Angiotensin II Causes Vascular Hypertrophy in Part by a Non-pressor Mechanism

Sheila A. Griffin, William C.B. Brown, Fiona MacPherson, John C. McGrath, Vincent G. Wilson, Niels Korsgaard, Michael J. Mulvany, and Anthony F. Lever

Angiotensin II, when given in low doses, raises blood pressure slowly. When tested in vitro on vascular smooth muscle cells, it has mitogenic and trophic effects; it is not known if it has these effects in vivo. Our purpose was to determine whether vascular hypertrophy develops during slow pressor infusion of angiotensin II and, if so, whether it is pressure induced. Three experiments were done in rats infused subcutaneously with angiotensin II (200 ng/kg/min) by minipump for 10-12 days. Experiment 1: Angiotensin II gradually raised systolic blood pressure (measured in the tail) from 143±2 to 208±8 mm Hg (mean±SEM), significantly suppressing plasma renin and increasing threefold (NS) plasma angiotensin II. There was no loss of peptide in the pump infusate when tested at the end of the experiment. Experiment 2: In the perfused mesenteric circulation, vasoconstrictor responses to norepinephrine, vasopressin, and KCl were enhanced in rats given a slow pressor infusion of angiotensin II, but sensitivity of responses was not altered. This combination of changes suggests that vascular hypertrophy develops during slow pressor infusion of angiotensin II. Experiment 3: Vessel myography was done after angiotensin II infusion with and without a pressor response. Angiotensin II raised systolic blood pressure, increased heart weight, and produced myographic changes of vascular hypertrophy in the mesenteric circulation, increasing media width, media cross-sectional area, and media/lumen ratio. Hydralazine given with angiotensin II prevented the rise of pressure and the cardiac effect but not the vascular changes. Two-way analysis of variance showed that angiotensin II significantly increased media width, media cross-sectional area, and media/lumen ratio, all independent of hydralazine. Thus, although hydralazine inhibits the pressor and cardiac effects of angiotensin II, suggesting a pressor mechanism for the cardiac change, it does not inhibit structural vascular change, which suggests that at least part of the effect has a non-pressor mechanism. (Hypertension 1991;17:626-635)

Increased thickness of the smooth muscle layer of resistance vessels, which is common in hypertension, may be a consequence and a contributory cause of increased arterial pressure.\(^1,2\) According to Folkow\(^1\) and Korner and his colleagues\(^3\), the two processes interact as a vascular amplifier raising arterial pressure. It has been suggested since that the amplifier is set in motion or modified by growth factors altering blood vessel structure.\(^4\)

Angiotensin II (Ang II), a well-recognized vasoconstrictor, may also act as a growth factor. Tested in vitro, it has mitogenic\(^5-7\) and trophic\(^8,9\) actions on vascular smooth muscle cells. It has not been shown to have these effects in vivo, although it may promote new vessel formation.\(^10\)

Infused in animals at a dose below the threshold of its acute vasoconstrictor effect, Ang II raises blood pressure slowly and progressively.\(^11,12\) The mechanism of this response is uncertain (see Discussion). Our aim here was to determine whether vascular hypertrophy develops during the slow response and, if so, whether it is a consequence of increased arterial pressure. Two techniques were used to assess hypertrophy: vascular perfusion,\(^13\) which is an indirect test of hypertrophy in the vasculature as a whole, and myography,\(^14\) which measures structure and function directly but only in small sections of the vasculature. The mesenteric circulation was chosen because it has receptors for Ang II\(^15\); its smooth muscle cells in culture show a mitogenic response to the peptide.\(^6,7\)
and in renal hypertension, mesenteric vessels undergo vascular and cellular hypertrophy.\textsuperscript{16}

Preliminary accounts have been given of the mesenteric perfusion studies\textsuperscript{17} and of the myographic investigation.\textsuperscript{18}

\section*{Methods}

\subsection*{Techniques for Intravenous and Subcutaneous Infusion of Angiotensin II}

