Effect of Chronic Dihydropyridine (Isradipine) on the Large Arterial Walls of Spontaneously Hypertensive Rats

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Background The effect of genetic hypertension and of chronic therapy by calcium entry blocker (CEB, isradipine) on the function and structure of large arteries has been studied in adult spontaneously hypertensive rats (SHR, n=30) and in their normotensive control Wistar-Kyoto (WKY) rats (n=30).

Methods and Results Fifteen-week-old rats were randomly allocated to treatment with isradipine (3 mg/kg subcutaneously once a day) or to placebo and followed for 12 weeks. Hemodynamic parameters, including instantaneous pressure and aortic velocity, were recorded under anesthesia at the end of the treatment period. Passive mechanical properties of carotid arteries were measured in situ in the presence or the absence of smooth muscle cell activity (potassium cyanide poisoning). Histomorphometric parameters of the carotid and aortic media, including cross-sectional area, medial thickness, nucleus density and size, and medial contents of proteins of interstitial matrix, were measured by an automated morphometric system. Untreated SHRs had greater peripheral resistance, stiffer and thicker arterial walls because of smooth muscle cell hyperplasia (thoracic aorta and carotid artery) and/or hypertrophy (thoracic aorta), and increased collagen content than did normotensive control rats. SHRs showed a significant left ventricular hypertrophy. For the whole duration of treatment, treatment with CEB normalized the arterial pressure in SHRs. We observed a significant decrease in peripheral resistance, increased cardiac output, and left ventricular contractility without significant reduction in left ventricular hypertrophy. Increases in diuresis and natriuresis were associated during the last week of treatment in both treated strains with marked increases in plasma renin activity; in contrast, urinary aldosterone was increased by treatment in WKY rats but not in SHRs. Arterial compliance was significantly increased by CEB under control and passive conditions. CEB induced a significant reduction in the medial hypertrophy of the aortic walls of SHRs and WKY rats associated with a reduction in medial hyperplasia. In the carotid artery, CEB reduced smooth muscle cell hypertrophy but did not affect the smooth muscle cell hyperplasia. Isradipine significantly reduced the arterial wall collagen contents in both strains, with marked increases in the elastin content in the carotid but not in the aortic wall.

Conclusions These results suggest that (1) despite normalization of arterial pressure, chronic treatment with CEB in SHRs does not significantly reduce left ventricular hypertrophy, probably because of increase in myocardial contractility and/or increase in plasma renin activity; (2) mechanical properties of the arterial wall are normalized by treatment; and (3) remodeling of the arterial wall by CEB is not uniform according to the studied vessel.

Key Words • arteries • mechanics • elastin • collagen • muscle, smooth

Hypertension has long been considered a disease of the cardiovascular system that affects almost exclusively the arterioles and the heart. However, damages to the large arteries are clearly involved in cardiovascular morbidity and mortality associated with hypertension.1 Numerous animal and human studies have shown that sustained hypertension is associated with structural and functional alterations to both large arteries and arterioles. There is good evidence that hypertension is associated with increased arterial wall thickness1-3 and alterations to the structural composition of the arterial wall,4,5 leading to altered arterial function.5-6 It has been suggested that smooth muscle cell hypertrophy, accompanied by polyplidism, contributes to the increase in smooth muscle cell mass in several animal models of hypertension.7 Successful treatment of patients with arterial hypertension requires that the drug used not only normalizes blood pressure but also counteracts the structural alterations of the cardiovascular system related to hypertension.

Calcium entry blockers (CEBs) are now widely used to treat patients with several cardiovascular diseases including ischemic heart disease and arterial hypertension.8 Their effectiveness in these disease states is based on a range of hemodynamic actions including effects on the heart and kidneys as well as different neural and endocrine mechanisms involved in cardiovascular homeostasis. The ultimate hemodynamic effect of a drug used to treat hypertension is the result of its primary pharmacological effect and the reactions of the cardiovascular system to the perturbations induced by the drug. This could mean that there are important differences between the in vitro or acute in vivo effects of a cardiovascular drug and its long-term actions in an experimental animal or human. An increase in large artery diameter after acute administration of CEBs has been reported in humans,9 and changes in mechanical properties of the arterial wall have been reported in animals.10,11 However, the long-term effects of CEBs on
changes in vascular structure are controversial. Nyborg and Mulvany\textsuperscript{12} reported no changes in mesenteric resistance vessel morphology during long-term treatment of spontaneously hypertensive rats (SHRs) with felodipine. Similarly, Mulvany’s group (Aalkjaer et al)\textsuperscript{13} reported that the media to lumen ratio of cutaneous resistance arteries from patients given CEBs for 1 year was not normalized. In contrast, Lundin and Hallback-Nordlander\textsuperscript{14} found that felodipine reversed resistance vessel hypertrophy in SHRs. However, there has been no study, to our knowledge, on the chronic effects of CEBs on the structure and function of large arteries in experimental models of hypertension.

The goal of this study was to determine whether prolonged treatment with a CEB of the dihydropyridine class (isradipine) modified (1) the hemodynamics, mechanical, and histomorphological features of the large arteries and (2) the associated left ventricular hypertrophy in an experimental model of genetic hypertension (SHR) and its normotensive control Wistar-Kyoto (WKY) strain.

