Angiotensin II, Sodium, and Cardiovascular Hypertrophy in Spontaneously Hypertensive Rats

Stephen B. Harrap, Glenn A. Mitchell, David J. Casley, Christine Mirakian, and Austin E. Doyle

Angiotensin II (Ang II) may cause cardiovascular hypertrophy as a consequence of increased blood pressure or possibly by direct trophic actions. To dissociate Ang II and blood pressure in young spontaneously hypertensive rats (SHR), we used sodium loading during angiotensin converting enzyme inhibitor treatment. Animals were treated between 6 and 10 weeks of age with perindopril to lower Ang II and blood pressure, or with perindopril and 1% saline drinking fluid or perindopril and aldosterone infusion to lower Ang II but maintain high blood pressure. Blood pressure, heart weight, and media/lumen ratio of mesenteric resistance arteries were studied while rats were on treatment at 10 weeks of age and 15 weeks after treatment at 25 weeks of age. Perindopril lowered blood pressure and inhibited the development of cardiovascular hypertrophy. Saline or aldosterone restored high blood pressure during perindopril treatment and resulted in increased heart weight/body weight and resistance artery media/lumen ratios in direct proportion to the elevation of blood pressure. Because increased structure occurred despite perindopril treatment, we conclude that direct trophic actions of Ang II are not essential for the development of cardiovascular hypertrophy in young SHR and that the antitrophic actions of angiotensin converting enzyme inhibitors depend more on changes in blood pressure than on Ang II. However, restoration of blood pressure and structure by sodium during perindopril treatment raises the possibility that the design of the cardiovascular system and blood pressure may depend indirectly on Ang II through effects on sodium metabolism. (Hypertension 1993;21:50-55)

**KEY WORDS** • salt • angiotensin II • blood pressure • hypertrophy • angiotensin converting enzyme inhibitors • rats, inbred SHR

It has been suggested that the effects of angiotensin II (Ang II) on cardiovascular structure are important determinants of blood pressure in spontaneously hypertensive rats (SHRs).\(^1\)\(^-\)\(^4\) The actions of Ang II may be especially important before adulthood because treatment of young SHRs with angiotensin converting enzyme (ACE) inhibitors disrupts the development of hypertension and reverses cardiovascular hypertrophy. A 4-week course of treatment resets the tracking of SHR blood pressure to approximately 20–30 mm Hg lower than untreated control SHRs.\(^1\) A specific effect of Ang II is suggested by the observations that nonpeptide Ang II antagonists have the same blood pressure and structural effects as ACE inhibitors\(^5\) and that replacement of Ang II during ACE inhibitor treatment in young SHRs restores hypertrophy and prevents resetting of SHR blood pressure.\(^1\)

However, in most previous SHR studies, changes in Ang II, blood pressure, and structure tend to occur in parallel, and it has not been possible to ascribe hypertrophy to a direct effect of Ang II rather than to a nonspecific adaptation to high pressure levels. It has been shown that sodium loading antagonizes the antihypertensive effects of ACE inhibitors.\(^6\) The aim of this study was to suppress Ang II while preventing the fall in blood pressure, by the combined use of sodium loading and the ACE inhibitor perindopril, and then to examine the effects on cardiovascular structure.

**Methods**

Male SHRs from a colony maintained in the Genetic Physiology Unit were used in these experiments. These SHRs were derived originally from National Institutes of Health SHR stock and are subject to regular tests with polymorphic markers to confirm their inbred status. All experiments were approved by the Austin Hospital Animal Welfare Committee.

Five groups of male SHRs were studied when rats were between 5 and 25 weeks of age, and treatment was administered between 6 and 10 weeks of age. Treatment consisted of 1) water by gavage once a day with tap water as drinking fluid \((n=27)\); 2) perindopril \((3 \text{ mg/kg/day})\) by daily gavage with tap water as drinking fluid \((n=17)\); 3) perindopril \((3 \text{ mg/kg/day})\) by daily gavage with 150 mmol/l sodium chloride solution as drinking fluid \((n=17)\); 4) perindopril \((3 \text{ mg/kg/day})\) by daily

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Supported by a grant from Institut de Recherches Internationales Servier, Courbevoie, France. S.B.H. was supported as an R. Douglas Wright Fellow of the National Health and Medical Research Council of Australia.

