Blood Pressure–Independent Cardiac Hypertrophy Induced by Locally Activated Renin-Angiotensin System

Lucia Mazzolai, Jürg Nussberger, Jean-François Aubert, Dorette B. Brunner, Giulio Gabbiani, Hans R. Brunner, Thierry Pedrazzini

Abstract—Cardiac hypertrophy is frequent in chronic hypertension. The renin-angiotensin system, via its effector angiotensin II (Ang II), regulates blood pressure and participates in sustaining hypertension. In addition, a growing body of evidence indicates that Ang II acts also as a growth factor. However, it is still a matter of debate whether the trophic effect of Ang II can trigger cardiac hypertrophy in the absence of elevated blood pressure. To address this question, transgenic mice overexpressing the rat angiotensinogen gene, specifically in the heart, were generated to increase the local activity of the renin-angiotensin system and therefore Ang II production. These mice develop myocardial hypertrophy without signs of fibrosis independently from the presence of hypertension, demonstrating that local Ang II production is important in mediating the hypertrophic response in vivo. (Hypertension. 1998;31:1324-1330.)

Key Words: heart ■ hypertrophy ■ renin ■ angiotensinogen ■ mice, transgenic

Cardiovascular hypertrophy is frequently associated with chronic hypertension. These structural adaptations are thought to represent a compensatory response to enhanced blood pressure and wall stress. However, they also represent a primary risk factor for heart failure. The RAS, and more precisely its biologically active hormone Ang II, is pivotal in the regulation of blood pressure.\(^1\) It also plays an important role in the pathogenesis of hypertension, as shown by the beneficial effect of drugs that inhibit Ang II production or its binding to specific receptors in hypertensive patients. Normalization of blood pressure is usually followed by a regression in cardiovascular hypertrophy,\(^2\) indicating that wall stress is a major inducer of hypertrophy. In addition to this load-induced hypertrophic response, Ang II may directly contribute to the development of hypertrophy via its growth factor properties on smooth muscle and cardiac cells.\(^3,4\) In the heart, Ang II also has been shown to play a role in the development of cardiac fibrosis via induction of fibroblast proliferation and collagen disposition.\(^6,7,9-11\) Hypertrophied cardiomyocytes are characterized by the reexpression of genes encoding protein isoforms that are normally not expressed in the adult ventricle.\(^12,13\) Along this line, Ang II has been demonstrated to induce the rapid activation of immediate-early genes in cardiac myocyte, as well as the expression of genes such as those for the \(\beta\)-MHC and ANF.\(^6,7,10,14\) Therefore, whether the development of cardiac hypertrophy is primarily the result of increased blood pressure or also due to Ang II–mediated cell growth remains unclear.

AGT is the direct precursor of Ang II and is rate limiting in the renin reaction.\(^1\) Plasma AGT concentrations correlate with blood pressure in humans and animals, indicating that high AGT levels may predispose to hypertension.\(^15,16\) These observations suggest that AGT may be a modulator of the activity of the RAS. The major source of plasma AGT is the liver. However, AGT mRNA has also been detected in other tissues, including the heart.\(^17\) Upregulation of the AGT gene in hypertrophied heart has been observed in animal models of pressure overload.\(^1\) Moreover, AGT expression is activated by stretch in isolated cardiomyocytes.\(^18\) Stretched cells release Ang II, which may act as an autocrine/paracrine factor on cardiac cells.\(^19\)

To investigate whether local activation of the RAS could trigger the development of cardiac hypertrophy independently of hemodynamic changes, transgenic mice overexpressing the rat AGT gene specifically in the heart were generated to increase the local activity of the RAS and, in turn, Ang II production. The cardiac-specific promoter of the \(\alpha\)-MHC gene was used to direct AGT synthesis in cardiomyocytes.\(^20,21\) Mice, unlike humans or rats, carry either one- or two-renin genes.\(^22\) The \(Ren-1\) gene is the human counterpart and is primarily expressed in the kidneys. Some strains carry an additional renin gene, named \(Ren-2\), which is also expressed in the kidneys but mainly in the submaxillary glands.\(^22\) The transgenic animals described in the present report develop cardiac hypertrophy and, depending on whether they carry one- or two-renin genes, have either normal or high blood pressure.
Methods