\textbf{Intravenous infusion.} Our original aim was to infuse Ang II intravenously in rats as before\textsuperscript{12} but with implanted minipumps rather than external pumps. Using ether anesthesia, 14-day Alzet mini-osmotic pumps (model 2002, Alza Corp., Palo Alto, Calif.) were implanted subcutaneously behind the neck. Ang II was infused into the jugular vein via Silastic tubing (0.064 cm i.d., Dow-Corning, Midland, Mich.). Pressor responses varied, possibly because reflux of blood peptides into the catheter or pump sometimes destroyed infusate Ang II. Ang II concentration in pump infusate after the experiment was often reduced. Priming pumps by immersion in 0.9\% NaCl at 37°C for 14–16 hours after they had been filled with infusate prevented this loss. Intravenous infusion of Ang II ([\textsuperscript{125}I]-Ang II amide, Hypertensin, Ciba-Geigy, Basel, Switzerland) at 40 and 80 ng/kg/min raised systolic blood pressure gradually over 10 days to 179±10 (n=5) and 233±8 mm Hg (n=12), respectively.

\textbf{Subcutaneous infusion.} Subcutaneous infusion of Ang II from minipumps was also tested. Because it gave consistent responses, comparable in timing and magnitude to those produced by intravenous infusion from external pumps,\textsuperscript{12} subcutaneous infusion was used in the experiment to be described.

Pumps were filled with Ang II in 0.9\% NaCl solution at a concentration between 7 and 8 mg/ml depending on the weight of the rat. Pump capacity was 226±0.6 \textmu l (mean±SD), and rate of infusion was 0.5±0.02 \textmu l/hr SD. Thus, a pump containing 8.0 mg Ang II in a rat of 330 g will deliver peptide at 200 ng/kg/min. The rate of infusion was tested in vitro using six primed pumps filled with [\textsuperscript{14}C]inulin (3×10\textsuperscript{6} counts/ml); the increase of effluent radioactivity (and thus volume infused) was accurate and linear for 14 days. The manufacturers note that subcutaneous infusion was likely to be between 170 and 200 ng Ang II/kg/min.

Systolic blood pressure was measured in the tail by recorder (model 8005, W & W Recorders Ltd., Basel, Switzerland). Rats were warmed at 37°C for 15 minutes before measurement; each value used in analysis was the mean of three estimates. Details of the technique and of its validation by comparison with simultaneous intra-arterial recording of pressure is given by Webb et al.\textsuperscript{19}

Using these methods, three experiments were done in male Sprague-Dawley rats aged 10–12 weeks.

\section*{Experiment 1. Subcutaneous Infusion of Angiotensin II: Effects on Arterial Pressure and on Plasma Concentrations of Angiotensin II and Renin With Measurement of Angiotensin II in Minipump Infusate at Conclusion of Experiment}

Twelve rats were infused subcutaneously with Ang II in 0.9\% saline at 200 ng/kg/min; six control rats received saline. On the 10th day, the rats were anesthetized with ether, the abdomen was opened, and a 10 ml blood sample was taken within 30 seconds from the abdominal aorta for estimation of plasma concentrations of Ang II\textsuperscript{20} and renin.\textsuperscript{21} Rats were then killed, pumps were removed, and samples of infusate were taken from the pump for assay of Ang II.

\section*{Experiment 2. Changes in the Isolated-Perfused Mesenteric Circulation After Infusion of Angiotensin II}

Eight rats were infused subcutaneously with Ang II for 10 days as in experiment 1. Eight control rats received saline. On the 10th day rats were anesthetized with pentobarbitone (60 mg/kg) and mesenteric vessels were prepared for perfusion by the method of MacGregor.\textsuperscript{13} Modified Krebs-Henseleit saline solution (KHS, composition below), oxygenated with a 95\% O\textsubscript{2}–5\% CO mixture and warmed to 37°C, was infused intra-arterially by pump (type 502S, Watson Marlow, Falmouth, England) at 3–4 ml/min to achieve a perfusion pressure of 30±2 mm Hg. Change of perfusion pressure was measured by transducer (type 751, Elmocatic, Neilston, Glasgow, Scotland) and blood pressure recorder (type 720, Elmocatic). After a 45-minute equilibration period, 10 \mu M norepinephrine solution in KHS was infused for 4-minute periods until a consistent vasoconstrictor response was obtained. After three satisfactory responses, KHS was perfused for 20 minutes and observations began: noncumulative concentration–response curves were obtained for norepinephrine (0.5–20 \mu M, Sigma Chemical Co., St. Louis, Mo.), arginine vasopressin (0.5–10 nM, Sigma), and KCl (25–125 mM exchanged with NaCl on an equimolar basis). The order of testing was the same in all preparations: norepinephrine, vasopressin, KCl. The “sensitivity” of a preparation to a vasoconstrictor agent was taken as the molar concentration producing 50\% of the maximum pressor response to that agent.