Methods

Twenty-four 15-week-old SHRs and 24 age-matched WKY rats were randomly assigned to one of four groups: two CEB-treated and two untreated groups. Isradipine (Sandoz), a dihydropyridine CEB acting for over 24 hours, was given daily by subcutaneous injection at 3 mg/kg in 1 mL of saline for 12 weeks. The untreated rats were given subcutaneous injection of 1 mL of saline daily over the same period. From the first to the 12th week of treatment, systolic blood pressure (SBP) was measured once a week by a tail-cuff method (BP reporter 8005, W+W Electronic). The conscious rats were warmed, with the cuff and the pulse wave transducer set around the tail about 10 minutes before SBP measurements. Body weight (BW) was measured weekly. At the end of the last week of treatment (12th week), the rats were placed in metabolic cages overnight for 12-hour urine collection. Blood samples were taken by retro-orbital puncture in unanesthetized rats on the same day for plasma renin hormone determination.

Electrolyte concentrations were measured on fresh urine samples with an ion-selective electrode (Beckman). Urinary aldosterone was determined by direct radioimmunoassay.\textsuperscript{15} Plasma renin activity was determined by immunomassay of angiotensin I generated after incubating the plasma for 1 hour at 37°C.\textsuperscript{16} At the end of the treatment period, the rats were anesthetized with pentobarbital and used for a hemodynamic study. They were then killed for morphological study.

Hemodynamic Study

The surgical procedure and hemodynamic measurements have been described in detail elsewhere.\textsuperscript{17} Briefly, rats were anesthetized with 50 mg/kg IP pentobarbital and placed on a thermostored regulated heating pad, intubated, and ventilated with a rodent respirator (frequency, 50 beats per minute; tidal volume, 4.5 mL) (model 680, Harvard Apparatus). A Teflon catheter (0.9 mm id), filled with saline and coupled to a Statham P23ID pressure transducer (Gould Statham), was inserted through the right carotid artery and the tip placed into the ascending aorta. The catheter manometer system was checked for time and frequency and showed a flat response beyond 60 Hz. A midventral thoracotomy was performed, and the ascending aorta was dissected free. An adapted Doppler probe was positioned around the vessel to measure mean (cardiac output minus coronary blood flow) and phasic aortic blood flow. The system was allowed to stabilize for 10 minutes before aortic blood flow and pressure were recorded and processed by a microcomputer system (Vector 2821, Hewlett-Packard) with an analog-to-digital converter (Metabyte, Data Translation). All parameters were calculated on a beat-to-beat basis for 30 seconds and then averaged.

The basic parameters studied were the SBP, diastolic blood pressure (DBP), mean arterial blood pressure (MBP), cardiac output (CO), and heart rate (HR). Total peripheral resistance (TPR) was determined as the quotient of MBP and CO.

Determination of Carotid Artery Compliance

The static mechanical properties of the carotid artery\textsuperscript{18,19} were measured at the end of the hemodynamic study. The distal end of the left carotid artery was dissected out and cannulated with the tip of a nondistensible nylon catheter (length, 10 cm; id, 0.6 mm) filled with Tyrode’s solution containing 4% albumin and 0.03% Evans blue. Albumin was added to the solution to preserve the endothelium and to maintain a physiological osmotic gradient across the arterial wall. The tube was connected by a three-way connector to a pressure transducer (P50, Gould) and to a syringe driven by a computer-controlled automatic pump (760, Harvard). The root of the carotid artery was then dissected free, and a removable clamp was positioned at the junction of the carotid artery and the aortic arch. This preparation allowed us to isolate, in situ, 22 to 23 mm of nonexposed carotid artery. The pressure in the artery was controlled by negative feedback. The arterial pressure was measured using the pressure transducer and digitized using the analog-to-digital converter board and the microcomputer system. A BASIC program was developed to compare the measured pressure (Pmeas) frequently (three times per second) to a user-specified reference pressure (Pref) and to command the computer-controlled pump to adjust the flow rate into the artery until Pref and Pmeas were equal. Thus, the flow rate was determined by the difference between the reference and measured values.

Flow rate = constant (Pref - Pmeas)

The constant, equal to the gain of the feedback loop, determined the dynamic response of the system. It was chosen to be large enough to ensure that the response time of the feedback loop (less than 1 second) was much faster than the intrinsic response time of the artery but small enough to prevent overshoot of the arterial pressure above the reference pressure (the pump could not apply suction). The reference pressure was changed at fixed intervals (every 5 minutes), and the volume of fluid pumped (net displacement of the syringe) was stored periodically (every 3 seconds) as a function of time in the computer. To start the measurements, the segment of isolated artery was placed at atmospheric pressure for 5 minutes; pressure was then increased by a 25-mm Hg step. Inflow was rapid for the first 30 to 45 seconds and then became constant with time. The initial rapid increase in volume with pressure was assumed to result from the viscoelastic behavior of the tissue and the relaxation of the vascular smooth muscle. The later constant flow into the isolated artery after the initial increase in arterial volume was attributed to fluid filtration across the vascular wall. The static increase in volume, free of fluid filtration, was estimated by extrapolating the linear portion of the volume curve to the time when the pressure step was applied (Fig 1). The slope of the late linear portion of the injected volume versus time relation represents the filtration rate of fluid across the wall of the carotid artery.\textsuperscript{20} These measurements were repeated for pressures from 25 to 200 mm Hg in 25-mm Hg steps. The static compliance of the isolated segment of carotid artery was automatically calculated at each pressure, as the quotient of the extrapolated volume increase and the pressure step imposed (25 mm Hg). Preliminary experiments showed that the carotid compliance values were similar when measured for increasing pressure (25 to 200 mm Hg) and decreasing pressure (200 to 25 mm Hg). Once the control measurements were complete, the carotid clamp was removed, and the artery was rinsed with the Tyrode’s solution. The clamp was then replaced and the artery
filled with saline containing 100 mg/L potassium cyanide (KCN). The KCN solution was left in the vessel for 30 minutes to completely poison the smooth muscle mass. The vessel was then rinsed and refilled with modified Tyrode’s solution and the compliance measurements repeated to determine carotid compliance in the fully relaxed state, depending only on passive elastic properties. Preliminary experiments, in which carotid compliance was measured 1 hour later, showed that the duration of total protocol did not affect the static compliance values.

