Brief Angiotensin Converting Enzyme Inhibitor Treatment in Young Spontaneously Hypertensive Rats Reduces Blood Pressure Long-term

Stephen B. Harrap, Walter M. Van der Merwe, Sheila A. Griffin, Fiona Macpherson, and Anthony F. Lever

Our study examines the long-term cardiovascular effects after a brief period of angiotensin converting enzyme (ACE) inhibitor treatment in young spontaneously hypertensive rats (SHR). SHR were treated with perindopril (3 mg/kg/day) by gavage from 2 to 6, from 6 to 10, or from 2 to 10 weeks of age. Systolic blood pressure was measured in the tail weekly until 25 weeks of age. Corresponding control groups received distilled water for the same periods. In each treatment group blood pressure was reduced significantly during treatment, rose when treatment stopped, but plateaued significantly below control SHR thereafter. This difference in blood pressure at 25 weeks of age was due to reduced total peripheral resistance as determined by microsphere methods, but plasma renin activity and angiotensin II concentrations were not different. Cardiac hypertrophy was also reduced in treated SHR. In a separate experiment, perindopril treatment from 6 to 10 weeks of age resulted in a significant reduction in the media/lumen ratios of mesenteric resistance vessels at 32 weeks of age. Concomitant administration of angiotensin II with perindopril from 6 to 10 weeks of age not only prevented the long-term effects on blood pressure seen with perindopril treatment alone but was associated with cardiovascular hypertrophy in excess of untreated control SHR. Finally, perindopril given for a shorter period (6 to 7 weeks) or later in life (20 to 24 weeks) had no significant long-term effects on blood pressure. These results demonstrate that a 4-week period of ACE inhibitor treatment in young SHR is sufficient to prevent the full expression of genetic hypertension and cardiovascular hypertrophy and that angiotensin II might be important in the development of hypertension in this model, its role in later life being less important. (Hypertension 1990;16:603-614)

The blood pressure of spontaneously hypertensive rats (SHR) reaches stable hypertensive levels by about 16–20 weeks of age. A wide variety of antihypertensive agents can prevent the rise in blood pressure in SHR during development. However, the effects after treatment is stopped seem to depend on the particular class of treatment. For example, if β-blockers, vasodilators, or calcium antagonists are given throughout development and then discontinued in adult life, blood pressure of SHR returns to high levels equivalent to that seen in untreated animals. However, some antihypertensive regimes do reduce blood pressure long after treatment is discontinued. It has been shown that extended treatment throughout development and into maturity with reserpine, hydralazine, and chlorothiazide or guanethidine and hydralazine, exerts persistent antihypertensive effects. More recently, long-term therapy with angiotensin converting enzyme (ACE) inhibitors has been shown to have similar actions. These observations suggest that during development, some drugs interfere with the factors responsible not only for the rise in blood pressure in young rats but also for hypertension in adult life, although the details of this interaction are unclear. The present study was designed to examine in detail the long-term cardiovascular effects of a brief period of ACE inhibitor treatment in young SHR.

Previously, most preventive treatment schedules have spanned the developmental phase of hypertension, starting in young SHR and continuing until adulthood (i.e., beyond 16 weeks of age). In the
present study ACE inhibitor treatment was restricted to relatively short periods during or after the developmental phase of hypertension to determine whether long-term effects on blood pressure depend on the age at which treatment is commenced or on its duration. We also characterized the basic cardiovascular mechanisms associated with persistent hypotensive effects of ACE inhibitors, assessing the systemic and regional hemodynamics, cardiac weight, and resistance vessel structure. Finally, we tested the long-term effects of replacing angiotensin II during the period of ACE inhibitor treatment in young SHR.

Methods

SHR used in these studies were derived from five breeding pairs purchased from Harlan Olac (Oxfordshire, UK). The average systolic blood pressures for male and female breeding SHR at 16 weeks of age were 229±5 mm Hg and 186±5 mm Hg (mean±SEM), respectively. Once these breeding pairs achieved the consistent production of healthy litters, female pups and occasional runts were culled within a few days of birth. Only healthy male pups were used in these experiments. The male pups were weaned at 4 weeks of age, after which they received standard laboratory chow (RMM, Labsure, Cambridgeshire, UK) and water. All experimental procedures were approved by the British Home Office.

Experiment 1: Effects on Blood Pressure, Growth, and Cardiovascular Hemodynamics

In the first series of experiments, three different treatment periods during development were tested to determine whether the age at which treatment began or its duration was important for the long-term blood pressure effects and to define a "window" period of treatment suitable for later studies.

SHR randomly assigned to receive active treatment were given the ACE inhibitor perindopril (3 mg/kg/day) by gavage each morning. Pups weighing less than 50 g received a daily gavage of perindopril as a volume of 2.5 μl/g body wt (perindopril concentration of 1.2 mg/ml), and pups greater than 50 g received 1.25 μl/g body wt (perindopril concentration of 2.4 mg/ml). The first group received perindopril from 2 to 6 weeks of age (n=8), the second group from 6 to 10 weeks of age (n=10), and the third group from 2 to 10 weeks of age (n=9). Control groups composed of the corresponding littersmates of each treatment group (n=8, 9, and 8, respectively) received an equivalent volume of distilled water. Control and experimental groups were studied concurrently. All rats entering the study survived.

