Enalapril Can Prevent Vascular Amplifier Development in Spontaneously Hypertensive Rats

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Three groups of spontaneously hypertensive rats (SHR) were given enalapril (25 mg/kg/day) from 4 to 9 weeks, 4 to 14 weeks, and 14 to 20 weeks of age. The drug was stopped and observations continued for another 16–21 weeks. At selected times, we measured blood pressure, in vitro hindquarter vascular resistance properties, left ventricular weight/body weight ratio, and skeletal muscle vessel norepinephrine kinetics in treated and untreated SHR and in Wistar-Kyoto (WKY) rats. At the end of each treatment period, all cardiovascular variables were close to values of WKY rats and well below those of untreated SHR, and the norepinephrine or fractional rate constant was about 25% below those levels. After enalapril was stopped, blood pressure and left ventricular weight/body weight ratio increased in parallel to levels ranging from 30% to 50% of the normal difference between untreated SHR and WKY rats. However, in SHR treated from 4 to 9 weeks and from 4 to 14 weeks of age, hindquarter resistance properties remained close to WKY rat levels for the entire observation period of 16–21 weeks after treatment, suggesting suppression of the enhanced resistance responses of SHR (amplifier properties). In SHR treated from 14 to 20 weeks of age, suppression of amplifier properties was more transient, and they redeveloped partially 5–6 weeks after cessation of therapy. When enalapril was given up to 14 weeks of age, the long-term suppression of amplifier properties was probably mainly through prevention of smooth muscle hypertrophy in resistance vessels and possibly through other mechanisms (e.g., “rarefaction”). After that age, its therapeutic action was similar to that of other antihypertensive drugs where, after drug withdrawal, there is a slow pressure rise in parallel with redevelopment of vascular and cardiac hypertrophy. (Hypertension 1990;16:252–260)

In earlier studies in patients with chronic human hypertension, we observed an inverse relation between the duration of treatment and the rate of redevelopment of hypertension after cessation of therapy.1–4 The findings suggested that the longer the period of treatment, the greater the degree of regression of cardiovascular hypertrophy.5,6 Normally, in chronic hypertension, hypertrophy is associated with the well-known structural changes of the resistance vessels, with narrowing of the lumen and an increase in wall/lumen ratio.6–8 As a result, during constriction, there is greater narrowing of the lumen than in normal vessels, with a greater rise in vascular resistance. The hypertensive vessels are sometimes said to “amplify” the resistance changes.3,4,8 Presumably, the reason for the slow redevelopment of hypertension in patients after therapy is stopped is a very gradual redevelopment of the amplifier properties after partial regression of structural changes in both vessels and heart.3–5 This is in accord with findings after long-term treatment in the spontaneously hypertensive rat (SHR) with certain drugs.9,10

In primary hypertension in young SHR, different types of antihypertensive drugs can readily restore blood pressure to the levels of normotensive Wistar-Kyoto (WKY) rats. After therapy is stopped, there is a spectrum of responses with different agents, but in general, blood pressure tends to rise more slowly after treatment of the hypertension with angiotensin converting enzyme (ACE) inhibitors than with other drugs.11–15 Long-term suppression of hypertension has also been produced by immunosympathectomy during early life.16,17 The long-lasting nature of some forms of therapy has led to the speculation that the
renin-angiotensin system or the sympathetic nervous system plays a causal or reinforcing role in the pathogenesis of primary hypertension.

Our recent findings in SHR, that the amplifier properties of the hindquarter resistance vessels are already established at 4 weeks (i.e., before the rise in blood pressure), suggest that structural changes develop very early in life and are probably the cause of hypertension. Accordingly, the purpose of the present investigation was to determine whether, in SHR, a short period of therapy with an ACE inhibitor (enalapril) at particular phases of development could prevent or attenuate the subsequent development of hypertension and vascular or cardiac hypertrophy. We used a constant dose of enalapril in SHR that, during the treatment period, brought blood pressure and hindquarter vascular resistance to levels of age-matched WKY rats. The drug was given over three periods: 1) from 4 to 9 weeks, when the initial rise in blood pressure normally occurs in untreated SHR; 2) from 4 to 14 weeks, when most of the rise in blood pressure occurs in untreated SHR; and 3) from 14 to 20 weeks, by which time adult levels of blood pressure have been reached in untreated SHR. Enalapril was stopped at the end of each period and blood pressure and hindquarter vascular resistance properties were measured at selected times over the next 16–21 weeks and compared with those of untreated SHR and WKY rats.