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Received July 2, 1992; accepted in revised form September 21, 1992.
gavage, plus aldosterone (10 μg/kg/day) delivered subcutaneously via osmotic minipumps, with tap water as drinking fluid (n=18); the rate of aldosterone infusion was chosen to approximate normal aldosterone levels based on the results of previous studies; and 5) water by gavage once a day with 150 mmol/L sodium chloride solution as drinking fluid (n=18). Rats from these five groups took part in either short- or long-term studies.

**Short-term Experiments to 10 Weeks of Age**

SHRs were studied from 5 until 10 weeks of age. While rats were still on treatment at 10 weeks of age, indirect systolic blood pressure, mean arterial pressure, cardiovascular structure, and the plasma renin-angiotensin system were assessed.

All blood pressure measurements were made in the morning, approximately 2 hours after gavage. Measurements of systolic blood pressure were made by the tail-cuff method (model 12-28 BP system, ITTC Inc., Life Science Instrumentation, Woodland Hills, Calif.) after rats had been accustomed to measurement procedures for 2 weeks. Mean arterial pressure was measured in conscious SHRs. Rats were anesthetized briefly with methohexital (40 mg/kg i.p.) for insertion of polyethylene catheters (PE-50) into the left carotid artery. Catheters were exteriorized in the interscapular region, and rats were allowed to recover in individual cages overnight with free access to food and water. The following morning, between 9 AM and 11 AM, rats remained in their own cages and a blood pressure transducer (model DPT 3003-S, Peter von Berg, Munich, FRG) was attached to the intra-arterial catheter with an additional length of PE-50 tubing. Transducer signals were preamplified through a preamplifier (model 7C, Grass Instrument Co., Quincy, Mass.) before analog-digital signal conversion (Maclab/8, Analog Digital Instruments Pty. Ltd., Castle Hill, NSW, Australia) for the recording, storage, and off-line analysis of data. Up to four animals were studied at one time, and the calibration of each transducer was checked daily. Once the animals were resting quietly and blood pressure appeared stable, recordings were made for half an hour with continuous sampling, and the average of these readings was used to estimate mean arterial blood pressure.

After direct blood pressure measurements, blood was collected from the arterial line. The first aliquot of 1.35 ml from each rat was allowed to drain freely into a tube with 0.15 ml angiotensinase inhibitor/anticoagulant solution for measurement of plasma Ang II and plasma renin activity (PRA). The second 1.35 ml aliquot was collected into a tube containing 16 μl of 5,000 IU/ml heparin for measurement of plasma aldosterone (Coat-a-Count Direct RIA, Diagnostic Products Corp., Los Angeles). Plasma for Ang II was extracted on the day of sampling and then stored with plasma for aldosterone and renin determination at −20°C until assayed.

Finally, rats were anesthetized with pentobarbital (50 mg/kg intra-arterially) so that the bowel and mesenteric vasculature could be excised intact. Small arteries of between approximately 150 and 350 μm in external diameter were then selected for morphological study. In both age groups, vessels were selected from the same anatomic site, i.e., the last arterial branch before the mesenteric arterial arcade. The details of these methods using the Mulvany-Halpern small-vessel myograph have been published previously. Media cross-sectional area, lumen diameter, thickness of the media, and the media/lumen ratio were determined. Hearts were removed at the time rats were killed and were weighed fresh.

**Long-term Experiments to 25 Weeks of Age**

In long-term studies, treatment was stopped after the end of the 10th week, and all rats were then given normal laboratory chow and water. Systolic blood pressure measurements were begun at 4 weeks of age and recorded each week between 5 and 25 weeks of age. Body weight was also measured each week. At 25 weeks of age, mean arterial pressure and cardiovascular structure were measured as described above.

