Effects of Arterial Vasodilators on Cardiac Hypertrophy and Sympathetic Activity in Rats

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SUMMARY In spontaneously hypertensive rats (SHR), the progression (or absence of regression) of cardiac hypertrophy despite adequate blood pressure (BP) control by arterial vasodilators has been attributed to increased cardiac sympathetic activity. We evaluated changes in indices of general and cardiac sympathetic tone in relation to changes in cardiac anatomy during treatment of normotensive rats and SHR with hydralazine, 120 mg/L, or minoxidil, 120 mg/L of drinking water. In SHR, both vasodilators reduced BP rapidly and consistently. Significant increases in heart rate and plasma norepinephrine were observed only in the initial 2 days of arterial vasodilator treatment. After 5 weeks of treatment, marked increases in left and right ventricular sympathetic activity (as assessed by norepinephrine turnover rates) were present, but no increase was seen in heart rate and plasma norepinephrine. Intravascular volume expansion was observed on Day 14 of minoxidil and Day 35 of hydralazine treatment. Prolonged treatment with minoxidil induced significant increases in left ventricular internal diameter, as well as in left and right ventricular weights, but not in the wall thickness of the left ventricle. Treatment with hydralazine did not affect left ventricular weight and caused a small increase in the weight of the right ventricle. In normotensive rats, both vasodilators initially decreased BP, but tolerance developed within 1 to 2 weeks of treatment. Plasma norepinephrine and heart rate showed increases only at Day 1 of either treatment, whereas cardiac sympathetic hyperactivity persisted at 2 and 5 weeks of treatment. Changes in cardiac anatomy were qualitatively similar to those observed in SHR. We conclude that, during treatment of normotensive rats and SHR with arterial vasodilators, cardiac sympathetic hyperactivity persists and may be involved in the cardiac effects of arterial vasodilators. However, other mechanisms, such as chronic cardiac volume overload, may also play an important role, particularly with minoxidil. (Hypertension 11: 376-386, 1988)

KEY WORDS • hydralazine • minoxidil • cardiac hypertrophy • cardiac sympathetic activity • blood volume

IN spontaneously hypertensive rats (SHR), but also in other hypertensive models (e.g., two-kidney, one clip [2K1C]) as well as in normotensive rats, long-term treatment with arterial vasodilators such as hydralazine and minoxidil is associated with progression (or absence of regression) of cardiac hypertrophy.1-4 Chronic sympathetic hyperactivity has been implicated in this effect on the heart.7-8 It has been assumed that cardiac sympathetic hyperactivity during long-term treatment of SHR with arterial vasodilators persists based on 1) the measurement of myocardial catecholamine content showing a 20% increase in myocardial catecholamines in SHR treated with hydralazine for 6 weeks8 and 2) pharmacological intervention studies showing that the association of the sympatholytic agent methyldopa with minoxidil reversed cardiac hypertrophy in SHR3-5 and prevented the minoxidil-induced cardiac hypertrophy in normotensive rats.3 However, this represents rather indirect and circumstantial evidence for sympathetic hyperactivity.

Whereas an acute reflex-mediated increase in sympathetic activity following the administration of an arterial vasodilator has been well documented,6-11 one might expect resetting of the baroreceptors12 during long-term treatment and therefore a return of sympathetic tone toward baseline. However, except for heart rate, no data in SHR are available in this regard. In SHR the (presumably baroreceptor reflex-mediated)
increases in heart rate and cardiac index disappeared within 2 hours after a single administration of hydralazine despite a persistent antihypertensive effect. Following long-term treatment of SHR with hydralazine for 3 to 24 weeks, increases in heart rate (by 5–10%) were reported in some studies but not in other studies. Similar discrepancies are evident in normotensive rats. Kohlmann et al. reported that, after 2 weeks of hydralazine treatment, a small blood pressure (BP)–lowering effect persisted and plasma catecholamines, heart rate, and the turnover rate of norepinephrine in the heart were all increased. However, other studies failed to find an increase in heart rate despite a small reduction in BP following long-term hydralazine administration. The reason for these inconsistent findings is not obvious. However, the validity of heart rate as an index of cardiac or general sympathetic activity is limited. To the best of our knowledge, no studies have assessed cardiac or even general sympathetic activity more directly during long-term treatment of SHR with either hydralazine or minoxidil. To provide a clearer insight into the long-term effects of arterial vasodilators in normotensive rats and SHR on cardiac sympathetic activity and cardiac hypertrophy, we evaluated the time course of changes in general sympathetic activity (plasma catecholamines and BP response to hexamethonium) versus cardiac sympathetic activity (heart rate and left [LV] and right ventricular [RV] norepinephrine turnover rate) in relationship to changes in intravascular volume and cardiac anatomy during long-term treatment of normotensive rats and SHR with the arterial vasodilators hydralazine and minoxidil.