**Methods**

**Animals and Blood Pressure Measurements**

The treated group consisted of male SHR, bred at the Baker Medical Research Institute from stock supplied by Professor Y. Yamori in 1986. Their responses were compared with age-matched untreated SHR and WKY rats. The rats were housed one to a cage in a room in which temperature was controlled between 23 and 25°C, and a 12-hour light/dark cycle was maintained. Food and water were supplied ad libitum to both treated and untreated rats.

Systolic blood pressure and heart rates were measured in conscious rats as described previously using a tail-cuff and a photoelectric pulse detector. Blood pressure measurements were made at weekly intervals during the study, which included animals from 4 to 40 weeks of age.

**Enalapril Treatment and Groups**

The rats received enalapril maleate in their drinking water at a dose, which was based on published reports, of 25–30 mg/kg/day. In preliminary experiments in 14-week-old SHR, we found that this dose readily reduced systolic blood pressure to levels of WKY rats. During the therapy period, we monitored the water consumption of the rats and adjusted the concentration of enalapril to maintain approximately constant the ingested dose per unit body weight.

The three groups of enalapril-treated rats comprised 48 SHR treated from 4 to 9 weeks, 52 SHR treated from 4 to 14 weeks, and 48 SHR treated from 14 to 20 weeks of age. They were compared with 72 untreated SHR and 85 WKY rats of appropriate ages.

**Hindquarter Perfusion and Left Ventricular Weight**

We used a slight modification of the method of Folkow et al., as described previously. Briefly, two rats of similar age (one treated with enalapril and one not) were anesthetized with sodium pentobarbital (60 mg/kg i.p.). The lower abdominal aorta was exposed at the iliac bifurcation through a midline abdominal incision. Heparin was administered to each rat (1,000 IU/kg), and the aorta was cannulated so that the tip of the cannula lay just proximal to the iliac bifurcation. After ligation of the middle caudal and the caudal mesenteric arteries and transection of the spinal cord and inferior vena cava, the hindquarter perfusion was performed at a flow rate of 10 ml/100 g hindquarter wt, calculated from the relation of hindquarter weight to total body weight at different ages; enalapril had an effect on body weight (see Results), but this affected the above relation only minimally within each group. The perfusate consisted of 1.5% dextran-containing Tyrode’s solution at 37°C of the composition previously described.

The hindquarter bed was maximally dilated by an infusion of papaverine HCl (3.7 mg/100 g hindquarter wt) 5 minutes after the start of perfusion. The perfusion pressure at maximum vasodilatation (PPmax dl) was recorded after 20 minutes of perfusion. Next, we derived cumulative dose–response curves to the α1-adrenergic receptor agonist methoxamine by stepwise increases of the infusion rate. A PP plateau was obtained with each dose before the next dose was infused until there was no further elevation of PP. Lastly, we tested whether this PP corresponded to the maximum constrictor response (PPmax con) by administering angiotensin II (Ang II) at a dose of 50 μg.

PPmax dl and PPmax con were obtained directly from the pressure tracings. We also derived logistic functions that, in addition to PPmax dl and PPmax con included the concentration of methoxamine to reach 50% of the range between them (EC50) and the maximum slope, as described earlier.

We have assumed that the differences in hindquarter vascular resistance during maximum constriction and maximum dilatation between untreated SHR and WKY rats mainly reflect their normal differences in vascular smooth muscle mass (see References 5, 18, and 21 and Discussion section). The responses of untreated SHR and WKY rats provide a scale for assessment of the effects of treatment.

We also weighed the left ventricle wall plus septum and, after dividing these by body weight, derived the left ventricle wall plus septum/body weight (LVS/BW) ratio. We have used assumptions similar to the above for assessing the changes in left ventricular hypertrophy from the LVS/BW ratios.
Hindquarter Norepinephrine Content and Turnover

The forelimb muscle (caput longum of triceps brachii) was taken from treated and untreated rats before the perfusion experiment. In this tissue, catecholamines are largely associated with the vascular innervation. Norepinephrine tissue concentration \([\text{NE}]\) was measured as described below. Norepinephrine fractional rate constant \((K)\) was determined by inhibiting the enzyme tyrosine hydroxylase by \(\alpha\)-methyl-DL-p-tyrosine methyl ester HCL (AMPT) (300 mg/kg i.p.) followed by a second injection after 4 hours to suppress norepinephrine biosynthesis. Eight hours after the first injection, the rats were killed, and all muscle specimens were weighed, rapidly frozen in liquid nitrogen, and then stored at \(-80^\circ\text{C}\) until analyzed.