**Statistical Analysis**

Longitudinal systolic blood pressure and weight data were compared between groups using repeated-measures analysis of variance (MANOVA, SPSS/PC+). Cross-sectional comparisons were made initially with one-way analysis of variance, followed by Student-Newman-Keuls (SNK) range test for individual group comparisons where the data described a normal distribution. For cross-sectional analysis of PRA, plasma Ang II, and aldosterone, the nonparametric Kruskal-Wallis one-way analysis of variance (ANOVA) was used, and differences between groups were analyzed by the Mann-Whitney U test. A value of p<0.05 was considered significant.

**Results**

**Short-term Experiments**

Perindopril treatment resulted in a substantial reduction in pressure compared with control and saline-treated SHRs (Table 1). Mean arterial pressures of perindopril-treated rats that also received saline or aldosterone were significantly higher than those of SHRs that were given perindopril alone. However, aldosterone resulted in significantly higher pressures than saline in the perindopril-treated rats (Table 1). Systolic and mean arterial pressures of control and saline-drinking SHRs were similar (Table 1).

At 10 weeks of age, the heart weight/body weight ratio of perindopril-treated rats was significantly lower (ANOVA, F_{1,8}=16.2, p<0.0001; SNK, p<0.05) than that of control SHRs but also lower (SNK, p<0.05) than that of perindopril-treated SHRs that received saline or aldosterone (Table 1). Saline alone did not influence heart size. A similar pattern occurred in the media/lumen ratio of mesenteric small arteries (ANOVA, F_{1,8}=3.9, p<0.01), with the smallest values seen in perindopril-treated SHRs (SNK, p<0.05), greatest in control and saline-drinking SHRs, and intermediate levels in perindopril-treated SHRs given saline or aldosterone (Table 1). In perindopril-treated rats, aldosterone resulted in slightly greater media/lumen and heart weight/body weight ratios than saline (Table 1). Although the media cross-sectional area of perindopril-treated rats was lower than any other group, none of the differences was statistically significant (Table 1).

During treatment at 10 weeks of age, perindopril resulted in lower plasma Ang II and aldosterone levels and higher PRA than control SHRs, consistent with the
effects of ACE inhibition (Table 2). However, the reduction in plasma Ang II was not statistically significant. This may reflect insufficient statistical power, but it is also likely that the reactive increase in renin and angiotensinogen was sufficient to overcome the inhibition of circulating ACE during the processing of samples, leading to artificially high plasma concentrations of Ang II. Rats that received saline and perindopril had slightly lower Ang II and aldosterone levels than those that received perindopril alone. The addition of aldosterone to perindopril treatment resulted in the lowest average levels of plasma Ang II, but plasma aldosterone was restored to levels roughly similar to those in control SHRs. Saline treatment alone did not significantly affect plasma Ang II level, PRA, or aldosterone level.

When the mean values of each group at the end of the treatment period were compared, there were significant correlations (Figure 1) between mean arterial pressures and both the heart weight/body weight ratios (Figure 1, top panel: \( r^2 = 0.989, p < 0.01 \)) and media/lumen ratios (Figure 1, bottom panel: \( r^2 = 0.902, p < 0.01 \)). However, no significant correlations were observed between plasma Ang II and these indexes of cardiac and resistance vessel structure (data not shown).

**Long-term Experiments**

Figure 2 shows the body weight of SHRs from 4 to 25 weeks of age. All groups were well matched before treatment (MANOVA, 4–6 weeks of age: \( F_{1,37} = 1.42, p = 0.239 \)). During treatment, the growth rate of the perindopril-treated SHRs slowed significantly compared with control rats (MANOVA, 7–10 weeks of age: \( F_{1,38} = 20.1, p < 0.0001 \)), as seen previously. However, perindopril-treated rats on saline or aldosterone grew normally compared with control or saline-drinking SHRs (MANOVA, 7–10 weeks of age: \( F_{1,38} = 1.08, p = 0.366 \)). After treatment, the perindopril-treated rats regained their weight deficit, and all groups grew at a similar rate; at 25 weeks of age, apart from a slightly higher weight of rats treated with perindopril and aldosterone, no significant differences were observed.