Generation of Transgenic Mice

The transgene, composed of a 1.9-kb rat AGT cDNA (a kind gift of Dr A.R. Brasier, University of Texas, Galveston) coupled to the 5.8-kb mouse α-MHC gene promoter (kindly provided by Dr J. Robbins, University of Cincinnati, Ohio) and containing 1.6 kb of the rabbit bIVS2 intronic sequences, was microinjected into the pronucleus of fertilized eggs from either NMRI (two-renin gene strain) or C57BL/6 animals to generate one-renin gene mice carrying the same transgene number as transgenic NMRI. Animals were handled in accordance with institutional guidelines.

Southern Blot Analysis

For transgenic detection, tail DNA was digested with Bg/II, separated on a 0.7% agarose gel, and transferred by capillarity onto nitrocellulose membrane (Hybond N+, Amersham). Blots were hybridized at 42°C in 50% formamide, 5× SSC, 10× Denhardt, 50 mmol/L phosphate buffer, pH 7.9, 10 mmol/L EDTA, and 1% SDS containing 100 µg/mL denatured salmon sperm DNA, using a randomly radiolabeled DNA probe spanning bIVS2 sequences. Ren-1 and Ren-2 genes were detected by hybridization with a complete mouse renin cDNA after PvuII digestion of tail DNA.

Reverse Transcription–Polymerase Chain Reaction

Transgene expression was assessed by RT-PCR. Total RNA, purified from frozen tissues by the guanidine thiocyanate/cesium chloride gradient technique as described,24 was reverse transcribed into cDNA amplified using transgene-specific primers. Amplification of the mouse GAPDH was used as control. Primer sequences were as follows: transgene, forward 5'-TGT GGT GTG ATG CCT CCT and backward 5'-GCC AAA ATG ATG (AGT-specific) and backward 5'-GCC AAA ATG ATG (AGT-specific); GAPDH, forward 5'-AAG CCC ATC ACC ATC TTC CAG CAG and backward 5'-AGC CCT TCC ACA ATG CCA AAG.

Western Blot Analysis

Hearts were homogenized in 50 mmol/L Tris-HCl, pH 7.5, 150 mmol/L NaCl, 1% NP-40, and 0.1% SDS containing a cocktail of protease inhibitors (Complete, Boehringer), as well as 100 µg/mL TPCK and 100 µg/mL pepstatin A (Sigma). Homogenates were centrifuged at 3000 rpm for 10 minutes at 4°C. Supernatants were collected and spun again at 14 000 rpm for 10 minutes at 4°C. Soluble proteins (40 µg) were separated in 10% SDS-polyacrylamide gels and transferred to nitrocellulose membranes (Hybond ECL, Amersham). Blots were blocked with 10% nonfat dry milk incubated with diluted rabbit anti-rat AGT polyclonal antibody (a kind gift of Dr J. Menard, Paris, France) and peroxidase-conjugated secondary anti-rabbit IgG antibody. Bands were revealed using a specific chemiluminescent detection system (ECL, Amersham).

Some samples were first deglycosylated by incubation for 3 hours at 37°C with 2 U/mL of N-glycosidase F (Boehringer) in 100 mmol/L phosphate buffer, pH 7.5, 25 mmol/L EDTA, 0.1% SDS, and 0.5% NP-40.