**Materials and Methods**

Male Wistar-Kyoto rats (WKY) and SHR were obtained from Taconic Farms (Germantown, NY, USA) at 16 weeks of age. Male Wistar rats weighing 250 to 260 g were obtained from Charles River Breeding Laboratories, Montreal, Canada. Rats were housed two to a cage and given food (Purina rat chow, St. Louis, MO, USA; 180 µmol Na/g food) and water ad libitum and kept on a 12-hour light/dark cycle. Following a 5-day acclimatization period, WKY and SHR were randomized into four groups (n = 8–10/group): untreated normotensive (WKY), untreated hypertensive, hypertensive treated with hydralazine, 120 mg/L, and hypertensive treated with minoxidil, 120 mg/L of drinking water. In separate experiments, normotensive Wistar rats were randomized into three groups (n = 10–12/group): untreated, hydralazine-treated, or minoxidil-treated. Fluid intake and body weight were monitored regularly, and the animals were handled twice weekly.

Different groups of rats were treated for either 2, 14, or 35 days. Two days before the end of the 14- and 35-day treatments, a PE-50 (Clay Adams, Parsippany, NJ, USA) catheter was inserted into a carotid artery for monitoring of BP and heart rate for the next 2 days. In the 2-day experiment, a carotid artery was cannulated 48 hours before the beginning of drug treatment and measurements were done on Days 0, 1, and 2 of treatment. Hemodynamic studies were performed in the morning under the same environmental conditions in a quiet study room. BP and heart rate were recorded in conscious, unrestrained animals after 30 minutes of rest. On the last day of each treatment period, after recording resting BP, blood samples were taken in all groups from the carotid catheter for determination of plasma catecholamines in duplicate by radioenzymatic assay and in SHR alone for plasma renin activity (PRA) by an antibody-trapping technique. For this, whole arterial blood (400 µL) was allowed to flow directly into chilled microcentrifuge tubes containing 0.26 M EGTA and 0.2 M glutathione and an additional 400 µL was collected into 0.0026 M EDTA (for PRA). Subsequently, plasma and blood volume were determined by the radioiodinated human serum albumin–iodine-131 technique requiring 300 µL of blood, as described previously. Following the collection of blood samples, the arterial catheters were reconnected to the pressure transducer and BP was monitored while the animals recovered for 10 minutes. Then, hexamethonium (30 mg/kg) was injected through the carotid catheter and maximal decreases in BP were noted.

At the end of an experiment, the animals were anesthetized with chloroform. The hearts were excised and immediately placed in ice-cold saline to arrest the heart in diastole and to remove blood. LV and RV weights were determined as described by Fenje and Leenen. After the weighing procedure, a transverse mid-level slice of LV was obtained by two transverse cuts at one third and two thirds of the length. This slice was viewed under a light microscope using a calibrated ocular lens (Macrometer, Olympus, Tokyo, Japan). The LV wall thickness was measured at 8 to 10 points around the circular section, and the average was calculated. The internal diameters of the slices were measured from the farthest points of the major (anterior–posterior) and minor (septal–lateral) internal diameters. In the 5-week experiments, dry LV and RV weights were determined after drying the ventricles for 24 hours in an oven at 37°C.