[\text{NE}] was measured by high performance liquid chromatography (HPLC) with electrochemical detection. Briefly, the procedure consisted of homogenizing the tissue in 0.4 M perchloric acid (PCA) that contained the chromatographic internal standard 3,4-dihydroxybenzylamine (0.1–5 \(\mu\text{g}\)). The precipitated protein was removed by low speed centrifugation and norepinephrine extracted from the supernatant onto 300 mg alumina (active/neutral form, Merck) using 1.5 M tris[hydroxymethyl]amino methane (Tris) buffer, pH 8.6. Norepinephrine was eluted from the alumina with 700 \(\mu\text{l}\) of 0.1 M PCA and quantified by HPLC plus electrochemical detection, using an Altec Ultrasphere-ODS column (3 \(\mu\text{m}\); 46×7.5 cm) and a mobile phase consisting of sodium dihydrogen phosphate (0.05 M), sodium citrate (0.05 M), EDTA (2 mM), and octyl sodium sulfate (100 mg%), dissolved in methanol/water (3–10%, vol/vol) and adjusted to pH 4.5. Peak areas were quantified on a Shimadzu data system (C-R3A, Shimadzu Science Instruments, Columbia, Md.) and corrected for recovery of the internal standard. The slopes of the linear regression functions relating the logarithms of [NE] to time after AMPT administration were used to calculate \(K\) for norepinephrine in muscle.

Statistical Methods

The significance of differences within and between strains was assessed by one-way analysis of variance (ANOVA) or analysis of covariance, and the age-slope data was examined by linear regression. Results have been expressed as mean±SEM or as the difference between mean±SED.

Results

Effect of Enalapril on Body Weight and Blood Pressure

At 4 weeks of age, there was little difference in body weight between SHR subsequently treated with enalapril and those not given the drug. By 9 weeks of age, the treated SHR weighed about 8% less than untreated SHR \((p<0.05)\), and the difference had increased to about 12% \((p<0.025)\) by 14 weeks of age (Figure 1). After stopping enalapril, the body weight difference diminished gradually and was no longer apparent at 30 weeks. In SHR given enalapril from 14 to 20 weeks of age, treatment had no significant effect on body weight; at 20 weeks of age, the enalapril-treated SHR weighed 393±4 g compared with 389±5 g in untreated SHR, and the subsequent body weight changes were closely similar in both groups. The age–body weight relation of WKY rats was closely similar to that of untreated SHR, which is in agreement with previous findings.

Systolic blood pressures at 4 weeks were similar in WKY rats (101±5 mm Hg), in SHR subsequently given enalapril (99±5 mm Hg), and in untreated SHR (104±6 mm Hg) (Figure 2). In all three groups of treated SHR, enalapril maintained systolic blood pressures close to those of age-matched WKY rats (Figure 2); in the SHR treated from 14 to 20 weeks of age, blood pressure remained slightly above the levels of the latter group \((p<0.05)\). After enalapril was stopped, blood pressure rose relatively rapidly over the next 2–4 weeks and then settled at an approximately steady level for the remaining 16–21 weeks. The initial rise was greatest in SHR treated from 4 to 9 weeks of age, where the peak systolic blood pressure was 178±4.5 mm Hg, which was not significantly different from the blood pressure of untreated SHR (185±6.0 mm Hg) (Figure 2, top left). In SHR treated from 4 to 14 weeks of age and from 14 to 20 weeks of age, the peak value during the initial rise remained significantly below the values in age-matched untreated SHR (Figure 2, middle and lower left panels) \((p<0.001)\). Subsequently, the steady-state blood pressure in all three previously treated groups remained between those of untreated SHR and WKY rats (Figure 2, left panels).