Northern Blot Analysis

Total cardiac RNA, extracted as described,24 was separated on a 1.2% agarose-formaldehyde gel (10 µg per lane), transferred to a nitrocellulose membrane (Hybond-N, Amersham), UV cross-linked, and hybridized at 42°C in 50% formamide solution containing 100 µg/mL denatured salmon sperm DNA, using a randomly radiolabeled ANF cDNA. Blots were subsequently stripped and reprobed for GAPDH. For quantification analysis, signals were quantified using an Instant Imager (Hewlett Packard). Results were normalized for the GAPDH signal.

Blood Pressure Measurements

Mice were anesthetized by halothane inhalation (1% to 2% in oxygen). The right carotid artery was exposed through cervical incision. A catheter was inserted into the artery and tunneled subcutaneously to exit at the back of the neck. After 4 hours, blood pressure and heart rate were recorded in conscious mice for 15 minutes by connecting the catheter to a pressure transducer, using a computerized data-acquisition system.

Hormone Measurements

Blood was collected through the arterial catheter into chilled tubes containing EDTA. Plasma was frozen in liquid nitrogen and stored at −70°C until used. Plasma AGT concentrations were measured using an indirect method.25 Briefly, plasma aliquots were diluted in 20 mmol/L phosphate buffer, pH 6.0, containing 30 mmol/L EDTA and 5 mmol/L p-phenanthroline and incubated for 60 minutes at 37°C in the presence of an excess of semipurified mouse submaxillary renin. The concentrations of Ang I produced were determined using a sensitive radioimmunoassay.26 PRCs were measured using a modified microassay based on Ang I trapping by antibody.26 Briefly, aliquots of plasma samples were incubated for 15 minutes at 37°C in the presence of nephrectomized rat plasma as a source of AGT and anti-Ang I rabbit antiserum. Ang I concentrations produced were determined by radioimmunoassay.26 PRA was similarly measured but without AGT addition.

CWI, Histological Analysis, and AT₁ Antagonist Treatment

Heart wet weight was determined without the atria. CWI was calculated as the ratio of ventricle wet weight (milligrams) to body weight (grams). Tissues were either snap-frozen in liquid nitrogen for RNA purification or fixed in 10% neutral buffered formalin. For histological examination, sections (4 µm) were stained with hematoxylin and eosin or Masson’s trichrome. For immunohistochemistry analysis, sections were stained with a monoclonal anti–Ang II antibody followed by polyclonal antibodies conjugated to FITC.

Ang II blockade was obtained by giving the mice 1.005 mg/mL of losartan in drinking water for 4 weeks.

Statistical Analysis

Results are expressed as mean±SE. Statistical analysis was performed by ANCOVA.

Results

Generation of Transgenic Mice

To increase cardiac angiotensin production, transgenic mice that overexpress AGT specifically in the heart were generated. The TG153 line was produced in a (C57BL/6×BALB/c) background, and these mice carry a single renin gene (Ren-1). In contrast, the TG101 line was established in

---

Selected Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGT</td>
<td>angiotensinogen</td>
</tr>
<tr>
<td>AT₁</td>
<td>angiotensin II type 1 receptor</td>
</tr>
<tr>
<td>ANF</td>
<td>atrial natriuretic factor</td>
</tr>
<tr>
<td>BW</td>
<td>body weight</td>
</tr>
<tr>
<td>CFI</td>
<td>cardiac function index</td>
</tr>
<tr>
<td>MHC</td>
<td>myosin heavy chain</td>
</tr>
<tr>
<td>PRA</td>
<td>plasma renin activity</td>
</tr>
<tr>
<td>PRC</td>
<td>plasma renin concentration</td>
</tr>
<tr>
<td>RAS</td>
<td>renin-angiotensin system</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>reverse transcription–polymerase chain reaction</td>
</tr>
</tbody>
</table>

---

Mazzolai et al June 1998 1325
NMRI mice, which are prototypes of two-renin gene animals (Ren-1, Ren-2). The Southern blot presented in Figure 1B demonstrates the presence of a 1.6-kb transgene-specific band in DNA from the two lines of transgenic animals. Quantitative analysis demonstrated that five copies of the transgene were integrated in TG153 transgenic mice and 30 copies in TG101 animals. A third transgenic line, named TG1306, was created by crossing TG101 with C57BL/6 mice to generate one-renin gene animals bearing a high copy number of the transgene. All transgenic mice appeared to develop normally. The percentage of transgenic mice among the population, as well as that of males and females, suggests a mendelian type of inheritance.