The integrity of the endothelial cells was verified at the end of each experiment by excising and longitudinally opening the carotid artery. The segment was washed and examined for bound Evans blue. No bound dye indicated that the endothelial surface remained unaltered. Experiments in which the endothelial surface was stained at the end of the experiment were rejected.

**Morphological Study**

The right carotid artery and a 2- to 3-cm segment from the descending thoracic aorta were removed, fixed at operating pressure (corresponding to the mean arterial pressure of each rat) in 10% formaldehyde in saline, and embedded in paraffin. Three successive 3-μm sagittal sections were treated by specific staining for the various structures in the media. Collagen fibers were stained with Sirius red, elastin with orcein, and nuclei with hematoxylin after periodic acid oxidation (Fig 2).

Morphometric analysis was performed with an automated image processor (NS 15000, Microvision). This processor is based on morphological mathematical principles and is software controlled. Specific algorithms were used to analyze each of the three stained structures. The images were sent to the processor by a video camera and examined on a video monitor. Luminosity was automatically adjusted by the software to obtain similar levels of contrast, taking into account the total light transmitted by the video camera. The analog image was then digitized. Each elementary point (pixel) was automatically compared with a threshold; if the gray level of the pixel exceeded this threshold, the pixel was assigned a value of 1; otherwise, it was given the numeric value of 0. Threshold determination was a complex operation involving pixel ensembles. The threshold was determined using the top-hat algorithm to minimize variations in staining and background. This binary image was then processed to eliminate background and artifacts, delineate the zones of interest and the reference zone, and extract and measure the parameters from the various zones of interest. The first algorithm analyzed the mean medial thickness by measuring of the distance between the internal and external elastic laminae (70 measurements in each section). The medial elastic network was analyzed in terms of the relative area and mean thickness; the measurements and calculations were made for 12 fields in each section. The second algorithm analyzed the collagen matrix by measuring of the relative area/density in 20 contiguous fields in each Sirius red-stained section. Elastin and collagen densities were defined as the ratio of the area stained by orcein or Sirius red to the area of the studied field. The total elastin and collagen contents per millimeter of aortic section were calculated as the product of the media surface area per millimeter of longitudinal section (medial thickness [micrometers]×1000) and the elastin and collagen densities, respectively. The third algorithm counted the number of nuclei within 20 fields of about 7000 μm² area on each section and measured the mean area of each nucleus. A two-step procedure (conditional opening, then conditional closing) eliminated all particles under a predetermined size. The final result is the images of the nuclei without any “holes” or deformations due to the structuring element (hexagon). The image processor automat-

![Fig 1. Volume-time relation in the carotid artery. Graph illustrates the pressure-volume relation in the carotid artery, including the effect of fluid filtration across the arterial wall. V2S through V200 are the successive volumes, free of viscoelastic and filtration effects, induced by the pressure steps.](image)

![Fig 2. Typical examples of specific staining of the thoracic aorta for histomorphometric measurement on elastin (A), collagen (B), and smooth muscle cell nuclei (C).](image)
ically eliminates "borderline" nuclei before measuring the number of nuclei per unit area. As for the elastin and elastin contents, the number of nuclei per millimeter of longitudinal section was calculated from nuclei densities and medial surface area. Repeat measurements were performed, pooled, and averaged for the three algorithms in the corresponding stained sections of the aortic wall media of each rat. The morphological analyses were performed twice by two independent researchers using a single-blind protocol.

Statistical Analysis

Results are expressed as mean±1 SD.23 The experimental design allowed us to use a two-way ANOVA to show differences due to the strains and/or treatment and interaction. The differences between groups were evaluated using Scheffe's F test. Nested ANOVA was performed to compare the morphological results of the thoracic aorta, allowing for the within-group variability (field, section, rat).

Results

The noninvasively measured systolic pressures in the two groups of rats throughout the 12 weeks of treatment are shown in Fig 3. Blood pressure was significantly higher in SHRs than in WKY rats in both treated and untreated groups. Isradipine significantly decreased blood pressure in both strains throughout the treatment. The systolic pressure of treated SHRs was not significantly different from that of untreated WKY rats after the first week.

Table 1 reports the body weights and left ventricular weights at the end of the experiments. The SHRs were significantly lighter than the WKY rats. Isradipine did not affect body weights of either WKY rats or SHRs and did not significantly affect the left ventricular weight (P=.79) or the left ventricular-to-body weight ratio (P=.37) despite a significant drop in arterial pressure in both treated groups.

The diuresis in SHRs and WKY rats were similar, but CEB treatment significantly increased diuresis in both strains, more in WKY rats than in SHRs (Table 2). The diuretic effect of treatment was associated with a significant natriuretic effect, with significant reductions in kaliuresis and urinary osmolality.