Norepinephrine turnover rates were estimated after 1, 14, or 35 days of treatment in normotensive Wistar rats and after 35 days of treatment in SHR. Turnover rate was determined by the decline of endogenous norepinephrine in the left and right ventricles after its synthesis had been inhibited by metyrosine (α-methyl-DL-p-tyrosine methyl ester hydrochloride). Metyrosine was dissolved in distilled water and administered subcutaneously at 200 mg/kg body weight, with a second dose of 100 mg/kg body weight given 4.5 hours later. For the experiments in normotensive rats, six rats per group (untreated; hydralazine-treated, 120 mg/L; and minoxidil-treated, 120 mg/L) per treatment period (1, 14, and 35 days) were decapitated 0, 4.5, and 9 hours after the first dose. Rats were allocated in groups of nine (one for each of the three treatments at the three time points), providing six disappearance curves per treatment group for statistical analysis. In the SHR experiment, six rats per group (untreated WKY, un-
treated SHR, and SHR treated for 5 weeks with either hydralazine, 120 mg/L, or minoxidil, 120 mg/L) were decapitated 0, 4.5, and 9 hours after the first dose. Rats were allocated in groups of 12 (one for each of the four groups at the three time points), providing six disappearance curves per group for statistical analysis. The hearts were quickly removed, rinsed in cold saline, blotted dry, and dissected free of atria and great vessels. Left and right ventricles were separated, frozen in dry ice, stored at −70°C, and processed for assay within 24 hours. The frozen ventricles were weighed, and 100 mg each of RV and LV tissue (cut from the apex) was homogenized in 2 ml of iced 0.2 N acetic acid with a Polytron homogenizer (Brinkmann Instruments, Westbury, NY, USA). The homogenate was centrifuged at 5000 g for 20 minutes at 4°C. The extraction of norepinephrine from the supernates and subsequent analysis by high performance liquid chromatography with electrical chemical detection were performed as described by Shum et al.24 and Sole et al.25 The turnover rate constant ($k$) was calculated from the rate of decline of the logarithm of the tissue norepinephrine concentration (regression coefficient). Half-life and turnover rate were calculated from the following equations: half-life = $0.693/k$ and turnover rate = [norepinephrine] $\cdot k$, where [norepinephrine] is the concentration of norepinephrine in each tissue before inhibition of synthesis.

Results are presented as means ± SEM. Statistically significant differences between groups at a given treatment period were analyzed by analyses of variance. The least significant difference approach was used to locate significant differences; a $p$ level below 0.05 was considered significant.

Results

Blood Pressure

Treatment of normotensive rats induced a clear drop in BP during the initial days of treatment (Table 1). However, tolerance developed within 1 to 2 weeks of treatment, and a small decrease in BP persisted only for hydralazine.

Treatment of SHR with either hydralazine or minoxidil resulted in a significant fall in mean arterial pressure (MAP = diastolic BP + 1/3 pulse pressure) from 170-180 (pretreatment level) to 110-120 mm Hg in the initial days of treatment. Prolonged treatment (14 or 35 days) kept MAP at or slightly above the level seen in untreated WKY.

General Sympathetic Activity

Plasma Catecholamines

Both vasodilators significantly increased plasma norepinephrine in normotensive rats on Day 1 of treatment (Figure 1). However, after 2, 14, and 35 days of either treatment, norepinephrine levels were similar to control values. Plasma norepinephrine concentration of untreated SHR was significantly higher than that in WKY. Treatment of SHR for 1 or 2 days resulted in a significant increase in plasma norepinephrine (more marked in the hydralazine group) compared with untreated SHR. After 14 and 35 days of treatment, however, norepinephrine levels were not significantly different between treated SHR and untreated SHR.

WKY and untreated SHR showed similar values for plasma epinephrine of around 100 pg/mL. Plasma epinephrine concentration was not affected by treatment in SHR and normotensive rats (data not shown).

BP Response to Hexamethonium

In normotensive rats, both vasodilators significantly potentiated the BP-lowering response to hexamethonium after 1 day of treatment but not after long term treatment (Figure 2). Ganglionic blockade caused significantly larger decreases in BP of untreated SHR as compared with untreated WKY. Treatment of SHR with hydralazine or minoxidil significantly reduced the BP response to hexamethonium. Differences in percent changes were less marked, and the inhibitory effect of treatment on the response to hexamethionum