Rats were followed from birth until 25 weeks of age. Body weight was measured each week from 2 weeks of age, and systolic blood pressure was determined weekly by an indirect tail-cuff technique (W & W recorder, model 8005, Basel, Switzerland) from 4 weeks of age in lightly restrained, conscious animals that had been preheated in a 37°C warming chamber. At 25 weeks of age, systemic and regional hemodynamics were assessed in conscious resting rats by standard radiolabeled microsphere techniques, with modifications according to Russell et al. Two rats, one treated and one control, were studied during each experimental session, and the order of study at each session was alternated to avoid any potential systematic bias. All rats underwent brief methohexitone anesthesia (40 mg/kg) for insertion of polyethylene (PE-10) tail artery and left ventricular catheters. The left ventricle was approached from the right carotid artery, and correct placement within the ventricle was confirmed at the time of insertion by the appearance of a consistent characteristic left ventricular pressure tracing. Placement was adjusted to avoid cardiac dysrhythmia. The position of left ventricular catheters was checked at the end of each experiment. Rats were allowed to recover for 4 hours in a clear Perspex restraining cage. Mean arterial pressure was measured via the tail artery catheter just before the injection of microspheres. The reference sample for the calculation of cardiac output was collected from the tail artery catheter, which was allowed to drain freely into a preweighed tube. Approximately 5 seconds after commencing collection of the reference sample, about 400,000 cobalt-59–labeled microspheres (15 μm in diameter, New England Nuclear Research Products, Boston, Mass.) in a total volume of about 50 μl were injected rapidly into the left ventricle. The reference sample was collected over a period of 1 minute, yielding an average volume of 0.245±0.012 ml (mean±SEM) with a total radioactivity of at least eight times background. Blood was then collected via the tail artery catheter for measurement of plasma angiotensin II10 and plasma renin activity.11 Rats were then killed with an overdose of pentobarbitone so that the kidneys, small intestine, diaphragm, spleen, heart, and brain could be removed, cleaned and weighed, and the organ radioactivity determined. Cardiac index was calculated as (total counts injected/counts in the reference sample) × (reference sample flow rate/body weight) and total peripheral resistance as (direct mean arterial pressure/cardiac index). The percentage distribution of the cardiac output received by individual organs was calculated as (counts in organ/total counts injected) × 100). Individual organ blood flow rates were calculated as (counts in organ/counts in reference sample) × (reference flow rate) and organ arterial vascular resistance as (direct mean arterial pressure/organ flow rate). Both organ blood flow and vascular resistance were corrected according to the weight of the organ in grams.

Experiment 2: Effects of Short or Late Angiotensin Converting Enzyme Inhibitor Treatment

Because of the similarity of long-term effects (see below) in the three treatment groups in the first series of experiments, a second series of experiments was performed to determine whether a very short course of
ACE inhibitor treatment in young rats, or 4 weeks of treatment in mature rats would have similar effects.

The first experiment comprised five SHR given perindopril (3 mg/kg/day) for 1 week only, from 6 to 7 weeks of age; this group was compared with six control littermates receiving distilled water over the same period. In these groups weight and blood pressure were recorded every week from 5 to 10 weeks and then every fourth week until 25 weeks of age.

The second experiment compared SHR given perindopril (3 mg/kg/day) from 20 to 24 weeks of age with controls given distilled water. Unfortunately, because of limited availability of older SHR, only three rats were used in each group. However, the longitudinal repeated-measures experimental design and analysis provided increased statistical power over isolated cross-sectional studies for the same number of subjects. Blood pressure was measured weekly from 19 to 35 weeks of age and finally under general anesthesia (pentobarbitone 40 mg/kg) at 52 weeks of age. Cardiac mass was recorded at this time.

**Experiment 3: Long-term Effects on Blood Pressure and Vascular Structure and Function of Angiotensin II Replacement During Angiotensin Converting Enzyme Inhibitor Treatment**

In the final series of experiments, we tested the effects of replacement of angiotensin II during ACE inhibitor treatment on long-term blood pressure and cardiovascular structure. Based on the results of foregoing experiments the treatment period of 6–10 weeks of age was chosen.