The plasma renin activities in untreated WKY rats and SHRs were similar. Chronic CEB significantly increased plasma renin activity in both strains; this effect was significantly more marked in SHRs than in WKY rats (Table 3). In contrast, there was a significant strain effect on the urinary aldosterone; it was higher in WKY rats than in SHRs. Chronic CEB significantly increased urinary aldosterone in WKY rats but not in SHRs.

Hemodynamics

The open-chest hemodynamic values measured in the four experimental groups are shown in Table 4. The arterial blood pressures (systolic and diastolic) were significantly higher in SHRs (treated and untreated) than in WKY rats. CEB treatment significantly decreased systolic pressure in WKY rats (−20%) and SHRs (−28%) and diastolic pressure in WKY rats (−30%) and in SHRs (−37%). Cardiac output was slightly but not significantly lower in SHRs than in WKY rats. Isradipine significantly increased cardiac output in hypertensive rats (+42%) but not in WKY rats (+3%). The aortic peak flow velocity, an index of left ventricular contractility, was not significantly different in both strains but was significantly increased in CEB-treated rats (+21% in WKY rats; +45% in SHRs). Heart rate was similar in both strains and was not affected by isradipine. TPR was significantly greater in untreated SHRs than in untreated WKY rats; it was decreased by isradipine (−28% in WKY rats and −50% in SHRs).

Mechanical Properties of the Carotid Artery

Fig 4 shows the pressure-compliance curves of in situ isolated carotid, measured for pressures from 25 to 200 mm Hg, in the four experimental groups. Minimal carotid compliance values were obtained for the lower and higher pressures, corresponding to "flat" parts of the pressure-volume relations. Maximal values for carotid compliance were obtained in the operating pressure range of 75 to 125 mm Hg for WKY rats and 100 to 150 mm Hg for SHRs.

Under control conditions (untreated WKY rats), carotid compliance was 0.097±0.02 to 0.22±0.06 μL/

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**Table 1. Body Weight and Left Ventricular Weight of Isradipine-Treated and Untreated Groups of WKY Rats and SHRs**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>WKY Rats</th>
<th>SHRs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated</td>
<td>Treated</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>374±42</td>
<td>376±30</td>
</tr>
<tr>
<td>Left ventricular weight, mg</td>
<td>805±83</td>
<td>782±23</td>
</tr>
<tr>
<td>Left ventricular weight/body weight, mg/g</td>
<td>2.16±0.15</td>
<td>2.09±0.62</td>
</tr>
</tbody>
</table>

WKY indicates Wistar-Kyoto; SHRs, spontaneously hypertensive rats.
n=15 in each group; duration of treatment, 12 weeks; age at the end of treatment, 27 weeks.
mm Hg. The carotid compliance of CEB-treated WKY rats was significantly larger, from 0.036±0.01 to 0.252±0.09 μL/mm Hg. The carotid compliance of untreated SHRs was 0.08±0.02 to 0.15±0.04 μL/mm Hg; and for CEB-treated SHRs, it was 0.05±0.01 to 0.18±0.05 μL/mm Hg.

The carotid compliance measured after poisoning the smooth muscle cells with potassium cyanide was 0.03±0.01 to 0.23±0.05 μL/mm Hg in the untreated WKY rats. It was 0.036±0.01 to 0.168±0.05 μL/mm Hg in the CEB-treated normotensive rats and 0.06±0.01 to 0.18±0.04 μL/mm Hg in the untreated hypertensive rats. In the CEB-treated SHRs, it was 0.04±0.01 to 0.21±0.06 μL/mm Hg.

The carotid compliance values over the whole pressure range were smaller in SHRs than in WKY rats in treated and untreated groups (P<.01). ANOVA indicated a global effect of isradipine treatment on carotid compliance (P<.01). It significantly increased carotid compliance in both WKY rats and SHRs. Carotid compliance was also significantly larger in CEB-treated than in untreated normotensive and hypertensive rats after KCN poisoning (P<.01). This indicates that, under passive conditions when mechanical properties of the arterial wall are mainly due to its extracellular matrix, treated rats had more compliant arteries than did untreated rats.

**Histomorphometry**

The aortic and carotid media thicknesses were significantly greater in SHRs than in WKY rats (Table 5). Isradipine decreased the aortic media thickness in WKY rats (−14%) and in SHRs (−32%). Similarly, carotid media thickness was significantly decreased by isradipine in both strains (−12% in WKY rats and −17% in SHRs).

The aortic elastin contents of the strains were similar and were not significantly affected by chronic treatment with CEB. In contrast, the carotid elastic content in SHRs was significantly larger than in WKY rats. Isradipine significantly increased the carotid elastic content in both strains (+18% in WKY rats and +49% in SHRs).