Figure 3 shows systolic blood pressures. Perindopril treatment was associated with a large reduction in blood pressure compared with controls (MANOVA, 7–10 weeks of age: \( F_{1,38} = 200, p < 0.0001 \)). In SHRs that received saline or aldosterone, the hypotensive effect of perindopril was not as large (MANOVA, 7–10 weeks of age: \( F_{1,38} = 63.5, p < 0.0001 \)), although their systolic blood pressures were significantly lower than those of control SHRs (MANOVA, 7–10 weeks of age: \( F_{1,38} = 41.3, p < 0.0001 \)).

The systolic blood pressures of SHRs that received saline alone were slightly but significantly higher than those of control SHRs during the treatment period (MANOVA, 7–10 weeks of age: \( F_{1,38} = 7.41, p = 0.01 \)). After treatment, the blood pressures of perindopril-treated rats rose but remained significantly below those of control SHRs (MANOVA, 14–25 weeks of age: \( F_{1,38} = 142, p < 0.0001 \)). The systolic blood pressures of perindopril-treated rats that received saline or aldosterone were similar (MANOVA, 11–25 weeks of age: \( F_{1,38} = 2.28, p = 0.151 \) and seemed to rise slowly throughout the posttreatment period, remaining significantly higher than those of perindopril-treated SHRs (MANOVA, 11–25 weeks of age: \( F_{1,38} = 36.5, p < 0.0001 \)) but less than those of control SHRs (MANOVA, 11–25 weeks of age: \( F_{1,38} = 43.7, p < 0.0001 \)).

**Table 1.** Variables Measured at 10 Weeks of Age in Five Groups of Male Spontaneously Hypertensive Rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n=8)</th>
<th>Perindopril (n=8)</th>
<th>Perindopril+saline (n=7)</th>
<th>Perindopril+aldosterone (n=8)</th>
<th>Saline (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mm Hg)</td>
<td>179±5</td>
<td>119±6*</td>
<td>155±5</td>
<td>174±7*</td>
<td>185±3</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>168±6</td>
<td>110±8*</td>
<td>129±5</td>
<td>145±4</td>
<td>158±6</td>
</tr>
<tr>
<td>HW/BW (g/kg)</td>
<td>3.70±0.05</td>
<td>3.00±0.03*</td>
<td>3.35±0.05</td>
<td>3.54±0.11</td>
<td>3.71±0.10</td>
</tr>
<tr>
<td>Lumen (μm)</td>
<td>264±20</td>
<td>225±25</td>
<td>258±11</td>
<td>271±12</td>
<td>222±15</td>
</tr>
<tr>
<td>Media (μm)</td>
<td>12.3±1.2</td>
<td>9.3±0.6</td>
<td>10.4±0.5</td>
<td>11.2±0.6</td>
<td>11.2±0.6</td>
</tr>
<tr>
<td>MLR (%)</td>
<td>4.15±0.22</td>
<td>3.22±0.27*</td>
<td>3.70±0.16</td>
<td>3.97±0.22</td>
<td>4.32±0.19</td>
</tr>
<tr>
<td>Area (μm²×10³)</td>
<td>12.5±1.9</td>
<td>9.2±1.0</td>
<td>9.8±0.7</td>
<td>10.3±1.1</td>
<td>9.8±1.1</td>
</tr>
</tbody>
</table>

*Significant difference between perindopril and all other groups.
†Significant difference between perindopril/aldosterone and perindopril/saline, Student-Newman-Keuls test.