The composition of KHS was (mM) NaCl 118.4, KCl 4.7, CaCl\textsubscript{2} 2.5, MgSO\textsubscript{4}•7H\textsubscript{2}O 1.2, NaHCO\textsubscript{3} 24.9, KH\textsubscript{2}PO\textsubscript{4} 1.2, and glucose 11.1. Na\textsubscript{2}EDTA (23 \mu M) was included in all experiments to prevent oxidative degradation of norepinephrine.

\section*{Experiment 3. Effect of Angiotensin II With and Without Increase of Arterial Pressure on Structure of Mesenteric Resistance Vessels}

Comparison was made of systolic blood pressure, of structure and function of resistance vessels, and of cardiac mass in four groups of rats all receiving subcutaneous infusion for 10 days. Rats in group A
received infusion of 0.9% saline; group B received Ang II in saline; group C received infusion of saline and, in addition, during the infusion hydralazine hydrochloride (Apresoline, Ciba) was added to their drinking water in a dose (220 mg/l) similar to that used by Jespersen et al.\(^{22}\) to lower pressure in spontaneously hypertensive rats (SHR). Rats of this size drink approximately 30 ml water/day, a value we confirmed. Rats of group D were given hydralazine, as above, but with subcutaneous infusion of Ang II in saline. Rats of all groups were killed on the 10th day and myography was done as follows.

Immediately after the rats were killed, the mesenteric bed was rapidly excised and the segment of vasculature supplying the jejunum 8–10 cm distal to the pylorus was placed in ice-cold KHS. Two 1.5–2.5 mm segments of mesenteric vessel, usually a third-order branch, were prepared for myography\(^{14}\); these were mounted on the myograph and set to an internal circumference, \(L_1\), corresponding to 90% of the internal circumference that vessels would have had when relaxed and subjected to a transmural pressure of 100 mm Hg.\(^{14}\) Lumen diameter, \(l_1\), was taken as \(L_1/\pi\). The corresponding media thickness, \(m_1\), was measured by light microscopy of the mounted vessel.\(^{23}\) Media cross-sectional area, \(A\), was calculated from

\[
A = \pi \times m_1 \times (l_1 + m_1)
\]

Active responses were determined from the fifth response of vessels to control activating solution, 10 \(\mu\)mol/l norepinephrine (Sigma) in KHS where KCl was substituted for NaCl on an equimolar basis. This response was expressed as 1) active tension (increase in force divided by wall length), 2) active media stress (active tension/\(m_1\)), and 3) effective active pressure (pressure against which vessels could contract, equal to active tension divided by \(l_1/2\)). Only segments with an effective active pressure greater than 100 mm Hg (13.3 kPa) were accepted for further study\(^{14}\) of 132 segments tested in a preliminary experiment and in experiment 3, only five were rejected for this reason. Where two segments were examined from one animal, the mean value was used in analysis. The sensitivity of vessels to norepinephrine was assessed by performing norepinephrine concentration-response curves as described previously.\(^{24}\) These responses were tested in the presence and absence of the neuronal amine uptake blocker cocaine (3 \(\mu\)mol/l, Sigma).

**Results**

**Experiment 1. Subcutaneous Infusion of Angiotensin II: Effects on Systolic Blood Pressure, Plasma Angiotensin II, Plasma Renin, and Angiotensin II Recovery in Minipump Infusate**

Infusion of Ang II for 10 days produced a slowly progressive pressor response, systolic blood pressure rising by 52 mm Hg as compared with control rats (Figure 1). Plasma renin was markedly and significantly suppressed by the Ang II infusion and plasma.