<table>
<thead>
<tr>
<th>Variable</th>
<th>Untreated Wistar rats</th>
<th>Untreated WKY</th>
<th>Untreated Hydralazine</th>
<th>Untreated Minoxidil</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg) Day 0</td>
<td>106 ± 4</td>
<td>113 ± 4</td>
<td>111 ± 4</td>
<td>109 ± 6</td>
<td>175 ± 6</td>
</tr>
<tr>
<td>Day 1</td>
<td>102 ± 5</td>
<td>86 ± 3*</td>
<td>85 ± 2*</td>
<td>110 ± 5</td>
<td>172 ± 6</td>
</tr>
<tr>
<td>Day 2</td>
<td>113 ± 3</td>
<td>89 ± 3*</td>
<td>98 ± 5*</td>
<td>106 ± 6</td>
<td>167 ± 4</td>
</tr>
<tr>
<td>Day 14</td>
<td>109 ± 4</td>
<td>104 ± 5</td>
<td>109 ± 5</td>
<td>98 ± 4</td>
<td>169 ± 5</td>
</tr>
<tr>
<td>Day 35</td>
<td>120 ± 5</td>
<td>106 ± 4†</td>
<td>119 ± 9</td>
<td>112 ± 4</td>
<td>180 ± 5</td>
</tr>
<tr>
<td>Heart rate (beats/min) Day 0</td>
<td>404 ± 14</td>
<td>413 ± 11</td>
<td>424 ± 11</td>
<td>398 ± 17</td>
<td>414 ± 13</td>
</tr>
<tr>
<td>Day 1</td>
<td>390 ± 18</td>
<td>471 ± 9*</td>
<td>470 ± 13*</td>
<td>398 ± 19</td>
<td>415 ± 12</td>
</tr>
<tr>
<td>Day 2</td>
<td>428 ± 23</td>
<td>419 ± 16</td>
<td>444 ± 14</td>
<td>365 ± 11</td>
<td>384 ± 17</td>
</tr>
<tr>
<td>Day 14</td>
<td>390 ± 11</td>
<td>378 ± 12</td>
<td>387 ± 10</td>
<td>337 ± 14</td>
<td>349 ± 13</td>
</tr>
<tr>
<td>Day 35</td>
<td>429 ± 14</td>
<td>398 ± 16</td>
<td>411 ± 15</td>
<td>343 ± 13</td>
<td>385 ± 19</td>
</tr>
</tbody>
</table>

Values represent means ± SEM ($n = 8-10$ group for WKY and SHR; $n = 10-12$ group for normotensive Wistar rats). *$p < 0.01$, †$p < 0.05$ compared with untreated SHR or untreated normotensive Wistar rats. ‡$p < 0.05$, compared with minoxidil.
ARTERIAL VASODILATORS AND CARDIAC HYPERTROPHY/Tsoporis and Leenen

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Figure 1. Plasma norepinephrine in untreated normotensive Wistar rats (■), WKY (▲), and SHR (■), as well as normotensive Wistar rats treated with either hydralazine, 120 mg/L (■), or minoxidil, 120 mg/L (■), and SHR treated with hydralazine, 120 mg/L (■), or minoxidil, 120 mg/L (■). Bars represent means ± SEM (n = 10–12/group, top panel; n = 8–10/group, bottom panel). Single (p < 0.05) and double asterisks (p < 0.01) indicate significant difference compared with untreated rats. Double dagger indicates significant difference (p < 0.01) compared with hydralazine.

was only consistently significant for minoxidil (data not shown).

Cardiac Sympathetic Activity

Heart Rate

Both vasodilators significantly increased heart rate in normotensive rats on Day 1 of treatment, but this increase disappeared after 2 days and heart rate subsequently remained at control levels (see Table 1). Heart rate in untreated SHR tended to be higher than that in untreated WKY (p = NS). In the initial days of treatment, hydralazine and minoxidil induced similar increases in heart rate. Subsequently, heart rate returned to the level seen in untreated SHR and remained at or below control levels with prolonged treatment.

Norepinephrine Turnover Rate

Correlation coefficients (range, 0.92–0.99) for the linear regressions of norepinephrine versus time were all significant (p < 0.001). Both vasodilators significantly increased the three parameters of LV sympathetic activity in normotensive rats after 1, 14, and 35 days of treatment. In the right ventricle, minoxidil and hydralazine increased fractional turnover rate on Day 1 and absolute turnover rate and half-life of norepinephrine on Days 1 and 35 of treatment (Figure 3, Table 2).

A significant increase in the fractional turnover rate (k) of endogenous norepinephrine, absolute turnover rate, and decrease in half-life were observed in the left but not in the right ventricle of untreated SHR as compared with untreated WKY. Treatment of SHR with either hydralazine or minoxidil for 5 weeks significantly increased cardiac sympathetic activity in both ventricles in a similar manner, as assessed by fractional turnover rate, absolute turnover rate, and half-life of norepinephrine (Figure 4; see Table 2).

Plasma and Blood Volumes

In normotensive rats, both vasodilators significantly increased plasma volume after 2 and 5 weeks of treatment and blood volume after 5 weeks (Table 3). Untreated WKY and SHR showed small, nonsignificant differences in plasma and blood volume (see Table 3). After 14 days of treatment, an increase in plasma volume was observed only with minoxidil. Both vasodilators increased plasma volume after 35 days of treatment, but this increase was more pronounced in the minoxidil group. A significant increase in blood volume was seen only after 35 days of treatment with minoxidil.

