clearance rate to the level observed in normotensive rats. Despite the functional and morphological improvement, urinary protein excretion was not reduced by benidipine treatment. In this context, it is reported that in patients with diabetes mellitus proteinuria is somewhat enhanced by treatment with calcium channel antagonists. Although this discrepancy has been demonstrated with other long-acting calcium channel antagonists, the exact mechanism is still uncertain. If the attenuation of glomerular sclerosis is due to a reduction in glomerular capillary pressure, the glomerular permeability must be increased. The enhanced permeability might account for the insufficient reduction in proteinuria with benidipine treatment.

Finally, we demonstrated in the present study that long-term treatment with benidipine attenuates the development of hypertension, and that this is associated with regression of cardiac hypertrophy in Dahl salt-sensitive rats. The mechanism underlying the regression may be multifactorial; however, the marked improvement in cardiac injury with benidipine treatment has implications for clinical usage. Long-term clinical trials are needed to investigate these important questions.

**Acknowledgment**

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