Thus, all previously treated groups developed some hypertension after drug withdrawal, but the rise in blood pressure was attenuated when compared with untreated SHR. The attenuation was most pro-
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...nounced in SHR given enalapril from 4 to 14 weeks of age, in which systolic blood pressure during the last 4 weeks of observation leveled at 158 ± 4 mm Hg, compared with 206 ± 7 and 136 ± 5 mm Hg in age-matched untreated SHR and WKY rats, respectively, of the same age (p for differences < 0.025) (Figure 3). In SHR treated from 4 to 9 weeks and from 14 to 20 weeks of age, the subsequent steady-state systolic blood pressures were 172 ± 4 and 170 ± 6 mm Hg, respectively, which were significantly above blood pressures of SHR treated from 4 to 14 weeks of age (p = 0.05).

Hindquarter Resistance Properties

At the end of the treatment period, enalapril had brought PPmax con to values close to or slightly below those of WKY rats and well below those of untreated SHR (Figure 2, right panels). On stopping the drug in SHR treated from 4 to 14 weeks of age, PPmax con remained close to the values of WKY rats for the next 16 weeks and followed the pattern of normal age-related changes of WKY rats (Figure 2; right, top, and middle panels). In rats treated from 4 to 9 weeks of age, the pattern was similar, with none of the values of PPmax con significantly different from those of WKY rats; the difference at 30 weeks of age was 19 ± 11 mm Hg (NS), which was about 28% of the difference between untreated SHR and WKY rats. In SHR treated from 14 to 20 weeks of age, PPmax con had risen by 30 weeks of age by 20 ± 8.2 mm Hg (p < 0.05) above the value of WKY rats. By 40 weeks of age, PPmax con was 32 ± 7.5 mm Hg (p < 0.01) above values of WKY rats, which was 44% of the normal difference between untreated SHR and WKY rats (Figure 2, lower right panel).

The differences in PPmax di between untreated SHR and WKY rats were statistically significant, although much smaller than the corresponding differences in PPmax con. In all three groups of previously treated SHR after withdrawal of enalapril, PPmax di remained below values of untreated SHR and close to those of WKY rats (Table 1). The differences between groups were most obvious in the values of PPmax di averaged over the entire period after treatment (Table 1). For example, after stopping enalapril in SHR treated from 4 to 14 weeks of age, PPmax di averaged 20.5 ± 1.0

Figure 3. Bar graph showing average "steady-state" tail-cuff systolic blood pressures after treatment and SEM in spontaneously hypertensive rats previously treated with enalapril (SHR-R) from 4 to 9 weeks (stippled rectangle), 4 to 14 weeks (black rectangle), and 14 to 20 weeks of age (hatched rectangle). Values in untreated SHR (left) and WKY rats (right) are shown as open rectangles. Scale on left (mm Hg); scale on right, difference in pressures between untreated SHR and WKY rats equals 100%.
TABLE 1. Perfusion Pressure at Maximum Vasodilation at Different Ages in Untreated and Enalapril-Treated Rats

<table>
<thead>
<tr>
<th>Rat group</th>
<th>9 wk</th>
<th>14 wk</th>
<th>20 wk</th>
<th>30 wk</th>
<th>Average</th>
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<tr>
<td>SHR</td>
<td>23.3±1.3</td>
<td>24.2±0.9</td>
<td>23.8±0.9</td>
<td>31.0±1.7</td>
<td>25.1±0.9</td>
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<td>(6)</td>
<td>(5)</td>
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<td>(22)</td>
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<td>SHR_{4-9}</td>
<td>20.6±1.2</td>
<td>19.7±1.1*</td>
<td>23.6±0.7</td>
<td>25.8±0.8*</td>
<td>22.0±0.7*</td>
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<tr>
<td></td>
<td>(4)</td>
<td>(6)</td>
<td>(5)</td>
<td>(5)</td>
<td>(20)</td>
</tr>
<tr>
<td>SHR_{4-14}</td>
<td>...</td>
<td>21.2±1.1*</td>
<td>18.3±1.1*</td>
<td>22.0±1.6*</td>
<td>20.5±1.0*</td>
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<tr>
<td></td>
<td>(5)</td>
<td>(6)</td>
<td>(6)</td>
<td>(6)</td>
<td>(17)</td>
</tr>
<tr>
<td>SHR_{14-20}</td>
<td>...</td>
<td>...</td>
<td>22.8±1.2</td>
<td>22.5±1.4*</td>
<td>23.3±1.0*</td>
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<td>(5)</td>
<td>(6)</td>
<td>(6)</td>
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<td>(21)</td>
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<tr>
<td>WKY</td>
<td>18.0±0.6*</td>
<td>21.0±1.0*</td>
<td>22.0±0.7</td>
<td>23.0±1.0*</td>
<td>22.0±0.7*</td>
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<tr>
<td></td>
<td>(5)</td>
<td>(5)</td>
<td>(6)</td>
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Values are mean±SEM. Average, average value over all periods in each row; SHR, untreated spontaneously hypertensive rats; SHR_{4-9}, SHR_{4-14}, SHR_{14-20}, SHR treated from 4 to 9, 4 to 14, and 14 to 20 weeks of age, respectively; WKY, untreated Wistar-Kyoto rats. Number of rats is given in parentheses.