Three groups of SHR were included. The first group were SHR (n=7) receiving perindopril (3 mg/kg/day) orally as before and angiotensin II (200 ng/kg/min, Hypertensin Ciba, Ciba-Geigy Ltd., Basel, Switzerland) in subcutaneous minipumps. The dose of angiotensin II was selected after pilot studies in young normotensive Wistar-Kyoto rats showed that an infusion rate of 200 ng/kg/min produced a slow rise in arterial pressure over several weeks, similar to the spontaneous rise seen in young SHR. The osmotic minipumps were implanted in the interscapular region under brief methohexitone (40 mg/kg) general anesthesia at the beginning of the sixth week of age. On recovery from anesthesia, rats were given their first dose of perindopril. At 8 weeks of age, old minipumps were removed and replaced by new pumps at adjacent fresh sites. After the final perindopril dose at the end of 4 weeks treatment, all minipumps were removed. Angiotensin II was dissolved in 150 mmol/l NaCl solution, and the volume of solution and the concentration of angiotensin II remaining in the minipumps at the end of each 2-week period was checked to ensure adequate delivery and potency of the infusate. A second group of SHR (n=10) received perindopril (3 mg/kg/day) from 6 to 10 weeks of age, but minipumps contained only 150 mmol/l NaCl solution. A third group of control SHR (n=10) received distilled water and saline-filled minipumps from 6 to 10 weeks of age.

Blood pressure was measured in the tail from 4 until 24 weeks of age. At about 32 weeks of age, the rats were anesthetized with pentobarbitone (40 mg/kg) and direct blood pressure recordings were made from the left carotid artery under anesthesia. Hearts were removed, cleaned of blood, and weighed fresh. The abdomen was then opened, and the mesenteric vasculature and small bowel were excised whole. This tissue was used to select resistance vessels of about 150–300 μm in external diameter that were mounted as a ring preparation on an isometric small vessel myograph for measurement of vessel structure and function. Two vessel segments from each rat were tested together in each experiment. Medial cross-sectional area was determined and the vessel was then set to 90% of the internal circumference expected from a transmural pressure of 100 mm Hg (Ld). Lumen diameter was taken as

\[ l = L_d / \pi \]

and the corresponding media thickness \( m_1 \) was calculated on the basis that the medial cross-sectional area remained constant. Vessels were held relaxed in physiological saline solution (PSS) consisting of (mmol/l): NaCl 119, KCl 4.7, NaHCO3 25, CaCl2 2.5, KH2PO4 1.18, MgSO4 1.17, ethylenediaminetetraacetic acid 0.0026, and glucose 5.5. K-PSS was PSS in which NaCl was exchanged for KCl on an equimolar basis. Control activating solution was K-PSS containing 10 mmol/l L-norepinephrine-HCl (Sigma Chemical Co., St. Louis, Mo.), which gives near maximal response. All solutions were bubbled with 95% O2 and 5% CO2 to give a pH of 7.4. Solutions were maintained at 37° C. Isometric contraction responses to control activating solutions were determined. These responses were standardized as active wall tension (increase in wall force—resting wall force divided by 2 times segment length), active media stress (active wall tension/media thickness), and effective active pressure (change in effective transmural pressure in response to isometric contraction), which is an estimate of the pressure against which the vessels would have been able to contract.

Vessels were then exposed to graded concentrations of norepinephrine (0, 0.08, 0.16, 0.32, 0.63, 1.25, 2.5, 5.0, and 10.0 μmol/l) first with and then without cocaine hydrochloride (3 μmol/l), to determine dose–response relations for norepinephrine either in the presence or absence of blockade of neural reuptake. Dose–response curves were used to determine the threshold concentration of norepinephrine that produced a rise in vessel tension equivalent to 10% of the maximal contraction (TH10) and the concentration that resulted in 50% maximal contraction (effective dose, ED50).

**Statistical Analyses**

Longitudinal blood pressure and weight data were analyzed by multivariate analysis of variance (MANOVA) for repeated measures on the MANOVA...
program of SPSS/PC+. Cross-sectional data from experiment 1 were analyzed using the analysis of variance (ANOVA) program of SPSS/PC+ to address the following questions: Does perindopril treatment per se in young SHR effect variables at 25 weeks of age, and if so, does such an effect depend on the period of treatment? For this purpose, the six groups of rats were categorized according to two criteria: 1) the period for which rats were gavaged (i.e., 2–6 weeks, 6–10 weeks, and 2–10 weeks), which reflected different handling and different litters, and 2) the form of treatment given by gavage (i.e., vehicle or perindopril). ANOVA was then used to test for homogeneity between the different gavage periods (vehicle and perindopril rats combined) and for the overall effects (different gavage periods combined) of perindopril versus vehicle. Statistical tests of interaction between the gavage period and the form of treatment were used to determine whether the effects of perindopril were similar in the three groups (2–6 weeks, 6–10 weeks, 2–10 weeks).

Growth

Growth rate comparisons between treated and control SHR before treatment were available only in the group treated from 6 to 10 weeks of age and no difference was noted (Figure 1) ($F_{1,33}=0.12, p=0.729$). In the three perindopril-treated groups growth rate appeared to slow during treatment compared with controls, resulting in significantly lower body weights than controls at the end of treatment (Figure 1). In the rats that received only 4 weeks treatment, growth rate seemed to recover when treatment was stopped, and by the end of the experiment much of the deficit in weight had been regained (Figure 1, Table 1). In rats treated for 8 weeks, the effect on growth was more pronounced, and there was little tendency for these rats to catch up on lost growth after treatment was stopped (Figure 1, Table 1).