**FIGURE 1.** Experiment 1: Line graph showing systolic blood pressure (BP) measured in the tail of 18 rats (mean±SEM). On seventh day, subcutaneous osmotic minipumps were implanted. In 12 rats these pumps delivered angiotensin II (Ang II) at 200 ng/kg/min; in six control rats, they delivered 0.9% saline, the diluent for Ang II.

Ang II concentration was, on average, three times greater than that in saline-infused controls. However, somewhat surprisingly (see Discussion), the difference between groups did not reach significance; variation in the Ang II group was large (Table 1). Similar values of plasma Ang II with similar variability occur during intravenous infusion of Ang II (see Discussion). Infusate in pumps at the end of the experiment showed no loss of Ang II. The lowest recovery was 79%; the average was 110% (Table 1).

**TABLE 1. Effect of Angiotensin II on Systolic Blood Pressure and Plasma Concentration of Angiotensin II and Renin**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Saline-infused rats ((n=6))</th>
<th>Ang II-infused rats ((n=12))</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>322.2±7.9</td>
<td>311±5.2</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma Ang II conc. (pg/ml)</td>
<td>47.2±4.7</td>
<td>157±95</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma renin conc. (microunits/ml)</td>
<td>299.3±79.9</td>
<td>37.3±12.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ang II conc. in residual infusate ((\mu)g/ml)</td>
<td>8.8 mg/ml</td>
<td>Not tested</td>
<td></td>
</tr>
</tbody>
</table>

Comparison of rats receiving subcutaneous infusion of either saline (group A) or angiotensin II (Ang II) in saline, 200 ng/kg/min (group B). Data are mean±SEM and comparisons are by \(t\) test. The threefold increase of Ang II was also insignificant by Mann-Whitney \(U\) test. Pumps were removed after the experiment, and Ang II was measured in the infusate that remained. On average, it was 110% of initial value, which was 8.0 mg/ml in all pumps. The peptide was unmeasurable in control infusate.
TABLE 2. Effect of Angiotensin II on Systolic Blood Pressure and Response of Perfused Mesenteric Preparation

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Saline-infused rats (n=8)</th>
<th>Ang II–infused rats (n=8)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP</td>
<td>133.1±2.6</td>
<td>198±10.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sensitivity of responses in perfused mesentery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>2.04±0.45</td>
<td>2.59±0.70</td>
<td>NS</td>
</tr>
<tr>
<td>AVP</td>
<td>1.14±0.40</td>
<td>1.16±0.42</td>
<td>NS</td>
</tr>
<tr>
<td>KCl</td>
<td>58.9±3.6</td>
<td>59.9±3.9</td>
<td>NS</td>
</tr>
</tbody>
</table>

Comparison of systolic blood pressure (BP) (mean of last two measurements) in rats infused with saline (group A) or angiotensin II (Ang II) (group B). Values are mean±SEM. Sensitivity is expressed as the concentration (mM) required to produce 50% of the maximum pressor response and is insignificantly different for responses to norepinephrine, arginine vasopressin (AVP), and KCl.

Thus, subcutaneous infusion of Ang II produces a slow pressor response without loss of peptide in the pump, with suppression of plasma renin and with a variable increase of plasma Ang II. Reasons for this variability are discussed later.

Experiment 2. Changes in Isolated-Perfused Mesenteric Circulation Following Infusion of Angiotensin II

As in experiment 1, infusion of Ang II at 200 ng/kg/min raised systolic blood pressure slowly and progressively, on this occasion by 65 mm Hg (Table 2). In the isolated perfused mesenteric circulation, the rate of perfusion needed to maintain pressure in the range 30±2 mm Hg was not significantly different in the two groups (3.8±0.2 in Ang II rats and 3.8±0.3 ml/min SEM in saline rats). Maximal pressor responses to norepinephrine, vasopressin, and KCl were greater in the Ang II group (Figure 2). However, the sensitivity of these responses as assessed by the concentration needed to produce 50% maximal effect was not different (Table 2). This pattern (no change of sensitivity with increased maximal response to three vasoconstrictor agents, each with different mode of action) suggests that structural change develops in resistance vessels of rats infused with Ang II. Differences between groups were greatest at maximal response and least at minimal response (Figure 2), a pattern suggesting that the structural change is an increase of vessel wall thickness, without marked reduction of lumen diameter.25