*p<0.05 for significant difference from untreated SHR.

In addition, three rats were studied at 25 weeks and six at 40 weeks of age.

In addition, six rats were studied at 40 weeks of age.

mm Hg over the period 14–30 weeks of age, compared with 26.3±0.9 mm Hg in untreated SHR (p for differences <0.01) over a corresponding period.

The maximum slope (S) of the dose–hindquarter resistance curve increased with age in an approximately linear manner, in agreement with earlier findings; the regression equation was

\[ S = 92 + 9.98 \text{age (weeks)} \]

where SEM (intercept) was ±15 units [mm Hg/(µg/ml)] and SEM regression coefficient was ±1.98 units (p<0.001). From this equation, we calculated the slopes at 14, 20, and 30 weeks, which were, respectively, 232, 292, and 392; mean 305 units. In WKY rats, the relation was also linear, with the regression coefficient 6.13±0.82 units; the average mean value of the slope at the three ages was 80±21 units below that of untreated SHR (p<0.01). In SHR treated from 4 to 14 weeks of age, the calculated mean value from the regression lines at the above three ages was, on average, 55±22 units (p<0.05) below that of untreated SHR; in SHR treated from 14 to 20 weeks of age, the slope was 41±19 units lower than in untreated SHR (p=0.05), whereas in SHR treated from 4 to 9 weeks of age, the difference was small (14±15) and not statistically significant.

The \( \text{EC}_{50} \) parameter at a given age was not statistically different in untreated SHR and WKY rats between the ages of 9 and 40 weeks, as described previously. \( \text{EC}_{50} \) was inversely related to age in each strain; values in untreated SHR aged 14, 20, and 30 weeks were, on average, 5.8, 4.7, and 3.2 µg/ml. The effects of enalapril treatment were variable, with a tendency for \( \text{EC}_{50} \) to be lower immediately at the end of the treatment period than during subsequent weeks; thus, the normal age–\( \text{EC}_{50} \) relation was less well defined than in untreated SHR.

**Left Ventricular Weight**

In each group of treated SHR, the ratio of LVS/BW at the end of therapy was significantly lower than in age-matched untreated SHR and close to the ratio in WKY rats (Figure 4). Only in SHR treated from 14 to 20 weeks of age was reversal of hypertrophy apparent less complete, with LVS/BW ratio at 20 weeks greater than in WKY rats (ΔLVS/BW = 0.166±0.060; p=0.01) (Figure 4). Enalapril was stopped, some hypertrophy redeveloped in all three groups, with the LVS/BW ratio rising to values between those of untreated SHR and WKY rats (Figure 4).

The LVS/BW ratio averaged over all three groups of treated SHR at the end of the treatment period,
was 1.981±0.033 g/kg, which was significantly below that of age-matched untreated SHR (2.477±0.032 g/kg; p<0.001), but closely similar to that of WKY rats (1.986±0.020 g/kg). Thus, on that criterion, treatment had reversed left ventricular hypertrophy almost completely. By the time the rats were 30 weeks of age, some left ventricular hypertrophy had redeveloped, with LVS/BW ratio 2.273±0.029 g/kg compared with values in untreated SHR and WKY rats of 2.728±0.043 and 1.990±0.042 g/kg, respectively (p for differences <0.01). At 30 weeks, LVS/BW ratio of untreated SHR was 37% above that of age-matched WKY rats, whereas that of the treated groups averaged 14% above the value in the latter group.