In general, changes in the weight of individual organs in those SHR that received ACE inhibitor treatment reflected the changes in body weight, and the weight of most organs expressed per unit body weight was the same in treated and control SHR. This pattern was not seen, however, for the heart and kidneys. In each of the treated groups, heart weight expressed per kilogram of body weight was less than that of the corresponding controls, and the overall effect of treatment was statistically significant (Table 1). In contrast, the relative weight of the kidneys in treated SHR was greater than that of controls (Table 1).

Blood Pressure

As for weight, the three control groups of SHR did not differ significantly in the measurements of indirect blood pressure ($F_{1,22}=2.12, p=0.144$), and the groups were combined for the purposes of analysis.

Results

Experiment I: Effects on Blood Pressure, Growth, and Cardiovascular Hemodynamics

There was no significant difference in the body weight of the three control groups of SHR ($F_{2,22}=1.44, p=0.248$) nor in their change of weight with age ($F_{2,44}=1.11, p=0.310$). Therefore, these groups were combined into one control group to provide a more representative sample of untreated SHR and to increase the statistical power of the comparisons.
TABLE 1. Measurements at 25 Weeks of Age

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>Period of gavage (weeks of age)</th>
<th>ANOVA $p$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2–6</td>
<td>6–10</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>Control</td>
<td>411±8</td>
<td>402±12</td>
</tr>
<tr>
<td></td>
<td>Perindopril</td>
<td>396±14</td>
<td>385±10</td>
</tr>
<tr>
<td>Mean intra-arterial pressure (mm Hg)</td>
<td>Control</td>
<td>163±6</td>
<td>170±4</td>
</tr>
<tr>
<td></td>
<td>Perindopril</td>
<td>140±4</td>
<td>143±3</td>
</tr>
<tr>
<td>Cardiac index (ml/min/100g)</td>
<td>Control</td>
<td>15.8±0.7</td>
<td>18.9±1.7</td>
</tr>
<tr>
<td></td>
<td>Perindopril</td>
<td>19.2±4.0</td>
<td>18.8±1.8</td>
</tr>
<tr>
<td>Total peripheral resistance (units)</td>
<td>Control</td>
<td>10.49±0.63</td>
<td>9.42±0.74</td>
</tr>
<tr>
<td></td>
<td>Perindopril</td>
<td>8.26±1.00</td>
<td>8.14±0.69</td>
</tr>
<tr>
<td>Relative cardiac mass (g/kg)</td>
<td>Control</td>
<td>3.60±0.09</td>
<td>3.61±0.07</td>
</tr>
<tr>
<td></td>
<td>Perindopril</td>
<td>3.47±0.09</td>
<td>3.39±0.03</td>
</tr>
<tr>
<td>Relative renal mass (g/kg)</td>
<td>Control</td>
<td>5.60±0.14</td>
<td>5.65±0.15</td>
</tr>
<tr>
<td></td>
<td>Perindopril</td>
<td>6.19±0.15</td>
<td>5.79±0.16</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Variables measured at 25 weeks of age in spontaneously hypertensive rats (SHR) given vehicle (control) or perindopril for three different periods in youth. Probability values are given from analysis of variance (ANOVA) for tests of differences between control and perindopril groups overall (different gavage periods combined [Treatment]), for tests of homogeneity among the three periods of gavage (control and perindopril SHR combined [Period]), and whether the effects of treatment depended on the period for which it was given (Interaction).

Before treatment, systolic blood pressure of SHR subsequently treated from 6 to 10 weeks of age was not different from control SHR (Figure 2) ($F_{1,32}=1.88$, $p=0.180$). During perindopril treatment there was a reduction in blood pressure in each of the groups compared with controls (Figure 2), and the magnitude of this effect was much the same at any given age, despite differences in the start time or duration of therapy (Figure 2). Blood pressure rose when treatment was stopped, and the rate of rise of blood pressure in each of the treated groups was comparable with the changes in blood pressure seen in the untreated SHR between 5 and 9 weeks of age (Figure 2). However, when the treated groups...
Table 2. Measurements at 25 Weeks of Age of Calculated Arterial Resistance