PRA

Untreated WKY and SHR showed small, nonsignificant differences in PRA (Table 4). Both hydralazine and minoxidil caused moderate (50–100%) increases in PRA during the initial 2 days of treatment. However, during long-term treatment for either 2 or 5 weeks, PRA in treated and untreated SHR was very similar.

Cardiac Anatomy

Right and Left Ventricleal Weights

Minoxidil increased LV and RV weights in normotensive rats after 2 weeks of treatment, and this re-
**FIGURE 3.** Norepinephrine (NE) turnover rates in the left and right ventricles of normotensive Wistar rats, untreated (●) or treated with either hydralazine, 120 mg/L (△), or minoxidil, 120 mg/L (○), for 1, 14, or 35 days. K = slope = fractional turnover rate of norepinephrine. Points represent means ± SEM (n = 6/group). Single (p<0.05) and double asterisks (p<0.01) indicate significant difference compared with untreated Wistar rats.

**TABLE 2.** Half-life and Turnover Rate of Norepinephrine in the Left and Right Ventricles of Untreated Normotensive Wistar Rats, WKY, and SHR, as Well as Normotensive Wistar Rats and SHR Treated with Hydralazine or Minoxidil

<table>
<thead>
<tr>
<th>Variable</th>
<th>Left ventricle</th>
<th>Right ventricle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Half-life (hr)</td>
<td>Turnover rate (ng/g/hr)</td>
</tr>
<tr>
<td>Normotensive Wistar rats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10.6 ± 1.5</td>
<td>35 ± 8</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>8.3 ± 0.6*</td>
<td>49 ± 9</td>
</tr>
<tr>
<td>Minoxidil</td>
<td>5.7 ± 0.6*</td>
<td>66 ± 8*</td>
</tr>
<tr>
<td>Day 14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9.5 ± 0.7</td>
<td>47 ± 7</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>7.0 ± 0.7*</td>
<td>59 ± 6</td>
</tr>
<tr>
<td>Minoxidil</td>
<td>7.3 ± 0.5*</td>
<td>48 ± 3</td>
</tr>
<tr>
<td>Day 35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9.4 ± 0.5</td>
<td>44 ± 3</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>6.2 ± 0.4*</td>
<td>66 ± 11*</td>
</tr>
<tr>
<td>Minoxidil</td>
<td>6.6 ± 0.6*</td>
<td>59 ± 8*</td>
</tr>
<tr>
<td>Untreated WKY, Day 35</td>
<td>7.9 ± 0.8</td>
<td>60 ± 8</td>
</tr>
<tr>
<td>SHR, Day 35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>5.0 ± 0.2</td>
<td>84 ± 6</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>4.2 ± 0.2</td>
<td>104 ± 5*</td>
</tr>
<tr>
<td>Minoxidil</td>
<td>4.0 ± 0.4*</td>
<td>108 ± 13*</td>
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Values represent means ± SEM (n = 18/group).
*p<0.05, †p<0.01, compared with untreated SHR or untreated normotensive Wistar rats.
**Left Ventricular Wall Thickness and Dimensions**

The increase in LV wall thickness in untreated SHR as compared with untreated WKY was significant at each treatment period. Long-term treatment with either hydralazine or minoxidil had no effect on LV wall thickness of SHR or of normotensive Wistar rats (see Table 4).

LV weight of untreated SHR showed an increase of about 50% over that of untreated WKY. Minoxidil treatment caused a significant increase in LV weight after 14 days, and this response was more marked \((p<0.01)\) after 35 days of treatment. Treatment with either hydralazine or minoxidil had no effect on LV weight (Figure 5). RV weight was significantly increased in untreated SHR compared with WKY. Treatment with either hydralazine or minoxidil induced similar increases in RV weight after 14 days. After 35 days, treatment with minoxidil resulted in a more marked \((p<0.05)\) increase and was greater than that observed with hydralazine treatment \((p<0.05)\; \text{Figure 6}\).

Dry LV and RV weights of the various groups were determined in the 5-week experiments and showed a similar pattern to that seen with wet weights (see Table 4). Dry/wet ratios for LV and RV weights showed no significant differences between treated and untreated groups.

**Body Weight and Water Intake**

In normotensive Wistar rats, body weight increased to 327 ± 6, 334 ± 7, and 338 ± 8 g at the end of the 5-week experiment for the untreated, hydralazine-treated, and minoxidil-treated groups, respectively. At the end of this experiment, water intake amounted to 7 ± 1 ml/100 g/day in all three groups, resulting in a drug intake of 8.6 ± 1.1 mg/kg/day for hydralazine and 8.5 ± 1.0 mg/kg/day for minoxidil.