Sympathetic Function

In SHR treated from 4 to 9 weeks, skeletal muscle [NE] at the end of treatment was about 35% below that of untreated SHR (p<0.01). In the other two groups of SHR, enalapril appeared to have the opposite effect and, at the end of the treatment period, [NE] of SHR treated from 4 to 14 and SHR treated from 14 to 20 weeks of age, were 26% (p<0.025) and 22% (NS), respectively, above those of age-matched untreated SHR.

The fractional rate constant tended to be lower at the end of therapy than in age-matched untreated SHR (Table 2). When averaged over all three groups, K at the time enalapril was stopped was 0.04915±0.00514 hr⁻¹ compared with 0.0665±0.00533 hr⁻¹ in untreated SHR (p for difference <0.05). Thus, treatment lowered K by about 25%, with the difference most uniform in SHR treated from 4 to 9 weeks of age (Table 2).

We also measured [NE] and K at 30 weeks. At that time [NE] was 110±2 ng/g in the treated group, which was closely similar to the value of 110±4 ng/g in untreated SHR. The fractional rate constant of previously treated SHR was 0.0434±0.00416 hr⁻¹, slightly above that of untreated SHR (0.0344±0.00459 hr⁻¹), but the difference was not statistically significant (t for difference=1.46, NS).

Discussion

Circulatory Properties

In the present study we found that, during enalapril treatment, mean arterial pressure of SHR was brought close to that of WKY rats when treatment occurred up to 14 weeks of age, but in SHR treated from 14 to 20 weeks of age, it remained slightly higher. This may reflect age-related differences in plasma renin activity, which is higher in young than in adult rats. After withdrawal of enalapril, the development of hypertension and left ventricular hypertrophy was markedly attenuated. The “steady-state” blood pressure at the end of the observation period was lowest in SHR previously treated from 4 to 14 weeks of age, reaching only about 30% of the normal differences between SHR and WKY rats of corresponding ages. This was about 15–20 mm Hg below the corresponding rises in earlier studies with ACE inhibitors. In the other two groups of treated SHR, the rise in blood pressure was to about 50% of the normal strain difference, which was similar to the earlier findings.

The design of the latter investigations involved treating SHR over a single time span, from 4 to 6 weeks to about 20 weeks of age, in contrast to the approach used here, where the effects of 5–10 weeks of treatment were examined at three different stages of development of hypertension. We did not study the effects of enalapril in WKY rats, in view of the observations of Harrap et al with perindopril, who found that after drug withdrawal, long-lasting reduction in blood pressure and in resting renal vascular resistance occurred in SHR but not in WKY rats. From this, we have assumed that the long-term effects on vascular properties produced by ACE inhibitors predominantly affected SHR.

Our main finding was the long-lasting restoration after cessation of treatment of the hindquarter vascular resistance properties of SHR close to the values observed in WKY rats. This was assessed from the changes in PPᵣ max_con⁰ PPᵣ max_dib⁰ and in slope. In the two groups of SHR treated before 14 weeks of age, PPᵣ max_con remained either at, or slightly below, the levels observed in WKY rats through the entire period after treatment, but in SHR treated from 14 to 20 weeks of age, PPᵣ max_con increased to about 45% of the normal difference between untreated SHR and WKY rats. For PPᵣ max_dib the changes after treatment were similar, but at the perfusion rates used, the small magnitude of the difference in PPᵣ max_dib between untreated SHR and WKY rats provided a scale with less capacity for discrimination than the corresponding strain difference for PPᵣ max_con. The slope of the curve observed during and after enalapril was, as expected, significantly reduced in SHR treated from 4 to 14 weeks and from 14 to 20 weeks of age. However, in SHR previously treated from 4 to 9 weeks of age,
the age-slope relation after treatment differed little from that of untreated SHR. The reason for this is not clear; possibly the reduction in local [NE] at 9 weeks of age enhanced the responsiveness to methoxamine, thereby distorting the normal age-slope relation. However, in this group, the effects of treatment on $PP_{max}$ coo and $PP_{max}$ dii were similar to those in the other two groups, and we have assumed that enalapril had an essentially similar action. In any event, in SHR treated from 4 to 14 weeks and from 14 to 20 weeks of age, enalapril produced long-lasting suppression of all the amplifier properties, as assessed from the changes in the three curve parameters.