<table>
<thead>
<tr>
<th>Arterial resistance (units/g tissue)</th>
<th>Treatment</th>
<th>Period of gavage (weeks of age)</th>
<th>ANOVA p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2-6</td>
<td>6-10</td>
</tr>
<tr>
<td>Renal</td>
<td>Control</td>
<td>21.0±1.4</td>
<td>21.3±2.1</td>
</tr>
<tr>
<td></td>
<td>Perindopril</td>
<td>15.7±1.5</td>
<td>16.1±1.3</td>
</tr>
<tr>
<td>Coronary</td>
<td>Control</td>
<td>49.8±8.1</td>
<td>46.4±7.0</td>
</tr>
<tr>
<td></td>
<td>Perindopril</td>
<td>47.5±1.5</td>
<td>34.5±3.4</td>
</tr>
<tr>
<td>Cerebral</td>
<td>Control</td>
<td>168±9</td>
<td>197±23</td>
</tr>
<tr>
<td></td>
<td>Perindopril</td>
<td>142±16</td>
<td>148±11</td>
</tr>
<tr>
<td>Mesenteric</td>
<td>Control</td>
<td>87.0±6.0</td>
<td>96.1±11.1</td>
</tr>
<tr>
<td></td>
<td>Perindopril</td>
<td>71.3±9.7</td>
<td>69.5±4.2</td>
</tr>
<tr>
<td>Splenic</td>
<td>Control</td>
<td>193±24</td>
<td>220±50</td>
</tr>
<tr>
<td></td>
<td>Perindopril</td>
<td>108±19</td>
<td>126±21</td>
</tr>
<tr>
<td>Diaphragmatic</td>
<td>Control</td>
<td>254±68</td>
<td>219±37</td>
</tr>
<tr>
<td></td>
<td>Perindopril</td>
<td>215±36</td>
<td>190±28</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Calculated arterial resistance at 25 weeks of age in spontaneously hypertensive rats (SHR) given vehicle (control) or perindopril for three different periods in youth. Probability values are given for analysis of variance (ANOVA) for tests of differences between control and perindopril groups overall (different gavage periods combined [Treatment]), for tests of homogeneity among the three periods of gavage (control and perindopril SHR combined [Period]), and whether the effects of treatment depended on the period for which it was given (Interaction).

reached an average systolic blood pressure of about 195 mm Hg, blood pressure levels tended to plateau. Beyond 14 weeks of age, there was no tendency for treated or control SHR blood pressures to change, resulting in a difference of about 25 to 30 mm Hg for the remainder of the study (Figure 2). In contrast to the effects on weight, there was no detectable difference in the steady-state blood pressure levels between the rats that were treated for either 4 or 8 weeks (F₁,12=0.46, p=0.639). These findings were confirmed in the mean arterial pressure recordings made in conscious animals at 25 weeks of age (Table 1).

The persistent reduction in blood pressure seen in SHR treated with perindopril appeared to result from a reduction in total peripheral resistance (Table 1), and this effect on systemic resistance did not seem to depend on the timing of treatment in the three groups (Table 1).

The effects of treatment on the arterial resistance of individual organs is shown in Table 2. Although each of the organs showed a trend to reduced resistance, the effect was not uniform. Perindopril treatment was associated with the proportionally greatest change in organ resistance in the spleen (−37.8%), followed by the kidneys (−26.2%), small intestine (−25.5%), brain (−21.3%), heart (−13.1%), and the diaphragm (−9.9%). Effects of treatment on the arterial resistance of the coronary and diaphragmatic beds were not significant (Table 2). Because of the manner in which resistance is derived, this pattern was mirrored by the differences in organ blood flow (expressed per gram organ weight) between the treated and control groups (Table 3). Consistent and significant increases in blood flow were observed in the renal and splenic beds of the treated groups, and similar proportional differences were seen between treated and control SHR in the intestinal and cerebral beds, although these did not reach statistical significance (Table 3). The changes in coronary and diaphragmatic blood flows were neither consistent nor significant across the three treatment groups (Table 3).

The percentage of cardiac output delivered to each vascular bed was not significantly different comparing treated and control SHR, except for the kidneys where the percentage of cardiac output received by the renal bed was significantly higher in treated SHR than in controls (control versus treated: 2–6 weeks, 27.8±1.5 versus 30.4±1.5; 6–10 weeks, 25.6±1.7 versus 29.7±2.1; 2–10 weeks, 25.3±2.2 versus 28.8±2.4; p=0.033 by ANOVA for overall effect of treatment).

No significant changes were seen in either plasma renin activity (control versus treated, ng angiotensin I/ml/min: 2–6 weeks, 216±23 versus 177±26; 6–10 weeks, 172–21 versus 193±29; 2–10 weeks, 233±37

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versus 193±38; p=0.465 by ANOVA for overall effect of treatment) or angiotensin II (control versus treated, pg/ml: 2-6 weeks, 44±7 versus 76±22; 6-10 weeks, 58±17 versus 62±17; 2-10 weeks, 66±18 versus 55±17; p=0.620 by ANOVA for overall effect of treatment) at 25 weeks of age as a result of prior treatment with perindopril. Plasma concentrations of angiotensin II were related to plasma renin activity (r=0.411, p=0.018).