Experiment 3. Effect of Angiotensin II With and Without Increased Arterial Pressure on Structure of Mesenteric Vessels

Comparison of saline-infused and angiotensin II–infused rats. Infusion of Ang II for 10 days raised systolic blood pressure by 70 mm Hg on average (Figure 3). The rise occurred in all animals; the smallest increase (mean of two final measurements) in a rat of this group (group B) was to 185 mm Hg; the greatest increase in a saline-infused control rat was to 255 mm Hg. Average systolic blood pressure for saline controls (group A) remained unchanged (Figure 3). As compared with these controls, Ang II rats had increased heart weight and their mesenteric vessels showed increases in media thickness, media cross-sectional area, and media/lumen ratio; lumen diameter was not affected (Figure 4, Table 3). This pattern of vascular hypertrophy is similar to that suggested by the vasoconstrictor responses during mesenteric perfusion in experiment 2.

Rats receiving hydralazine with and without angiotensin II. In the second part of the experiment, groups C and D were compared. Hydralazine given with Ang II (group D) prevented the slow pressor response (Figure 3). Systolic blood pressure in hydralazine-treated control rats (group C) was slightly but consistently lower than that in saline controls (group A) and in hydralazine-Ang II rats (Figure 3). Compared with hydralazine controls (group C), hydralazine-Ang II–treated rats (group D) had increased media thickness, media cross-sectional area, and media/lumen ratio; lumen diameter was not affected (Figure 4, Table 3).
area, and media/lumen ratio (Figure 4). The statistical analysis of these differences is described below. Lumen diameter was not different (Table 3).

The first part of experiment 3 (group A compared with group B) shows that Ang II causes structural vascular change. It does not indicate whether or not the change is a consequence of increased arterial pressure. The second comparison (group C versus group D) raises the important possibility that structural vascular change is not wholly attributable to increased pressure. Two-way analysis of variance was done to pursue this (Table 3).

**Analysis of variance.** Interactions between Ang II and hydralazine were tested in a two-way analysis of variance (Table 3). First, where the interaction term $p_2$ is insignificant ($p_2 > 0.05$), there is no significant interaction of Ang II and hydralazine, and their effects are likely to be independent of each other and can be analyzed separately. These analyses are in columns headed "$p$ Ang II" and "$p$ Hydr" in Table 3. In this category it can be seen that: 1) Ang II causes significant increases in media thickness, media/lumen ratio, and media cross-sectional area together with a decrease of media stress, all independent of effects of hydralazine; 2) similarly, hydralazine causes an increase in media stress, independent of the effect of Ang II; and 3) neither Ang II infusion nor hydralazine treatment affected the ability of vessels to develop force (active tension), their ability to contract against pressure (effective active pressure), or their sensitivity to norepinephrine either in the absence or presence of cocaine.

However, Ang II decreased and hydralazine treatment increased the force development per unit area of media (active media stress). Second, where interaction of Ang
II and hydralazine is significant ($p < 0.05$). Ang II has different effects depending on whether hydralazine has been given. In this category it can be seen 1) that the blood pressure increase in Ang II-infused rats without hydralazine is significantly greater than that in Ang II-infused rats receiving hydralazine (Table 3); and 2) that heart weight increases in Ang II-infused rats without hydralazine (+11%), but not in Ang II-infused rats with hydralazine (−1%) (Table 3).

The principal conclusion from analysis of variance is that, although hydralazine inhibits the pressor effect of Ang II, it does not significantly inhibit the effect of Ang II on vascular structure. This suggests that Ang II alters structure by a nonpressor mechanism.
It does not follow that pressure has no effect on structure. Indeed, structural change is more marked in Ang II–infused animals with increased pressure than in Ang II–infused animals without increased pressure (compare groups B and D in Figure 4 and Table 3). Thus, there may be a second pressor component to structural vascular change, although it was not identified as such by an analysis that did suggest a non-pressor component.