What is the significance of the amplifier properties in untreated SHR from the viewpoint of vascular structure? Folkow proposed that the main determinant of the amplifier properties was an increased wall/lumen ratio in the resistance vessels, which in practice was associated with encroachment on the vessel lumen. Subsequently, Sivertsson suggested that the amplifier properties depended on an increase in wall/lumen ratio even without luminal encroachment. We have recently modeled the structural requirements of a vascular resistance amplifier and, in contrast to the above, found that the sole requirement for “physiological” amplification about resting vascular tone was narrowing of the vascular lumen. An increase in wall thickness without vascular narrowing accentuated the rises and falls in resistance changes at the two plateaus of the dose–resistance curve, but had little effect on slope (Reference 8 and J.A. Angus and P.I. Korner, unpublished data). On the other hand, narrowing of the lumen when the wall thickness remained normal increased all curve parameters, including $PP_{max}$ diib, $PP_{max}$ slope, and $PP_{max}$ coo, but the effect was smaller than in the presence of thickening of the vascular wall (J.A. Angus and P.I. Korner, unpublished data).

In the present study, we did not determine the morphological changes, but others have shown that, in untreated SHR, the resistance vessels of many vascular beds show an increase in wall thickness associated with some reduction in vascular lumen. This is probably the major mechanism underlying vascular amplification and enalapril could prevent or reverse the medial hypertrophy, leading to normalization of the vessel lumen. Other mechanisms that have been considered as possible sources of amplifier properties include certain types of vascular narrowing and “rarefaction” (reduction in microvascular density) that occur in hypertension. This is probably the major mechanism underlying vascular amplification and enalapril could prevent or reverse the medial hypertrophy, leading to normalization of the vessel lumen. Other mechanisms that have been considered as possible sources of amplifier properties include certain types of vascular narrowing and “rarefaction” (reduction in microvascular density) that occur in hypertension. The relative importance of all the above mechanisms is uncertain. Experimentally, acute microsphere-induced anatomic rarefaction in the hindquarter vascular bed of SHR caused elevation of $PP_{max}$ dii, but left $PP_{max}$ coo and slope unaltered, so that the “rarefied” bed did not function as a resistance amplifier. However, our recent model suggests that rarefaction can produce enhancement of resistance changes similar to lumen narrowing (J.A. Angus and P.I. Korner, unpublished observations).

Possibly the experimental conditions used by Hallbäck et al. did not simulate the physiological rarefaction in SHR.

Although the interpretation of the present experiments is not unequivocal, it seems highly likely that the major determinant of the amplifier properties of untreated SHR was medial thickening with luminal narrowing, with the other mechanisms playing a minor role. Enalapril would predominantly affect the former mechanism. This is in accord with the known actions of Ang II as a stimulus for growth of vascular smooth muscle cells in culture. The time at which rarefaction has been described in certain vascular beds is not in accord with the maximum action of enalapril in the present experiments. Thus, in the mesenteric bed of SHR, rarefaction was most pronounced in adolescent and adult animals rather than in young rats, whereas effects of enalapril on amplifier properties were greatest when given to young rats up to 14 weeks of age. Enalapril affected whole body growth when given to young SHR (Figure 1). We suggest it had a corresponding effect on excess smooth muscle growth over that found in WKY rats.

With the prolonged suppression of the vascular amplifier properties by enalapril, what was responsible for the rise in blood pressure in the two groups of SHR treated up to 14 weeks of age? The rise after drug withdrawal could have been due to elevation of cardiac output, as observed in human primary hypertension, where cardiac output was raised but total peripheral resistance was normal a few days after stopping all drugs after 1 year of therapy. Our earlier study has indicated that cardiac sympathetic activity was elevated in untreated SHR through much of the period between 4 and 50 weeks of age. This could have contributed to the relatively rapid initial rise in blood pressure soon after drug withdrawal, whereas later on, during steady-state conditions, the intrinsic enhancement of myocardial performance due to left ventricular hypertrophy would also have contributed to the redevelopment of hypertension. In the third group of SHR, treated from 14 to 20 weeks of age, the gradual redevelopment of vascular amplifier properties after treatment accounted for much of the steady-state rise in blood pressure (Figure 2), assuming that the changes were similar to those of other beds.

The action of the ACE inhibitors reduces production of Ang II in plasma and at local production sites, including the kidney and blood vessels. Unger et al. found that, in adult SHR, enalapril produced relatively long-lasting inhibition of ACE in vessels and other local sites, which was still substantial 1 week after stopping therapy. However, even this time does not account for the very long period of suppression of vascular hypertrophy in the two groups of SHR previously treated from 4 to 9 and from 4 to 14 weeks of age.