Experiment 2: Effects of Short or Late Angiotensin Converting Enzyme Inhibitor Treatment

Treatment with perindopril for 1 week, from 6 to 7 weeks of age, had no demonstrable effect on weight (MANOVA control versus treated from 8 to 24 weeks of age, F1,17=0.59, p=0.466), and although blood pressure was reduced significantly at the end of the treatment week (control 175±9 mm Hg versus treated 148±10 mm Hg, p<0.01), it returned rapidly to the normal developmental blood pressure track seen in untreated SHR (MANOVA control versus treated from 21 to 24 weeks of age, F1,14=7.5, p=0.001). However, when treatment was stopped blood pressure rose over the ensuing weeks and was not significantly different from control untreated SHR (MANOVA control versus treated from 25 to 36 weeks of age, F1,14=3.0, p=0.156). This finding was confirmed by direct arterial pressure recordings at 52 weeks of age (control 169±9 mm Hg versus treated 167±8 mm Hg), and the heart weight expressed per kilogram was also not significantly different (control 3.266±0.311 g/kg versus treated 3.122±0.226 g/kg), although the certainty of excluding a significant difference is limited in this study.

Experiment 3: Long-term Effects on Blood Pressure and Vascular Structure and Function of Angiotensin II Replacement During Angiotensin Converting Enzyme Inhibitor Treatment

Figure 4 shows the blood pressure of SHR receiving infused angiotensin II with perindopril from 6 to

### Table 3. Measurements at 25 Weeks of Age of Arterial Blood Flow of Individual Organs

<table>
<thead>
<tr>
<th>Organ blood flow (ml/min/g tissue)</th>
<th>Treatment</th>
<th>Period of gavage (weeks of age)</th>
<th>ANOVA p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2-6</td>
<td>6-10</td>
</tr>
<tr>
<td>Renal</td>
<td>Control</td>
<td>7.89±0.35</td>
<td>8.54±0.96</td>
</tr>
<tr>
<td></td>
<td>Perindopril</td>
<td>9.55±1.14</td>
<td>9.50±0.91</td>
</tr>
<tr>
<td>Coronary</td>
<td>Control</td>
<td>3.93±0.62</td>
<td>4.35±0.77</td>
</tr>
<tr>
<td></td>
<td>Perindopril</td>
<td>3.69±0.69</td>
<td>4.51±0.48</td>
</tr>
<tr>
<td>Cerebral</td>
<td>Control</td>
<td>1.09±0.17</td>
<td>1.01±0.08</td>
</tr>
<tr>
<td></td>
<td>Perindopril</td>
<td>1.92±0.11</td>
<td>1.90±0.18</td>
</tr>
<tr>
<td>Mesenteric</td>
<td>Control</td>
<td>2.22±0.34</td>
<td>2.13±0.14</td>
</tr>
<tr>
<td></td>
<td>Perindopril</td>
<td>0.95±0.12</td>
<td>1.16±0.31</td>
</tr>
<tr>
<td>Splenic</td>
<td>Control</td>
<td>1.70±0.39</td>
<td>1.42±0.24</td>
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<tr>
<td></td>
<td>Perindopril</td>
<td>0.93±0.17</td>
<td>0.96±0.17</td>
</tr>
<tr>
<td>Diaphragmatic</td>
<td>Perindopril</td>
<td>0.74±0.10</td>
<td>1.03±0.25</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Arterial blood flow of individual organs expressed per gram of tissue measured at 25 weeks of age in spontaneously hypertensive rats (SHR) given vehicle (control) or perindopril for three different periods in youth. Probability values are given for analysis of variance (ANOVA) for tests of differences between control and perindopril groups overall (different gavage periods combined [Treatment]), for tests of homogeneity among the three periods of gavage (control and perindopril SHR combined [Period]), and whether the effects of treatment depended on the period for which it was given (Interaction).
10 weeks of age. Compared with rats treated with perindopril alone, replacement of angiotensin II prevented the antihypertensive effect of perindopril during treatment (MANOVA perindopril versus perindopril plus angiotensin II from 7 to 10 weeks of age, $F_{1,13}=35.6$, $p=0.001$). In fact, the SHR receiving angiotensin II achieved blood pressure levels of about 220 mm Hg from the very first measurement 4 days after treatment began. More importantly, after the treatment period SHR that had received angiotensin II with the perindopril maintained a blood pressure significantly higher than perindopril-treated rats (MANOVA perindopril versus perindopril plus angiotensin II from 11 to 24 weeks of age, $F_{1,13}=32.9$, $p<0.001$) and no different from control untreated SHR (MANOVA control versus perindopril plus angiotensin II from 11 to 24 weeks of age, $F_{1,14}=0.03$, $p=0.856$).

Table 4 summarizes the findings in 32-week-old SHR treated between 6 and 10 weeks of age with vehicle, perindopril, or perindopril and angiotensin. Direct arterial pressure recordings showed differences consistent with tail-cuff readings at 24 weeks of age. The low blood pressure in perindopril-treated SHR was associated with the lowest heart weight corrected for body weight, as seen previously. Interestingly, the relative heart weight of SHR that had received replacement angiotensin II was not only significantly greater than either the SHR treated with perindopril alone but also greater than that seen in control untreated SHR.