Transgene Expression

Tissue-specific transgene expression was assessed by RT-PCR (Figure 2A). No transgene expression was observed in tissues from control mice. In contrast, the presence of a transgene-specific product was readily detected in the hearts of transgenic animals. None of the other tissues tested appeared to express the transgene, demonstrating cardiac specificity. Specific amplification of GAPDH mRNA ensured the presence of RNA in all samples.

Cardiac and Plasma AGT and Cardiac Ang II

Western blot analysis of total heart proteins demonstrated the presence of rat AGT in the heart of transgenic mice (Figure 2B). The intensity of the signal was stronger in samples from TG101 mice, which carry more transgene copies, than that observed in TG153 animals. The transgenic protein did not migrate in the gel at the same level as the naturally occurring peptide from rat plasma. To assess whether this was due to a difference in glycosylation, samples were treated with N-glycosidase F. In this case, the two proteins comigrated in the gel. Similarly analyzed plasma samples revealed the presence of the transgenic protein in the plasma of transgenic animals (data not shown). Furthermore, an increase in plasma AGT concentrations, although not significant, was constantly demonstrated in transgenic mice (Table 1). Finally, endogenous AGT expression did not appear to be upregulated in the liver of transgenic animals (data not shown).

To investigate whether increased AGT synthesis in the heart results also in increased cardiac Ang II levels, Ang II–specific immunohistochemical analysis was performed. Indeed, heart sections from transgenic mice demonstrated increased immunofluorescent staining to Ang II compared with control hearts (Figure 3).

Plasma Renin Values

Depending on the presence of one- or two-renin gene in their genome, mice demonstrated different plasma levels of renin. PRCs were always 10 to 25 times higher in control two-renin gene animals than those measured in mice carrying a single renin gene (Tables 1 and 2). In addition, a decrease in renin secretion was observed in one-renin gene transgenic mice compared with normal littermates (Tables 1 and 2). Because AGT has been shown to be rate limiting in the renin reaction, we studied the effect of increased plasma AGT levels on renin activity. One-renin transgenic mice have normal PRA despite increased plasma AGT levels. On the other hand, PRA is elevated in transgenic TG101 (Ren-1, Ren-2) mice (Table 1).
TABLE 1. Parameters in Mice With Cardiac AGT Overexpression and Normal Littermate Controls