Body weight was significantly higher and water in-
### Table 4. PRA and Parameters of Cardiac Anatomy in Untreated Normotensive Wistar Rats, WKY, and SHR, as Well as Normotensive Wistar Rats and SHR Treated with Hydralazine or Minoxidil

<table>
<thead>
<tr>
<th>Variable</th>
<th>Untreated WKY</th>
<th>SHR Untreated</th>
<th>Hydralazine</th>
<th>Minoxidil</th>
<th>Normotensive Wistar rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRA (ng Ang I/ml/hr)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Day 1</td>
<td>3.9±1.0</td>
<td>5.2±1.0</td>
<td>10.4±1.6*</td>
<td>7.1±2.0</td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
<td>4.8±1.0</td>
<td>6.1±1.7</td>
<td>10.4±2.6*</td>
<td>11.4±2.1†</td>
<td></td>
</tr>
<tr>
<td>Day 14</td>
<td>4.1±1.0</td>
<td>4.9±0.6</td>
<td>5.3±0.9</td>
<td>5.5±0.8</td>
<td></td>
</tr>
<tr>
<td>Day 35</td>
<td>3.1±0.8</td>
<td>2.7±0.4</td>
<td>2.1±0.2</td>
<td>3.2±0.5</td>
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<tr>
<td>Dry LV weight (mg/100 g body weight)§</td>
<td>53±1</td>
<td>76±2</td>
<td>75±2</td>
<td>91±2†</td>
<td>44±1</td>
</tr>
<tr>
<td>Dry RV weight (mg/100 g body weight)§</td>
<td>12±0.4</td>
<td>15±0.7</td>
<td>16±0.6</td>
<td>21±1.3†</td>
<td>9.3±0.4</td>
</tr>
<tr>
<td>LV wall thickness (mm)§</td>
<td>2.6±0.05</td>
<td>3.2±0.1</td>
<td>3.0±0.1</td>
<td>3.2±0.1</td>
<td>2.3±0.07</td>
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</tbody>
</table>

Values represent means ± SEM (n = 8-10/group for WKY and SHR, n = 10-12/group for normotensive Wistar rats). Ang I = angiotensin I; LV = left ventricular; RV = right ventricular.

* p<0.05, † p<0.01, compared with untreated SHR or untreated normotensive Wistar rats.
†† p<0.01, compared with hydralazine.
§ Day 35.

Discussion

Studies by Sen et al.4,5 established that BP control alone is not sufficient to bring about regression of cardiac hypertrophy in SHR. In agreement with these studies, our results show that long-term treatment of SHR with hydralazine or minoxidil normalizes BP but that hydralazine treatment results in the persistence of LV hypertrophy (LVH) whereas minoxidil treatment leads to a 20% increase in LVH. More prolonged treatment of SHR with hydralazine (3–9 months) does result in a small decrease in cardiac hypertrophy (about...
minoxidil treatment also resulted in the development of RV hypertrophy (RVH) and eccentric LVH, whereas hydralazine treatment also caused RVH but only a minor increase in LV internal diameter and no increase in LV weight. In 2K1C hypertensive rats, treatment with either hydralazine or minoxidil (5–8 weeks) decreased BP only temporarily but resulted in the potentiation of RVH and the development of eccentric LVH superimposed on the preexisting LVH. However, the heart of SHR appears more responsive to minoxidil, having more marked increases in LV and RV weights and LV dimensions despite normal BP than are seen in 2K1C hypertensive rats with persistence of hypertension. Increases in dry weight (see Table 4) and absence of changes in dry/wet ratios indicate that the increases in ventricular weight represent hypertrophy and not increases in myocardial water content (edema). However, morphometric measurements will be needed to confirm the actual type of hypertrophy.

Chronic cardiac volume overload could be responsible for these changes in cardiac anatomy. Minoxidil is known to induce salt and water retention in response to a lowered renal perfusion pressure, or activation of the renin-angiotensin system and renal sympathetic nerves, or both. The sodium-retaining effect of hydralazine is less obvious. Our results in SHR show increases in both plasma and blood volumes of about 5 to 8% and 15 to 20% following long-term (5 weeks) hydralazine and minoxidil treatment, respectively, and of about 10% in the normotensive rats. It is possible (but not very likely) that these small increases in blood volume together with a shift of blood to the central blood volume cause sufficient cardiac volume overload to explain the persistence or progression of cardiac hypertrophy despite BP control in SHR and the development of cardiac hypertrophy in normotensive rats during treatment with the two arterial vasodilators. Measurement of cardiac filling pressure and cardiac output will be needed to substantiate the extent of cardiac volume overload occurring during long-term treatment with arterial vasodilators. Only one study, showing a 20% increase in cardiac output after 3 weeks of hydralazine treatment in SHR, has been reported. In addition, minoxidil administration has been associated with a rise in pulmonary arterial pressure in hypertensive patients. Pulmonary hypertension would result in the development of RVH but might also cause an unexpected increase in LV weight.