No significant differences in lumen diameters of the mesenteric vessels were noted among the three groups. However, comparisons of control and perindopril-treated SHR showed that the persistent antihypertensive effect of perindopril was associated with
significant reductions of media thickness, media/lumen ratio, and a trend toward lower medial cross-sectional area (Table 4). Although the tension developed by the vessel segments in response to near maximal stimulation was not different when expressed per unit wall thickness (active media stress), the lower media thickness in perindopril-treated SHR was associated with smaller change in transmural pressure (Table 4); that is, reduced contractility compared with controls.

SHR that had received angiotensin II in addition to perindopril between 6 and 10 weeks of age seemed to exhibit a greater degree of cardiovascular hypertrophy than either of the other two groups, with the highest values of relative cardiac mass, media thickness, media/lumen ratio, and media cross-sectional area. In addition, the contractility of the vessels was significantly greater than the control and perindopril-treated groups (Table 4), which in view of the similar levels of active media stress, was probably the result of thicker vessel walls.

The dose–response relations for norepinephrine did not reveal any differences among the three groups (Table 5). The blockade of norepinephrine reuptake in the presence of cocaine lowered both TH_10 and the ED50 concentrations, but there was no differential effect of cocaine between the groups. These findings also suggest that the differences in contractility among the three groups were more likely to be the result of structural changes than changes in the sensitivity of smooth muscle cells to norepinephrine.

### Discussion

These studies have revealed that even relatively brief periods of ACE inhibitor treatment in growing SHR can have long-term consequences for the level of steady-state blood pressures in mature animals. It is worth noting that although the timing of ACE inhibitor administration is well defined, the period of enzyme inhibition, especially in target tissues such as the kidneys and blood vessels might extend a week or so beyond the cessation of treatment.15 Nevertheless,
treatment for only 4 weeks, beginning at either 2 or 6 weeks of age, was sufficient to cause an important reduction in systolic blood pressure of about 25–30 mm Hg that showed no signs of reversing throughout the follow-up, which consisted of as much as 22 weeks observation after treatment was stopped in experiment 3. Measurement of intra-arterial pressure at the end of the experiment confirmed these differences (Tables 1 and 4), showing that differences in systolic pressure were not an artifact of measuring pressure in the tail.

Treatment with ACE inhibitors for 8 instead of 4 weeks did not result in any greater reduction in blood pressure, and previous studies in which perindopril was given for 12 weeks from 4 to 16 weeks of age or for 20 weeks from 4 to 24 weeks of age produced a similar difference in arterial pressure comparing treated and control SHR. These observations suggest that in quantitative terms, the long-term effects on blood pressure are not directly proportional to the period of blood pressure reduction during treatment, nor as others have shown, to the degree to which blood pressure is lowered during treatment.

We did not test in this experiment the importance of reduced arterial pressure during treatment as a cause of the hypotension that persists when treatment is withdrawn. However, other workers have assessed this, comparing equipotent hypotensive agents of different class. Their results suggest that persistent hypotensive effects are more likely with ACE inhibitors than with other drugs.

Although there appears to be little difference in the long-term effects on blood pressure in SHR treated with ACE inhibitors for periods ranging from 4 to 20 weeks, no long-term effect was seen when perindopril treatment was given to young SHR for only 1 week, nor in animals that were treated for 4 weeks in later life, the latter consistent with previous observations. Possibly ACE inhibitor treatment must exceed a threshold, somewhere between 1 and 4 weeks, and be given during development to result in a persistent interference with the full expression of hypertension. The reasons for the apparent increased susceptibility of young SHR to the long-term effects of ACE inhibitors are uncertain, but perhaps it is more difficult to reverse the hypertensive process once present in older SHR than to prevent in young animals the full expression of genetic predisposition to high blood pressure.

The apparent all-or-nothing nature of this ACE inhibitor effect suggests that treatment negates a factor that exerts a qualitative rather than quantitative effect on blood pressure, for example, a genetic abnormality. Cross-breeding studies have identified several genetic factors such as vascular smooth muscle cell reactivity, sympathetic nerve activity, and renal hemodynamics, each of which might be negated by ACE inhibitor treatment. Interestingly some of these abnormalities are present only in the young SHR, and it has been suggested that as a result of developmental stage-specific regulation, genetic hypertensive factors are active preferentially in youth, accounting for the rapid rise in pressure, and that in mature animals, high blood pressure might be maintained by nonspecific structural adaptation of the cardiovascular system. In this case, prevention of the consequences of gene expression in youth might have far-reaching effects. If angiotensin II is an integral part of the genetic hypertensive mechanisms in young SHR, either directly or via interaction with the sympathetic nervous system, this might explain why brief ACE inhibitor treatment in the young SHR reduces blood pressure in the long-term and might also account for the apparent absence of long-term effects when treatment is given to older SHR.

Our third experiment tested the role of reduced angiotensin II in young perindopril-treated SHR. Infusion of the peptide at a dose that has only minor direct pressor action in the normal rat reversed completely the hypotensive effect of ACE inhibitor treatment but, more importantly, also reversed the persistent hypotensive effect. This suggests quite strongly a role for angiotensin II in causing the persistent effect, but again the point is not established. Other pressor agents should be tested to ensure that the effect is specific for angiotensin II.