<table>
<thead>
<tr>
<th>Line</th>
<th>Sex</th>
<th>Group</th>
<th>n</th>
<th>BW, g</th>
<th>CWI, mg/g</th>
<th>Plasma AGT, nmol/L</th>
<th>PRC, ng Ang I·mL⁻¹·h⁻¹</th>
<th>PRA, ng Ang I·mL⁻¹·h⁻¹</th>
<th>MBP, mm Hg</th>
<th>HR, bpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG153</td>
<td>Female</td>
<td>Control</td>
<td>16</td>
<td>21±1</td>
<td>3.8±0.1</td>
<td>300±10</td>
<td>700±200</td>
<td>7.4±0.8</td>
<td>118±2</td>
<td>700±20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Transgenic</td>
<td>10</td>
<td>22±1</td>
<td>3.9±0.1</td>
<td>300±10</td>
<td>700±200</td>
<td>7.4±1.0</td>
<td>113±2</td>
<td>700±30</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>Control</td>
<td>12</td>
<td>25±1</td>
<td>3.8±0.1</td>
<td>300±10</td>
<td>1400±300</td>
<td>7.0±2.0</td>
<td>115±3</td>
<td>680±20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Transgenic</td>
<td>11</td>
<td>26±1</td>
<td>3.9±0.1</td>
<td>300±10</td>
<td>300±80†</td>
<td>7.0±1.0</td>
<td>117±1</td>
<td>690±20</td>
</tr>
<tr>
<td>TG101</td>
<td>Female</td>
<td>Control</td>
<td>13</td>
<td>33±1</td>
<td>3.4±0.1</td>
<td>210±40</td>
<td>5600±2000</td>
<td>10.1±1.0</td>
<td>119±2</td>
<td>660±30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Transgenic</td>
<td>18</td>
<td>32±1</td>
<td>3.9±0.1†</td>
<td>290±30</td>
<td>2200±500</td>
<td>19.0±2.0*</td>
<td>130±3†</td>
<td>630±20</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>Control</td>
<td>12</td>
<td>37±2</td>
<td>3.7±0.1</td>
<td>80±30</td>
<td>19 000±7000</td>
<td>6.0±1.0</td>
<td>122±2</td>
<td>660±30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Transgenic</td>
<td>13</td>
<td>38±1</td>
<td>4.6±0.1†</td>
<td>130±50</td>
<td>15 000±3000</td>
<td>20.0±5.0†</td>
<td>160±7†</td>
<td>680±30</td>
</tr>
</tbody>
</table>

BW indicates body weight; MBP, mean blood pressure; and HR, heart rate.

*P<0.05; †P<0.01.

Blood Pressure and Heart Rate Measurements

The hemodynamic effects of cardiac AGT overexpression were assessed by measuring blood pressure and heart rate. Blood pressure remained normal in TG153 animals, whereas it was elevated in TG101 mice (Tables 1 and 2). Transgenic TG1306 mice carrying the Ren-1 gene only did not demonstrate a significant elevation of blood pressure despite the fact that they carried the same transgene number as TG101 mice (Table 2). Moreover, monitoring of blood pressure over 24 hours did not show any difference between TG1306 mice and their normal littermates (data not shown). No variations in heart rate were found in transgenic mice.

Cardiac Hypertrophy

The effects of AGT overexpression on the development of cardiac hypertrophy were investigated in adult mice of various ages up to 20 weeks. Hypertensive animals from the TG101 line present a significant increase in cardiac mass in both males (24% increase in CWI) and females (14% increase) when compared with matched control mice. Cardiac hypertrophy is less evident in normotensive TG153 mice (Table 1). However, when male mice at two different ages are compared (Table 2), a significant hypertrophy is observed in 20-week-old normotensive TG153 animals (10% increase in CWI) while no increase in CWI is seen at 8 weeks after birth. Interestingly, transgenic mice of the TG1306 strain, also carrying one renin gene only but a higher copy number of the transgene, develop a significant hypertrophy at as early as 8 weeks (18% increase in CWI) while blood pressure stays within a normal range (Table 2). Female mice follow the same pattern as male animals (data not shown). To test whether the development of cardiac hypertrophy was Ang II dependent, transgenic male mice from line TG1306 were treated or not treated with an AT1 receptor antagonist for 4 weeks. Treatment of transgenic mice resulted in a regression of cardiac mass to normal values (Figure 4). Cardiac hypertrophy in transgenic mice was characterized by enlarged cardiomyocytes. However, we have not seen any significant development of fibrosis after examination of hematoxylin and eosin–stained slides, even in 33-week-old hypertensive animals. This observation was confirmed by Masson’s trichrome staining. Moreover, interstitial fibroblasts did not show any expression of α-smooth muscle actin, the actin isoform typical of fibroblastic cells (data not shown). The induction of the ANF gene was readily demonstrated in the heart of transgenic mice (Figure 5A). Quantitative analysis showed that cardiac ANF expression was significantly increased in transgenic hearts compared with their respective controls (Figure 5B).