**Table 2.** Hormonal Variables Measured at 10 Weeks of Age in Five Groups of Male Spontaneously Hypertensive Rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Perindopril</th>
<th>Perindopril+saline</th>
<th>Perindopril+aldosterone</th>
<th>Saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ang II (pg/ml)</td>
<td>61.1±21.5</td>
<td>44.5±6.5</td>
<td>34.9±11.9</td>
<td>28.6±5.9</td>
<td>52.4±11.5</td>
</tr>
<tr>
<td>PRA (ng Ang I·ml⁻¹·hr⁻¹)</td>
<td>9.6±1.9*</td>
<td>85±17</td>
<td>69±15</td>
<td>21±12</td>
<td>7±2.1</td>
</tr>
<tr>
<td>Aldo (ng/dl)</td>
<td>69±21</td>
<td>24±8</td>
<td>18±4</td>
<td>40±5</td>
<td>48±22</td>
</tr>
</tbody>
</table>

Aldo, plasma aldosterone concentration. Values are mean±SEM.

*Significant difference between adjacent groups by Kruskal-Wallis and Mann-Whitney U tests.
systolic blood pressures of saline-treated SHRs were similar to those of control SHRs.

At 25 weeks of age, the direct mean arterial pressures of the perindopril-treated SHRs were lower than all other groups, but no significant differences were demonstrated between any of the other four groups (Table 3). The heart weight/body weight and media/lumen ratios and media cross-sectional area of SHRs that had received perindopril were lower than any of the other groups, although the differences were less marked than at 10 weeks of age (Table 3). Perindopril-treated rats that had received saline or aldosterone showed the same degree of cardiovascular hypertrophy as control SHRs (Table 3).

Discussion

It has been proposed that the development of cardiovascular structure before adulthood has a major influence on blood pressure in later life. Cardiovascular hypertrophy is characteristic of young SHRs and may be important in this regard. The structure of the heart and vessels in young SHRs seems to be out of proportion to the blood pressure, raising the possibility that nonpressor factors may be responsible for excessive growth of the heart and vessels. Ang II is a candidate here. ACE inhibitors and specific Ang II antagonists lower blood pressure in young SHRs and are effective in reversing hypertrophy. Although Ang II appears to be important, it is unclear to what extent its effects on cardiovascular structure are secondary to changes in blood pressure or are the result of direct trophic effects on cardiovascular cells.

Our study design dissociated changes in Ang II from the hypotensive effects of perindopril treatment by restoring blood pressure with saline or aldosterone. We found that heart weight/body weight ratio and media/lumen ratio of mesenteric resistance arteries were significantly greater when saline or aldosterone was given with perindopril than perindopril alone. In other words, when changes in Ang II and changes in blood pressure are dissociated, structure follows pressure. These findings suggest that, in terms of cardiovascular growth, the effects of perindopril on blood pressure are more important than its effects on Ang II.

When all treatment groups were considered, we found a close linear relation between cardiovascular structure and mean arterial pressure. These observa-
tions are consistent with a simple adaptive response by the heart and vessels to the prevailing blood pressure. The same relation between structure and blood pressure in rats receiving saline or aldosterone with perindopril argues against a major contribution of Ang II per se to structure in these experiments. Ang II production is reduced in perindopril-treated rats, and saline and aldosterone might be expected to enhance this effect. If direct trophic actions of Ang II modulate structure at a given pressure, there should have been less structure for a given pressure in the saline- or aldosterone-treated SHRs receiving perindopril. This was not the case. Other studies have also shown that in hypertensive models characterized by suppression (deoxycorticosterone acetate/salt) or activation (two-kidney, one clip) of the renin-angiotensin system, the degree of hypertrophy shows a consistent relation with blood pressure but not Ang II levels.

The incremental blood pressure effect of saline or aldosterone in perindopril-treated rats raises the possibility that at least part of the antihypertensive effect of ACE inhibitors in young SHRs is mediated through effects on sodium metabolism. We have suggested previously a developmental hypothesis for SHR hypertension, based on abnormalities of the renin-angiotensin system in young SHRs. Sodium retention is associated with increased PRA and reduced glomerular filtration rate and renal blood flow. It is possible to reduce blood pressure in young SHRs using severe sodium restriction. The antihypertensive effects of perindopril in young SHRs are associated with reduced accumulation of body sodium and increased glomerular filtration rate. Therefore, in young SHRs, angiotensin may affect blood pressure via effects on renal sodium metabolism.