The long-term effects of angiotensin replacement are found not only for blood pressure, but also for vascular structure. In fact, the replacement of angiotensin II (and blood pressure) in young SHR not only prevented the reduction in cardiovascular hypertrophy seen in later life when perindopril alone was given, but it also seemed to increase the average cardiac and resistance vessel wall size compared with untreated control SHR, despite similar levels of blood pressure. This supports further the contention that angiotensin in young SHR can affect cardiovascular growth independent of blood pressure, perhaps either directly or through interactions with the sympathetic nervous system.

The exact mechanisms resulting in the long-term hypotensive effects after ACE inhibitor treatment remain unclear. Previous studies have shown that sodium balance and cardiac output are normal during treatment and that when treatment is stopped total body sodium remains stable. The present studies reveal that long after treatment is stopped, cardiac output is not significantly altered and that the basis of the lower blood pressure is a reduction in vascular resistance. This might be the result of reduced tone in the vascular smooth muscle cells or reduced contractility as a consequence of structural changes.

Reduced vascular tone may result from changes in the stimulus to contraction or the sensitivity of vascular smooth muscle to pressor stimuli. In the present study, no long-term changes were detected in circulating elements of the renin-angiotensin system that might affect vascular tone, but data on local elements of this system and other vasoactive substances are not available. Changes in the local renin-
angiotensin systems in organs such as the brain, kidneys, or blood vessels\(^5\) might be important in this regard. For example, it has been demonstrated that SHR have increased levels of components of the renin-angiotensin system within the brain,\(^9\) and that intracerebroventricular infusion of captopril, at a dose that has no effect when administered intravenously, can prevent the development of hypertension.\(^30\)

In terms of local sensitivity to pressor substances, the dose–response relations of the mesenteric resistance vessels to norepinephrine do not suggest any alteration as a result of prior treatment with perindopril, a finding that has been noted in other studies after long-term ACE inhibition.\(^17\) This does not necessarily imply, however, that vascular sensitivity in other vascular beds or to other pressor stimuli is not affected in the long-term by brief treatment with ACE inhibitors in young SHR.

The results of this study do provide evidence in favor of the structural hypothesis. There are two elements to this hypothesis: structural vascular change may be produced by increased arterial pressure and, when produced, may raise pressure further, initiating positive feedback amplification.\(^31\) An ACE inhibitor may, by lowering pressure, interrupt progression of the hypertension after treatment with the inhibitor is stopped. A second possibility is that angiotensin II causes structural vascular change directly by a mitogenic or trophic action, which also initiates positive feedback as above\(^32\) and that ACE inhibitors halt the progression of the hypertension by reducing angiotensin II. Our results are compatible with these ideas, but we have not assessed the role of structural change in the elevation of arterial pressure, nor have we distinguished direct effects of infused angiotensin II on structure from secondary effects that are the consequence of increased pressure caused by angiotensin II.

The apparent lack of uniformity in the resistance changes in individual organ beds is interesting. The splenic arterial bed showed large proportional reductions in arterial resistance as a result of prior treatment, but its contribution to the total peripheral resistance is likely to be small in view of relatively low blood flow. However, the large changes in renovascular resistance, which have been reported previously using a different methodology,\(^8\) are important probably in the overall reduction in total peripheral resistance as the kidneys account for about 30% of the cardiac output. The absence of significant changes in the resistance of the coronary and diaphragmatic beds suggests that local factors might be important in determining the response of individual arterial systems to the long-term consequences of ACE inhibition in young SHR.

Unfortunately no data are available on resistance vessel structure in different arterial beds, which would be of particular interest in view of the lack of uniformity in the change in arterial resistance. If, for example, the vascular structure correlated with the individual organ resistance, then it would suggest that the structural changes were not just a generalized adaptive response to the lower blood pressure in treated SHR but might represent a vessel-specific sensitivity to ACE inhibitor prevention of vascular hypertrophy. This might be related, for example, to the activity of the local renin-angiotensin system or the sympathetic nerves.\(^27\)

These studies raise important questions regarding the ontogeny of blood pressure in the SHR, in particular the role of events in young developing rats that appear to depend on angiotensin II. Although our experiments have not established the mechanism of the hypotensive effect that persists on withdrawal of ACE inhibitors, in particular the primary or secondary role of cardiovascular hypertrophy, the existence of this effect is clear. These observations, and the definition of a convenient model in which to study these effects by treating SHR from 6 to 10 weeks of age are likely to provide stimulus for further studies of the ways in which ACE inhibitors interact with the genetic factors responsible for high blood pressure, increasing our understanding of the genesis of hypertension. These experiments raise the possibility that it might also be possible to prevent or reduce the severity of hypertension by treatment, which may not necessarily be life-long, although the long-term effects on stroke, heart failure, and mortality remain to be defined.

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