Changes of cardiac weight after Ang II infusion make an interesting contrast: heart weight increased when pressure rose with Ang II, but not when the rise was prevented by hydralazine (Figure 4). This and the analysis of variance (Table 3) suggest that the increase of cardiac weight is pressure induced. Neither of these analyses suggests an additional non-pressor component of the effect of Ang II on cardiac weight.

Discussion

Until recently, slow pressor responses to Ang II were produced by intravenous infusion either from external pumps,11,12,26 or from large (10 ml) portable pumps.27 Miniosmotic pumps are simpler for the experimenter and less restricting for the rat. After initial difficulties with minipumps (see Methods), consistent responses were obtained with intravenous infusion. However, subcutaneous infusion was also satisfactory and was chosen because the technique is simpler, the response is equally or more consistent and recovery of Ang II in pump infusate is good (Table 1). Other workers report slow pressor responses to Ang II with intravenous,28,29 subcutaneous,30,31 and intraperitoneal32,33 infusion from minipumps.

Ang II produced marked and consistent suppression of renin with smaller, less consistent elevation of plasma Ang II (Table 1). We have seen the latter during slow pressor infusions of Ang II given intravenously.12 A possible explanation for statistical insignificance of the threefold increase of plasma Ang II in the present experiment is that infusion from the pump is intermittent. Because the half-life of Ang II in plasma is brief (1 or 2 minutes), cessation of infusion will cause a rapid decrease of plasma Ang II, which will be particularly marked when endogenous renin and Ang II are suppressed. The decrease of blood pressure after slow pressor infusion of Ang II is much slower, taking more than 80 minutes to reach control values.12 It follows, if pump delivery is intermittently without marked change of pressure.

The mechanism of the slow pressor effect of Ang II is uncertain. A calcium-dependent process is suggested by the ability of calcium entry blockers to prevent the rise of pressure.31 Sodium retention may be important, but stimulation of the sodium-retaining steroid aldosterone by Ang II seems unimportant27,34; there is less information on the direct sodium-retaining action of Ang II during the slow pressor response. In early experiments, Yu and Dickinson35 prevented the response by blockade of sympathetic nerves. It has been recognized since that Ang II interacts with central and autonomic nervous systems at several levels.26,36,37 One of these interactions could be important in the slow pressor effect of Ang II.26,34,38,39 Not all of the evidence points in this way, however; rats sympathectomized with 6-OH dopamine have unimpaired slow responses to Ang II.29,33 Also, infusion of Ang II at pressor doses in the dog40 and at subpressor doses in humans41 elicits no sign of sympathetic nerve activation.

Apart from their effect on arterial pressure, nervous mechanisms may be involved more directly in causing vascular hypertrophy; intact sympathetic nerves are necessary for full development of vascular hypertrophy in hypertensive animals.42 Also, in the SHR, which has marked43 and early44,45 vascular hypertrophy, sympathetic nerve activity and adrenergic nerve density are increased.46,47 The slow pressor effect of Ang II is augmented in the SHR,29 although the augmentation appears to relate more to a renal than to a nervous action of the peptide.

Two related issues arise from our experiment: the mechanism of vascular hypertrophy developing during the slow pressor response to Ang II and the mechanism of the slow response itself, in particular the role in producing the response of autonomic nerves and vascular hypertrophy. Our data are mainly relevant to the first issue. Although we cannot say whether vascular hypertrophy developing during Ang II infusion contributes to the slow rise of pressure, the ability of hypertrophic vessels to amplify vasoconstrictor effects (which we do show in Figure 2) is part of the process by which vascular hypertrophy may cause hypertension.1,3,46

Our first main finding is that vascular hypertrophy develops within 10 days during continuous infusion of Ang II. The presence of hypertrophy was confirmed by two independent methods, both suggesting that Ang II has a greater effect on wall thickness than on lumen diameter. Schlegel and colleagues38 also show an effect of pressor infusions of Ang II on the response of the perfused mesenteric circulation. Structural vascular change was one interpretation of their findings, although the response to norepinephrine was not enhanced as it was in the present experiment.

Our second finding is that at least part of the hypertrophic response appears to result from a non-pressor mechanism, since preventing the rise of pressure did not inhibit development of significant structural vascular change (Figure 4, Table 3). For reasons given earlier, this does not exclude a second component of hypertrophy, which is a consequence of pressure.