The nature of the relation between sodium, blood pressure, and cardiovascular growth cannot be disentangled by these studies. However, both sodium restriction and perindopril cause reduced growth rates, and we have found that these latter effects are reversed entirely by saline or aldosterone. Just as sodium determines growth of the animal as a whole, so may it influence growth of the cardiovascular system. The level of sodium intake can exert important effects on heart weight in certain strains of young rats, in the absence of changes in blood pressure or cardiac output. Sodium loading in SHRs results in hypertrophy of the aorta and small intrarenal vessels, before any changes in blood pressure. The growth of vascular smooth muscle cells in culture is related to the uptake of sodium from the extracellular space, and the distribution of sodium between the intracellular and extracellular spaces affects both contractility and size of vascular smooth muscle cells. If Ang II does play an important role in sodium metabolism in young SHRs, it is possible that sodium may provide a link between Ang II and cardiovascular structure.

### Table 3. Variables Measured at 25 Weeks of Age in Five Groups of Male Spontaneously Hypertensive Rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n=19)</th>
<th>Perindopril (n=9)</th>
<th>Perindopril+ saline (n=10)</th>
<th>Perindopril+ aldosterone (n=10)</th>
<th>Saline (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>194±4</td>
<td>179±3*</td>
<td>196±3</td>
<td>193±6</td>
<td>192±3</td>
</tr>
<tr>
<td>HW/BW (g/kg)</td>
<td>3.54±0.06</td>
<td>3.26±0.10</td>
<td>3.44±0.06</td>
<td>3.45±0.10</td>
<td>3.58±0.08</td>
</tr>
<tr>
<td>Lumen (μm)</td>
<td>301±13</td>
<td>290±25</td>
<td>320±13</td>
<td>302±16</td>
<td>314±9</td>
</tr>
<tr>
<td>Media (μm)</td>
<td>13.0±0.6</td>
<td>10.9±1.6</td>
<td>12.5±0.7</td>
<td>12.5±0.4</td>
<td>12.5±0.6</td>
</tr>
<tr>
<td>MLR (%)</td>
<td>4.41±0.23</td>
<td>3.75±0.37</td>
<td>3.94±0.21</td>
<td>4.25±0.27</td>
<td>4.01±0.25</td>
</tr>
<tr>
<td>Area (μm²×10⁹)</td>
<td>13.2±1.0</td>
<td>10.9±2.2</td>
<td>12.2±1.1</td>
<td>12.4±0.8</td>
<td>13.0±0.7</td>
</tr>
</tbody>
</table>

MAP, mean arterial pressure; HW/BW, heart weight/body weight ratio; lumen, normalized lumen diameter; media, normalized media thickness; MLR, media/lumen ratio; area, media cross-sectional area. Values are mean±SEM.

*Significant difference between perindopril and all other groups, Student-Newman-Keuls test.
As observed previously, once blood pressure and cardiovascular structure are set in youth, they tend to be carried forward into adult life. The persistent blood pressure reduction after ACE inhibitors or angiotensin antagonists may be an example of this process. The pressure and structural effects of sodium loading during perindopril treatment persisted into adulthood and offset the long-term antihypertensive effects of perindopril. It is possible that at least part of the persistent blood pressure effects after perindopril treatment in young SHR may be the result of the induction of a state of relative sodium deficiency that has important effects on growth of the animal generally and the cardiovascular system in particular. Indirect, sodium-dependent growth effects of Ang II in young SHR may represent part of a series of developmental genetically determined events that set, at a critical period, both structure and blood pressure for the life of the animal.

References
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Hypertension. 1993;21:50-55
doi: 10.1161/01.HYP.21.1.50
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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