As an alternative to volume overload, previous studies have implicated the cardiac sympathetic nerves in the persistence or progression of cardiac hypertrophy in SHR. Our results clearly show that the increase in LV weight during long-term minoxidil treatment is associated with a significant increase in RV weight as well as LV internal diameters but not in LV wall thickness. In the case of hydralazine, LV weight and anatomy remained unchanged and only a small increase in RV weight was observed. Therefore, long-term minoxidil treatment of SHR initiated the development of eccentric LV hypertrophy superimposed on the preexisting hypertrophy. This response is not specific for SHR. In normotensive rats, long-term

**Figure 7.** Left ventricular (LV) internal diameter (major axis) of untreated normotensive Wistar rats (●), WKY (○), and SHR (■), as well as normotensive Wistar rats treated with either hydralazine, 120 mg/L (●), or minoxidil, 120 mg/L (■) and SHR treated with hydralazine, 120 mg/L (●), or minoxidil, 120 mg/L (■). Bars represent means ± SEM (n = 10–12/group, top panel; n = 8–10/group, bottom panel). Single (p < 0.05) and double daggers (p < 0.01) indicate significant difference compared with untreated rats. Single (p < 0.05) and double daggers (p < 0.01) indicate significant difference compared with hydralazine.
dose that reduced ventricular norepinephrine. However, ventricular norepinephrine content is an inaccurate assessment of cardiac sympathetic activity. Moreover, it is not known what type of hypertrophy (concentric vs eccentric) would be induced by chronic cardiac sympathetic hyperactivity.

In the present study, we obtained more direct measures of general and cardiac sympathetic activity. Sympathetic hyperactivity has been extensively documented in SHR versus WKY, and in the present study we observed increases in plasma norepinephrine, BP response to hexamethonium, and LV norepinephrine turnover rate and a trend toward increased heart rate. Treatment of SHR with either arterial vasodilator for 1 and 2 days increased general sympathetic activity, as reflected by increases in heart rate and plasma norepinephrine concentration. This activation of the sympathetic nervous system likely occurred secondary to the decrease in arterial pressure. Of interest, this sympathetic hyperactivity was associated with a decreased response to hexamethonium in SHR. Similar results were obtained in normotensive rats, except that the sympathetic hyperactivity was associated with the anticipated increased response to ganglionic blockade with hexamethonium. These results indicate that the vasodilators inhibited the pressor responses to increased sympathetic tone in SHR but not in normotensive rats. A similar — presumably reflex-mediated — increase in sympathetic drive (manifesting itself as increases in heart rate and plasma norepinephrine) associated with short-term arterial vasodilator treatment has been reported in normotensive and hypertensive humans, normotensive rats, SHR, and 2K1C hypertensive rats.

During long-term treatment one may expect resetting of the arterial baroreceptors and a return of sympathetic tone toward baseline. Indeed, in our studies, during long-term treatment (2 and 5 weeks) of both SHR and normotensive rats, heart rate returned to control values, as did plasma norepinephrine. Struyker-Boudier et al. reported that the increase in heart rate after hydralazine treatment of SHR was brief in comparison with the prolonged decrease seen in BP. Other studies also reported no increases in heart rate during long-term treatment of SHR with hydralazine. Others have reported small (5–10%) increases in heart rate following long-term (3–25 weeks) hydralazine treatment of SHR. Similar discrepant results have been reported for normotensive rats (as noted in the introductory section). Persistent sympathetic hyperactivity may partly relate to the mode of drug administration: through the drinking water or twice daily through a gastric tube. The latter may cause more intermittent BP effects and, possibly, less resetting of baroreceptors. Taking into account our results in SHR, normotensive rats, and 2K1C hypertensive rats as well as the balance of other studies, we believe that a baroreceptor reflex-mediated increase in general sympathetic drive occurs on initiation of arterial vasodilator treatment; however, during long-term treatment a resetting of the baroreceptors occurs and general sympathetic tone returns to control levels. However, absence of generalized sympathetic hyperactivity does not exclude selective, persistent increased activity to specific organs. Indeed, LV and RV norepinephrine turnover rates (both absolute and fractional) in SHR were significantly increased after 5 weeks of treatment with either minoxidil or hydralazine. In normotensive rats, increases were found both on Day 1 — when evidence of generalized sympathetic hyperactivity was present — and selectively on Days 14 and 35. To our knowledge, no previous studies have assessed the effects of arterial vasodilators on ventricular sympathetic activity in hypertensive rats. In normotensive rats, treatment with hydralazine for 2 weeks was associated with a significant increase in ventricular norepinephrine turnover rate. Of interest, despite significant increases in norepinephrine turnover rates, initial ventricular norepinephrine content did not differ, either between untreated WKY and SHR or in treated versus untreated SHR or normotensive rats. As outlined previously, changes in the turnover rate of norepinephrine are clearly a better indicator of sympathetic tone than changes in the ventricular content of the amine, which may remain constant, increase, or decline in the presence of an increased turnover rate.