One hypothesis for the long-lasting effect of enalapril on the resistance vessels is that the drug interfered with the mechanisms involved in the develop-
ment of excess vascular growth in SHR over that present in WKY rats. This is suggested by the more prolonged suppression of amplifier properties in SHR, treated up to 14 weeks of age, which corresponds to the end of the normal blood pressure rise. Ang II has many actions on cellular metabolism, some of which could affect vascular smooth muscle growth. Thus, in culture, Ang II increases mobilization of intracellular calcium and turnover of inositol triphosphate, activates the sodium-hydrogen antiport, and can increase ribosomal protein S6 phosphorylation. In addition, the actions of Ang II on proliferation of vascular smooth muscle require the presence of other growth factors, particularly platelet-derived growth factor b-b chain. Such a dual mechanism could explain why the action of enalapril on vascular hypertrophy had different long-term consequences when the drug was given early in postnatal life compared with its action when administered later. A role of the vascular renin-angiotensin system in the development and maintenance of hypertrophy of the resistance vessels in SHR is suggested by the higher vascular renin concentration compared with that in WKY rats.

If Ang II requires the presence of one or more growth factors to cause excess vascular growth in SHR (i.e., medial hypertrophy), its effectiveness as a growth potentiator could be determined entirely on the time of expression of the other, genetically regulated growth factors. Such timing during an early period of postnatal life is consistent with the findings of Adams et al that a substantial part of the vascular amplifier was already established by 4 weeks of age.

We suggest that enalapril given early in life interfered with normal formation in SHR of excess vascular growth and associated structural changes, leading to the prolonged normalization of resistance properties. When given later on, its actions were similar to those of other antihypertensive agents, which produce nonspecific reversal of cardiovascular hypertrophy through prolonged control of blood pressure. This has been observed with many drugs after prolonged periods of treatment, both in SHR and in human primary hypertension. In the two groups treated from 4 to 9 and from 4 to 14 weeks of age, the follow-up period after drug withdrawal was only 16–21 weeks. We do not know whether, in these groups, vascular hypertrophy would have developed eventually, as occurs in secondary hypertension.

**Sympathetic Function**

The present experiments have been the first to show in vivo that chronic administration of ACE inhibitors reduces regional sympathetic nerve activity, complementing previous findings under somewhat more controlled conditions, using central and peripheral nerve stimulation. At the end of enalapril treatment, the K in hindquarter muscle vessels was lower than that in untreated SHR, suggesting reduction in sympathetic neural activity. Thus, it is possible that, in untreated SHR, Ang II enhances transmitter release, as suggested by previous investigators. We do not know the source of Ang II, but since the reduction in K at the end of treatment was reasonably uniform in all three groups, it could have arisen from vascular renin-angiotensin system activity, which remains more uniformly elevated than in plasma in SHR of different ages. Earlier in vitro studies have shown that relatively large concentrations of angiotensin increase transmitter release from sympathetic nerve endings. The present findings suggest that the local renin-angiotensin system in the vascular wall in the normal SHR is closely associated with the sympathetic nerves and may modulate transmitter release under physiological conditions. In our experiments, neural activity was not measured in the early period after treatment, but by 30 weeks, K had returned to normal.

The effects of enalapril on [NE] differed in the three groups, with [NE] lower in SHR treated from 4 to 9 weeks of age than in untreated SHR, but with reversal of the difference in the other two groups (Table 2). The elevation of [NE] in young, untreated SHR has been considered to be due to a transient increase in vascular sympathetic innervation and, from our findings, Ang II may help to mediate the hyperinnervation response. However, we cannot say whether the observed changes in sympathetic function played a role in determining the hindquarter vascular resistance properties during and after treatment.

In conclusion, the long-term suppression of vascular amplifier properties after withdrawal of enalapril, in SHR treated up to 14 weeks of age, suggests that Ang II in conjunction with other growth factors is important in the early development of vascular hypertrophy in SHR. When treatment started after 14 weeks of age, withdrawal of enalapril produced more transient suppression of amplifier properties, similar to findings with other antihypertensive drugs. The actions of enalapril on left ventricular hypertrophy after cessation of treatment appeared to be largely parallel to the blood pressure changes.

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