The collagen content in the media of untreated SHRs was significantly greater than that of untreated WKY rats. Isradipine induced a significant reduction in collagen content in the aortic media in SHRs (−30%) but not in WKY rats (−6%). The global ANOVA indicated that the wall of the carotid artery from SHRs had a significantly greater collagen content than did that from

| TABLE 3. Metabolic Measurements Performed at the End (12th Week) of the Experiment in Isradipine-Treated and Untreated SHR and WKY Groups | ANOVA |
|-----------------------------|-----------------|--------|--------|
|                            | WKY Group       | SHR Group | Strain | Treatment | Interaction |
| Diuresis, μL/h              | 95±65           | 143±71   | NS     | P<.02     |
| Isradipine                  | 254±112         | 207±55   |        |           |
| Natriuresis, nEq/h          | 5.41±3.85       | 13.43±10.59 | P<.05 | P<.001   |
| Isradipine                  | 25.72±9.2       | 27.34±9.87 | NS     |           |
| Kaliuresis, nEq/h           | 18.97±4.83      | 16.90±5.40 | NS     | P<.001   |
| Isradipine                  | 10.81±3.49      | 8.99±2.46 |        |           |

See Table 1 for abbreviations.

n=15 in each group; age at the end of treatment, 27 weeks.

| TABLE 3. Plasma Renin Activity and Urinary Aldosterone Excretion at the Last Week of Experiment in Isradipine-Treated and Untreated Groups of WKY Rats | ANOVA |
|--------------------------------------------------------------------------------|--------|--------|--------|
|                             | WKY Rats       | SHRs    | Strain | Treatment | Interaction |
| Plasma renin activity, ng    | 6.4±2.0        | 5.4±2.2 | P<.05  | P<.001    | P<.05     |
| angiotensin l/mL per hour    |                 |         |        |           |           |
| Isradipine                  | 73.4±36.9      | 101.3±30.6 |       |           |           |
| Urinary aldosterone         | 646±331        | 393±170 | P<.001 | P<.001    | P<.01    |
| secretion, pg/h             | 1072±353       | 398±269 |       |           |           |

See Table 1 for abbreviations.

n=15 in each group; age at the end of treatment, 27 weeks.
TABLE 4. Hemodynamic Parameters Recorded After 12 Weeks of Treatment in Isradipine and Placebo WKY Rats

<table>
<thead>
<tr>
<th>Systolic pressure, mm Hg</th>
<th>WKY Rats</th>
<th>SHR</th>
<th>Strain</th>
<th>Treatment</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>129±11</td>
<td>221±19</td>
<td>P&lt;.001</td>
<td>P&lt;.001</td>
<td>NS</td>
</tr>
<tr>
<td>Isradipine</td>
<td>103±10</td>
<td>150±17</td>
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<tr>
<td>Diastolic pressure, mm Hg</td>
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<tr>
<td>Placebo</td>
<td>102±9</td>
<td>175±15</td>
<td>P&lt;.001</td>
<td>P&lt;.001</td>
<td>NS</td>
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<tr>
<td>Isradipine</td>
<td>72±8</td>
<td>109±14</td>
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<tr>
<td>Cardiac output, mL/min</td>
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<tr>
<td>Placebo</td>
<td>69±24</td>
<td>64±15</td>
<td>NS</td>
<td>P&lt;.05</td>
<td>P&lt;.05</td>
</tr>
<tr>
<td>Isradipine</td>
<td>71±32</td>
<td>91±26</td>
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<tr>
<td>Peak flow velocity, mL/min</td>
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<tr>
<td>Placebo</td>
<td>289±84</td>
<td>271±44</td>
<td>NS</td>
<td>P&lt;.01</td>
<td>NS</td>
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<tr>
<td>Isradipine</td>
<td>350±104</td>
<td>394±9</td>
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<tr>
<td>Heart rate, beats per minute</td>
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<tr>
<td>Placebo</td>
<td>345±32</td>
<td>336±28</td>
<td>NS</td>
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<tr>
<td>Isradipine</td>
<td>349±29</td>
<td>338±34</td>
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<tr>
<td>Total peripheral resistance, 10^5 dyne·s⁻¹·cm⁻²</td>
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<td>Placebo</td>
<td>149±52</td>
<td>256±52</td>
<td>P&lt;.01</td>
<td>P&lt;.01</td>
<td>NS</td>
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<tr>
<td>Isradipine</td>
<td>107±41</td>
<td>122±32</td>
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</table>

See Table 1 for abbreviations. n=15 in each group; age at end of treatment, 27 weeks.

WKY rats. Treatment with CEB significantly reduced the collagen content in the carotid arteries of both SHRs and WKY rats.

Morphometric measurements of nuclei in the aortic and carotid walls of untreated rats indicates more nuclei per millimeter length in SHRs than in WKY rats (+21%) in the aorta and (+18%) in the carotid artery, suggesting smooth muscle hyperplasia in untreated SHRs. Treatment reduced the number of nuclei per millimeter of aorta in WKY rats (−21%) and in SHRs (−17%). The number of nuclei in the carotid arterial wall was not significantly affected by treatment.

The mean cross-sectional area of nuclei in smooth muscle cells from the thoracic aorta was larger in untreated SHRs than in untreated WKY rats (+10%). In contrast, the mean nuclei cross-sectional areas in the carotid arterial walls of WKY rats and SHRs were similar. There was no significant difference in the cross-sectional area of nuclei in the aortic and carotid walls of isradipine-treated WKY rats and untreated WKY rats. In contrast, the cross-sectional area of nuclei in the aortic and carotid walls was smaller in treated (−26%) than in untreated SHRs (−8%).

**Discussion**

Various animal models have been used to study the pathogenesis of hypertension, including SHRs, which are compared with the normotensive WKY strain. Hypertrophy of the left ventricle, which has in SHRs both genetic and mechanical components associated with the development of hypertension, appears to be a compensatory mechanism and opposes increased vascular resistance. However, gradually, heart failure ensues.