The use of norepinephrine turnover rate as a direct measurement of cardiac sympathetic activity has been employed by a number of investigators. In the absence of an increase in general sympathetic activity, the question arises as to the mechanism responsible for maintaining ventricular sympathetic hyperactivity during treatment of SHR or normotensive rats with arterial vasodilators. A direct cardiostimulatory effect has been suggested for both arterial vasodilators. In addition, it is tempting to speculate that the observed intravascular volume overload and distention of the ventricles and thus activate ventricular wall mechanoreceptors and a cardiocardiac reflex, maintaining ventricular sympathetic hyperactivity. Persistent hyperactivity of the renin-angiotensin system also could contribute to increased norepinephrine release and cardiac hypertrophy. However, PRA increased only during the initial days of treatment and then normalized, thus reflecting the time course of generalized sympathetic activity.

Our results show that increased ventricular sympathetic activity accompanies the development and persistence or progression of cardiac hypertrophy in normotensive rats and SHR treated with either hydralazine or minoxidil. As such, these results would appear compatible with the previously stated hypothesis. However, assessment of the density and responsiveness of cardiac adrenergic receptors will be needed to exclude down-regulation (as a primary or secondary event) resulting in normal effective sympathetic activity. Moreover, both vasodilators increased ventricular sympathetic activity to a similar degree, and both normalized BP rather similarly in SHR; yet in SHR, hydralazine treatment maintained LVH at pretreatment levels and caused only a small increase in RV weight, whereas minoxidil caused marked increases in RV weight, LV
weight, and LV internal diameters. Similarly, in normotensive rats, long-term treatment with either arterial vasodilator increased LV norepinephrine turnover to a similar degree, yet only minoxidil increased LV weight. Therefore, it appears unlikely that there is a direct relationship between ventricular sympathetic activity and the trophic response of the heart to treatment with arterial vasodilators. Our results indicate that, if ventricular sympathetic activity is involved in cardiac hypertrophy, other mechanisms (e.g., extent of LV and RV volume overload) are needed for the full expression of the trophic response or other mechanisms (e.g., down-regulation of receptors) can prevent this effect.

The present results showed that long-term arterial vasodilator treatment of SHR with established hypertension produces a sustained decrease in BP, in agreement with previous studies with hydralazine, and minoxidil. In contrast, in normotensive rats and 2K1C hypertensive rats, tolerance develops to the BP-lowering effect of arterial vasodilators. Several mechanisms can be evoked to explain the different BP response of SHR as compared with the other models. It is tempting to speculate that the vascular smooth muscle of SHR is different and is not able to develop tolerance to the relaxant effect of the two arterial vasodilators. In support of this concept of different responses, long-term treatment with either vasodilator decreases the hypotensive response to ganglionic blockade only in SHR, suggesting a decreased responsiveness of vascular smooth muscle to pressor mechanisms.

In conclusion, the present study clearly shows that despite adequate BP control, long-term arterial vasodilator treatment of SHR at the doses used resulted in the persistence of cardiac hypertrophy with hydralazine and, in the case of minoxidil, potentiation of RVH and development of eccentric LVH. Normotensive rats exhibit similar cardiac effects. In both animal models, generalized sympathetic hyperactivity only occurs during the initial period but a selective increase in ventricular sympathetic activity persists during long-term treatment. Both ventricular sympathetic hyperactivity and volume overload may be involved as possible causal or contributory mechanisms for the cardiac effects of arterial vasodilators.

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