Discussion

The results presented here demonstrate that increased local AGT synthesis triggers the development of cardiac hypertrophy. Because both hypertensive and normotensive animals show cardiac hypertrophy, the hypertrophic response in these models does not depend on increased load. Cardiac AGT from transgenic origin appears differently glycosylated than the naturally occurring peptide from rat plasma. Nevertheless, the primary structure seems conserved, since the two proteins comigrate in SDS-gel after deglycosylation. Different glycosylated forms of AGT have been described, and glycosylation does not affect the rate of cleavage by renin. The transgenic mice with cardiac AGT overexpression also have a constant, although moderate, increase in plasma AGT concentrations. It is noteworthy that transgenic AGT is also detected in the plasma (data not shown), thus suggesting that cardiac production contributes to plasma levels in these models.

The amounts of AGT measured in the plasma depend on the rate of protein synthesis and enzymatic cleavage by renin. High renin concentrations are found in two-renin gene mice in which, unlike one-renin gene animals, submaxillary glands represent the main source of circulating enzyme. Renin secretion from the submaxillary glands is under the control of stimuli different from those known to stimulate renin release from the kidneys. These stimuli include stress induced by male aggressive behavior. High renin concentration is probably responsible for the lower plasma levels in TG101 mice, even though these animals carry more transgene copies. PRA depends on the levels of both renin and AGT. Transgenic one-renin gene animals show normal PRA, whereas, in the two-renin-gene strain, transgenic mice have increased PRA values (Table 1). It is likely that in two-renin gene mice, constitutive renin secretion from the submaxillary glands keeps levels of PRC and PRA high, whereas in one-renin gene animals, the Ang II–mediated feedback downregulates...
renin release from the kidneys, maintaining normal PRA values. Indeed, a decrease in renin secretion was observed in one-renin gene transgenic mice (Tables 1 and 2). These results demonstrate that no chronic activation of the RAS occurs in the plasma of transgenic one-renin gene mice.

Hypertension is observed in transgenic TG101 mice that demonstrate increased PRA values (Table 1). Blood pressure is particularly high in males. It is noteworthy that PRC was maximally increased in these animals (Tables 1 and 2). Transgenic TG1306 mice, despite the presence of the same transgene number as TG101 mice but carrying only one-renin gene, do not show a significant increase in blood pressure (Table 2). In addition, monitoring of blood pressure over 24 hours did not show any significant difference between values measured in control and TG1306 animals at any time considered (data not shown). These data demonstrate that plasma AGT alone is not sufficient to increase blood pressure to pathological values in the absence of stimulated renin secretion.

All transgenic mice described in the present report developed cardiac hypertrophy (Tables 1 and 2), which is characterized by the presence of enlarged cardiomyocytes. However, several studies have also pointed toward a role for Ang II in collagen accumulation and fibrogenesis.6,7,9 In our model, such an effect of Ang II has not been observed. This absence of Ang II–induced fibrosis needs to be investigated further. It would be of great interest to know whether the concentrations of the key determinant, ie, Ang II, are really enhanced in the

---

**Figure 3.** Immunohistochemical analysis. Hearts from TG153 (A and B) and TG101 (C and D) were immunostained with a mouse monoclonal anti-Ang II antibody. All pictures were taken using the same exposure time.
myocardium of transgenic mice and whether this is also the case in the presence of normal circulating Ang II levels. At present, a method to simultaneously measure Ang II in minute amounts of mouse cardiac and plasma samples is being established. Although the amounts of tissue and plasma Ang II were not measured, immunochemical analysis indicated that cardiac levels were indeed increased in the heart of transgenic animals. In addition, blockade of the AT1 receptor resulted in the regression of cardiac hypertrophy, indicating a direct role of Ang II in the development of cardiac hypertrophy in this model. Alterations also include the expression of genes that are normally not expressed in the adult ventricle.30 Indeed, ANF gene induction was demonstrated in the heart of transgenic mice (Figure 5). However, since the degree of ANF induction was similar in all lines, expression does not seem to depend on the levels of blood pressure in these transgenic models. In contrast, the onset of hypertrophy depends on the amount of AGT actually produced in cardiac tissues. TG153 mice demonstrate a significant increase in cardiac mass at 20 weeks of age only, whereas in TG1306 animals, carrying a higher transgene copy number, hypertrophy is already present at 8 weeks (Table 2). These results indicate that a local increase in AGT concentration is sufficient to induce a hypertrophic response in the absence of high plasma renin levels. Therefore, hypertrophy develops both in hypertensive TG101 animals bearing a high transgene copy