Animal experiments suggest that the pharmacological properties of arterial and arteriolar vessels differ along the arterial tree. As arteries become smaller, important differences develop; these include involvement of nonadrenergic neurotransmitters, increased dependency on external calcium, and increased range of trophic influence.
en. Such pharmacological abnormalities suggest that antihypertensive drugs and especially calcium entry blockers might have different consequences for small and large arteries.

**Main Findings of the Present Study**

First, untreated SHRs had greater peripheral resistance, stiffer and thicker arterial walls because of smooth muscle cell hyperplasia (thoracic aorta and carotid artery) and/or hypertrophy (thoracic aorta) and more increased collagen content than did 27-week-old normotensive control WKY rats. Arterial hypertension led to significant left ventricular hypertrophy in SHRs. Second, chronic treatment for 3 months with CEB (isradipine, 3 mg/kg) normalized the arterial pressure in SHRs after the first week of treatment. The CEB-induced normalization of arterial pressure was accompanied by a significant decrease in peripheral resistance, increased cardiac output, and left ventricular contractility (assessed by the ascending aortic peak blood flow velocity), without significant reduction in left ventricular hypertrophy. Increases in diuresis and natriuresis were associated during the last week of treatment in both treated strains, with marked increases in plasma renin activity. In contrast, urinary aldosterone was increased by treatment in WKY rats but not in SHRs. Furthermore, we observed a decrease in the K⁺ urinary excretion in rats receiving CEBs. Third, the large arteries were affected by chronic CEB treatment. Carotid compliance was markedly increased under control and passive conditions. Morphological analyses of the carotid artery and descending thoracic aorta suggest that treatment with CEB induces a significant reduction in the medial hypertrophy of the aortic walls of SHRs and WKY rats (as evidenced by the decrease in medial thickness and size of the nuclei) associated with a reduction in medial hyperplasia (suggested by the decrease in the number of nuclei per millimeter of aorta). Isradipine reduced the medial thickness in the carotid artery, with a reduction in smooth muscle cell hypertrophy and no significant change in the number of smooth muscle cell nuclei. Last, isradipine significantly reduced the arterial wall collagen contents in both strains, with marked increases in the elastin content in the carotid but not in the aortic wall.

**Hemodynamic and Hormonal Effects of Isradipine**

Studies on in vitro isolated vessel preparations have provided considerable evidence of CEB-induced vascular relaxation (see review in Struyker Boudier et al). It is clear that contracted large (conductance) arteries, small (resistance) arteries, and veins can be dilated by CEBs, although there may be quantitative differences in the degree of dilation. Effects on vascular tone should ultimately be reflected, in vivo, in hemodynamic changes in vascular resistance. In the present study and in published reports, CEBs cause a fall in mean arterial pressure and total vascular resistance. The effects of CEBs on cardiac hemodynamic variables are less consistent. Acute administration of dihydropyridines generally causes a rise in heart rate and cardiac output caused by an activation of the sinoaortic baroreflex. However, the reflex effects on cardiac output and heart rate disappear to a large extent under chronic administration of dihydropyridines. This is generally attributed to a gradual diminution of baroreflex influences subsequent to adaptation of the sinoaortic baroreceptors to the chronically prevailing arterial blood pressure. In the

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**Table 5. Histomorphometric Measurements of the Thoracic Aorta and Carotid From Isradipine- and Placebo-Treated WKY Rats and SHRs**

<table>
<thead>
<tr>
<th></th>
<th>Thoracic Aorta</th>
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<th></th>
<th>Carotid</th>
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</thead>
</table>
|                      | WKY Rats | SHRs | Strain | Treatment | Inter- | WKY Rats | SHRs | Strain | Treatment | Inter-
| Media thickness, μm |         |     |       | P<.001 | P<.001 | NS |       |       | NS | P<.01 | P<.01 | NS
| Placebo              | 119±9    | 159±11 |       |         |         | 68±6 | 83±9 |       |       | P<.01 |       | NS |
| Isradipine           | 102±13   | 109±11 |       |         |         | 60±7  | 69±8 |       |       |       |       |     |
| Elastin content, μm²/mm of section |         |       |       |         |         |       |       |       |       |       |       |     |
| Placebo              | 2573±5074 | 29257±4975 | NS | NS | NS | 13944±2360 | 15273±3017 | P<.01 | P<.01 | NS |
| Isradipine           | 31673±5951 | 29678±4587 | NS | NS | NS | 16947±3858 | 22733±7277 |       |       |     |
| Collagen content, μm²/mm of section |         |       |       |         |         |       |       |       |       |       |       |     |
| Placebo              | 18260±3168 | 26972±5463 | P<.05 | P<.001 | P<.01 | 8449±2468 | 10376±2812 | P<.01 | P<.01 | NS |
| Isradipine           | 17083±4098 | 18957±3833 |       |         |         | 7407±1064 | 9270±3004 |       |       |     |
| Nucleus content, nuclei/mm of section |         |       |       |         |         |       |       |       |       |       |       |     |
| Placebo              | 534±80    | 645±107 | P<.01 | P<.01 | NS | 168±21 | 199±36 |       |       |     |
| Isradipine           | 422±63    | 536±60  |       |         |         | 193±31 | 181±22 |       |       |     |
| Nucleus size, μm² |         |       |       |         |         |       |       |       |       |       |       |     |
| Placebo              | 7.88±0.87 | 8.65±0.86 | NS | P<.05 | P<.01 | 8.8±1.2 | 8.8±0.9 | NS | P<.05 | P<.01 |
| Isradipine           | 7.58±0.88 | 7.11±0.55 |       |         |         | 8.4±0.9 | 8.1±0.7 |       |       |     |