Four points are relevant to these conclusions. First, pressure-induced structural change in a small resistance vessel is caused by pressure in that small vessel. Our estimates are of pressure in larger vessels, and thus we have not excluded the possibility that Ang II or hydralazine influence pressure in small vessels in a way that is not reflected by pressure in larger vessels. A second possibility is that hydralazine
alters vessel structure by an action unrelated to its hypotensive effect (by an action in stimulating sympathetic nerves, for example). This is an unlikely explanation for the structural change produced by Ang II and hydralazine when given together since analysis of variance (Table 3) shows no sign of their interaction. A third possibility is that measurement of systolic blood pressure in the tail (as compared with intra-arterial measurement) overestimates the fall of pressure when Ang II is given with hydralazine (or underestimates the fall when hydralazine is given alone). This is unlikely partly for reasons given below and partly because tests by ourselves and others show good correspondence between tail-cuff and intra-arterial measurements in a variety of states. Fourth and finally, because measurements of pressure were made during the day, we have not excluded the possibility that the slow pressor effect and its response to hydralazine are different at night. We have shown previously, using continuous arterial recording, that the slow pressor response to Ang II is present during the day and night with slightly greater pressure at night. Also, the dose of hydralazine is unlikely to be lower at night, since this is the time at which rats drink most.

Ang II increased cardiac weight and hydralazine prevented increases of pressure and cardiac weight. This and the analysis of variance (Table 3) suggest that the increase of cardiac weight is mainly or wholly pressure induced. The contrast with vascular change is interesting and relevant to two of the arguments above: measurements of systolic blood pressure during the day and in the tail of the rat suggest, on the one hand, a pressure-induced effect of Ang II on cardiac mass and on the other a non-pressor effect on vessel structure. The same measurements can hardly be sufficient to support one conclusion without supporting the other.

Few experiments have tested the ability of Ang II to produce vascular hypertrophy in vivo. As noted earlier, the peptide may cause neovascularization in the cornea of rabbits. Other evidence is less direct: 1) An experiment of ours raises the possibility that vascular hypertrophy is present 1 day after a slow pressor infusion when arterial pressure has returned to control values and at a time when direct vasoconstrictor response to Ang II is greatly enhanced. 2) The Goldblatt two-kidney, one clip model of renal hypertension in the rat is said to depend on Ang II in its early stages. Plasma Ang II is raised at this time and vascular hypertrophy is demonstrable at early and late stages. 3) In the one-kidney, one clip model (less Ang II-dependent, probably), [3H]thymidine uptake into vessel DNA is increased before arterial pressure rises. 4) Angiotensin converting enzyme inhibitor restores both pressure and structure to values seen in the untreated control SHR.

Decreased active media stress of mesenteric small arteries was seen during Ang II infusion (Table 3) and has been seen in other models of hypertension. It is consistent with the possibility that cellular growth is associated with increased expression of a non-contractile "synthetic" phenotype of smooth muscle.

Further in vivo experiments are needed to test the mechanism of the non-pressor effect of Ang II on vessel structure. The mitogenic and trophic actions of the peptide demonstrated in cultured smooth muscle cells (see introduction) could be important as could an effect of Ang II on the intracellular matrix, since interaction of cells and matrix in the vessel wall may influence vessel structure. Another possibility is that Ang II acts indirectly by stimulating synthesis of new receptors for platelet-derived growth factor, thereby potentiating the mitogenic action of platelet-derived growth factor. It may be relevant here that the mitogenic action of Ang II in vitro occurs only in the presence of fetal calf serum, which contains platelet-derived growth factor. A related possibility is that Ang II causes hypertrophy or hyperplasia by stimulating expression of the platelet-derived growth factor gene.

In conclusion, Ang II given in low doses for 10 days in conscious rats raises arterial pressure slowly, causing structural change in resistance vessels and increased cardiac weight. The mechanism of the slow pressor effect is uncertain. The cardiac effect is a likely consequence of increased pressure, but the structural vascular change appears to result, at least partly, from a nonpressor action of Ang II. Although the mechanism of this has not been identified, in vitro studies suggest several possibilities, but these need testing in vivo.

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