See Table 1 for abbreviations.

n=15 in each group; duration of treatment, 12 weeks; age at the end of treatment, 27 weeks.
present experiments, CEB induced significant increases in cardiac output in hypertensive rats and increases in peak flow velocity with no change in heart rate in both strains. Under our experimental conditions, anesthesia with pentobarbital has a well-known negative inotropic effect and probably affects the peak flow velocity. However, this methodological issue is probably overcome by the use of control groups. Effects of CEBs on the cardiac function are consistent with those reported by Cody26 with verapamil, by Majid and De Jong27 with nifedipine, and by Fagard et al.,28 Lambert et al.,29 and Silke et al.30 using different types of dihydropyridines in patients with essential hypertension. Both a reduced afterload and a reflex increase in sympathetic activity may be involved in the increase in cardiac output and parameters for cardiac contractility. Acute systemic hemodynamic studies in experimental animals confirm these clinical observations. The dihydropyridines almost invariably increase cardiac output and heart rate (see review by Struyker Boudier et al.). Very few studies have been designed thus far to analyze the long-term hemodynamic effects of CEBs in animal models for hypertension. A 12-week treatment of SHRs with felodipine lowered blood pressure and peripheral resistance.31 Heart rate was not changed after this period treatment, but cardiac output was significantly increased. A 3-week treatment with nifedipine also lowered blood pressure and vascular resistance without significant changes in cardiac variables.32 Another study in which SHRs were treated with diltiazem for 3 weeks gave inconsistent results: Although blood pressure was slightly reduced, no other hemodynamic variable changed significantly.33

As for the vascular smooth muscle, cardiac muscle excitation-contraction involves the activation of a calcium inward current. The various groups of CEBs bind differently to the cell at or near the calcium channels. This probably is the basis for differing abilities of CEBs to influence the myocardium. Ljung33 reported that the rat vascular and myocardial muscles were equally sensitive to verapamil. Nifedipine and felodipine were 14 and 118 times more active, respectively, on vascular than on myocardial preparations. Chiu35 also reported that dihydropyridines are much less active on the myocardium than on the vascular smooth muscle. In the present study, the 12-week treatment of SHRs lowered blood pressure and total peripheral resistance and increased cardiac output without altering the heart rate. These in vivo results can be explained by the differential sensitivity of heart and vessels to isradipine. Reflex compensatory mechanisms via the sinoaortic baroreceptors also could compensate for or even exceed any potential direct negative inotropic effect. In addition, the reduction in afterload may also contribute to a positive inotropic effect.36 These mechanisms could explain the significant increase in aortic peak flow velocity recorded in CEB-treated WKY rats and SHRs in the present experiment. The parallel decrease in arterial pressure and increase in myocardial contractility could account for the nonsignificant reduction in left ventricular weight after 8 weeks of treatment with nifedipine despite normalization of arterial pressure.

Plasma renin activity usually remains unchanged during the chronic treatment of hypertensive patients with CEBs. This has been observed for several CEBs, including nifedipine, nicardipine, nitrindipine, diltiazem, and verapamil.3 However, Luft et al.38 reported that plasma renin activity remained elevated during the treatment of hypertensive patients with nitrendipine for 1 to 2 weeks. In the present study, we measured significant increases in plasma renin activity in both isradipine-treated WKY rats and SHRs for the last (12th) week of treatment. The elevation of renin secretion could be due to the association of three factors: persistence of marked hypotensive effect of isradipine, the activation of the sympathetic nervous system, and the increase in natriuresis. Therefore, persistent elevation of renin secretion could be one of the reactions of renovascular homeostasis to the hemodynamic changes induced by CEB. This counterregulation gives argument for the contribution of the renin-angiotensin system to the absence of the expected heart remodeling in response to large blood pressure reduction.

Interestingly, the present work shows in SHRs a divergent response of renin system components produced by chronic CEB treatment, ie, markedly elevated plasma renin activity contrasting with normal levels of aldosterone associated with a decrease in urinary K* excretion. Similar results have been reported previously in hypertensive patients treated with nifedipine.39 There is some evidence from studies by Kojima et al.39 on isolated adrenal glomerulosa cells that CEBs inhibit angiotensin II--induced aldosterone secretion. Angiotensin II regulates the secretion of aldosterone from adrenal glomerulosa cells by a calcium-dependent mechanism that involves both a CEB-sensitive uptake of calcium from the extracellular pool and the release of calcium from an intracellular pool.

Mechanical Properties of the Large Arteries in Hypertension and the Role of CEBs

Vascular hypertrophy in hypertension is widely considered to be an adaptation to increased arterial wall stress. The medial thickening, involving smooth muscle cell and extracellular matrix components, could have deleterious effects on the damping function of the arterial network and could contribute to arteriosclerosis. Increased arterial stiffness also participates in the increase in left ventricular afterload in hypertension. An experimental model has been developed in our laboratory for precise measurement of pressure-volume relations in the in situ isolated rat carotid artery.3.18.19 The carotid artery compliance was significantly lower in SHRs than in WKY rats for pressures from 50 to 200 mm Hg, indicating that there had been an intrinsic, pressure-independent change in the arterial wall mechanical properties in hypertensive rats. These may be structural (alterations in smooth muscle mass and in extracellular matrix) or due to altered arterial smooth muscle tone. Abolition of vascular smooth muscle tone by potassium cyanide poisoning resulted in a significant increase in compliance in both WKY rats and SHRs. The compliance of the totally relaxed carotid arteries was still smaller in SHRs than in WKY rats, suggesting that structural changes and not only vasomotor tone are
important in reducing the compliance observed in hypertensive animals. Several studies in humans have shown that the damping function of the arteries is altered in essential as well as in secondary hypertension and that age-related changes in the arterial wall are accelerated.\textsuperscript{40,42} These studies all suggest that arterial compliance is reduced in hypertension not only because of high blood pressure but also as the result of intrinsic alterations in the viscoelastic properties in the arterial walls.

**Response of the Large Arteries to CEBs**

There are few experimental reports in which the response of large arteries to CEB has been evaluated. The response usually evaluated is the aortic pressure-diameter relation during acute administration of drugs. Yano et al\textsuperscript{43} studied the effects of acute diltiazem treatment on the aortic pressure-diameter relation in anesthetized dogs. They reported that the aortic diameter and pressure were reduced by diltiazem and that the aortic pressure-diameter curve was shifted toward higher diameters for any given pressure. The significant increase in aortic wall distensibility was attributed to a decrease in aortic smooth muscle tone. Using our in situ model of isolated carotid arteries in normotensive and spontaneously hypertensive rats, we observed that calcium blockade by clentiazem induced a significant shift of the pressure-volume curve in both strains, so that volume was significantly higher after calcium blockade than under control conditions.\textsuperscript{11}

The fall in arterial pressure produced by antihypertensive drugs may be associated with active changes in the geometry of large arteries; this has been demonstrated in healthy volunteers as well as in hypertensive patients.\textsuperscript{9,44} In a double-blind, placebo-controlled study in healthy volunteers, acute administration of increasing doses of nicardipine increased the diameters of the carotid and brachial arteries in a dose-dependent manner. Systemic blood pressure did not significantly change, suggesting that the CEB acted on the arterial wall independently of transmural pressure-induced mechanical factors. When healthy volunteers were given verapamil, arterial diameter did not increase despite significant decrease in blood pressure, again suggesting a direct action on the arterial wall.\textsuperscript{45} Similar observations have been made on the brachial artery in essential hypertension with diltiazem.\textsuperscript{4} For an equipotent antihypertensive effect, dipyridamole reduced the diameter of the brachial artery, whereas diltiazem increased in the brachial arterial diameter. Acute oral administration of dipyridamole derivatives such as nifedipine, nicardipine, and nitrendipine caused a significant increase in arterial diameter in the brachial artery in patients with sustained essential hypertension.\textsuperscript{43} The increase in the brachial artery diameter persisted for several weeks when nicardipine was given orally.\textsuperscript{44} Because such findings were obtained in the presence of a significant reduction of blood pressure, it appears that the passive mechanical effect of blood pressure (decrease in diameter) was offset by the direct arterial dilation caused by the CEB.

**Histomorphological Properties of the Large Arterial Wall and Effect of CEBs**

The aortic and carotid wall thickness of untreated SHRs is significantly greater (+33\% and +22\%) than that of untreated WKY rats. These results are in agreement with preceding studies showing an increase in medial thickness in several models of hypertension.\textsuperscript{2,45} The number and size of smooth muscle nuclei, both greater in untreated SHRs than in WKY rats, suggest that the medial thickening of the thoracic aorta in 27-week-old SHRs is due to smooth muscle hyperplasia and hypertrophy. In contrast, the identical size of smooth muscle cell nuclei from the carotid wall suggests that smooth muscle cell hyperplasia is the predominant cause of medial thickening in this conductance artery.

The increase in nuclear cross-sectional area in the aortic wall of SHRs agrees with results reporting an increase in the DNA content and polyploidy in hypertension.\textsuperscript{46} This increase in the nucleus size could be related to protein synthesis by smooth muscle cells. It is well established that the arterial wall collagen content is increased in hypertension.\textsuperscript{2,45} Using the same morphometric methods as in the present study, we reported a similar increase in collagen content in hypertensive rats.\textsuperscript{3,18,47} As in our previous studies, the aortic wall elastin contents of untreated WKY rats and SHRs were similar. However, the elastin content of the carotid walls of untreated SHRs was significantly greater than in WKY rats.

The present experiment is the first, to our knowledge, to simultaneously study the effects of chronic treatment with a CEB on the functional (mechanical properties) and structural (histomorphological) properties of the arterial wall. Normalization of arterial pressure by CEBs induced significant reductions in medial thickness in both strains. This reduction in medial hypertrophy occurred simultaneously with large increases in plasma renin activity. Some experimental studies\textsuperscript{48-49} suggest that angiotensin II is a specific factor effecting smooth muscle hypertrophy. Our present results show that the marked decrease in arterial pressure and therefore the normalization in wall stress in the large arteries induced a normalization of medial thickness despite significant increases in plasma renin activity and probably in angiotensin II. Moreover, the arterial wall collagen contents were significantly lower in treated WKY rats and SHRs than in their untreated control groups. It has been demonstrated that angiotensin II stimulates collagen synthesis in vitro.\textsuperscript{50} Our in vivo results suggest that in the present experimental model, the distending pressure is a predominant factor influencing the collagen synthesis/degradation balance in the aortic and carotid wall.

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Effect of chronic dihydropyridine (isradipine) on the large arterial walls of spontaneously hypertensive rats.

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