<table>
<thead>
<tr>
<th>Line</th>
<th>Age, wk</th>
<th>Group</th>
<th>n</th>
<th>BW, g</th>
<th>CWI, mg/g</th>
<th>MBP, mm Hg</th>
<th>PRC, ng Ang I · mL⁻¹ · h⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG153 8</td>
<td>Control</td>
<td>13</td>
<td>23±1</td>
<td>3.9±0.1</td>
<td>114±3</td>
<td>1000±300</td>
<td></td>
</tr>
<tr>
<td>Ren-1</td>
<td>Transgenic</td>
<td>8</td>
<td>21±1</td>
<td>3.9±0.1</td>
<td>113±3</td>
<td>200±40†</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20 Control</td>
<td>8</td>
<td>28±1</td>
<td>3.7±0.1</td>
<td>115±4</td>
<td>1600±300</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Transgenic</td>
<td>8</td>
<td>30±1</td>
<td>4.1±0.2*</td>
<td>116±4</td>
<td>400±90†</td>
<td></td>
</tr>
<tr>
<td>TG101 8</td>
<td>Control</td>
<td>8</td>
<td>34±1</td>
<td>3.6±0.1</td>
<td>119±2</td>
<td>26 000±7000</td>
<td></td>
</tr>
<tr>
<td>Ren-1, Ren-2</td>
<td>Transgenic</td>
<td>8</td>
<td>35±1</td>
<td>4.4±0.1†</td>
<td>154±4†</td>
<td>10 000±3000*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20 Control</td>
<td>5</td>
<td>44±2</td>
<td>3.7±0.1</td>
<td>129±3</td>
<td>11 000±1400</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Transgenic</td>
<td>6</td>
<td>43±1</td>
<td>4.6±0.3†</td>
<td>169±10†</td>
<td>17 000±4000</td>
<td></td>
</tr>
<tr>
<td>TG1306 8</td>
<td>Control</td>
<td>7</td>
<td>26±1</td>
<td>3.8±0.1</td>
<td>117±4</td>
<td>1200±400</td>
<td></td>
</tr>
<tr>
<td>Ren-1</td>
<td>Transgenic</td>
<td>13</td>
<td>27±1</td>
<td>4.5±0.1†</td>
<td>125±5</td>
<td>300±100*</td>
<td></td>
</tr>
</tbody>
</table>

BW indicates body weight; MBP, mean blood pressure.
*P<0.05; †P<0.01.

Figure 4. AT1 inhibitor treatment. Male TG1306 mice were either treated or not treated for 4 weeks with losartan. n=5 in all groups. **P<0.01 compared with control mice.

Figure 5. ANF expression in the heart. A, Northern blot analysis of ANF induction in hearts of control (lanes 1, 3, and 5) and transgenic (lanes 2, 4, and 6) mice; 20-week-old TG101 mice, lanes 1 and 2; 8-week-old TG1306 mice, lanes 3 and 4; 20-week-old TG153 mice, lanes 5 and 6. EtBr indicates ethidium bromide stain. B, Quantification of ANF expression: ANF signal was normalized to GAPDH expression. Results are expressed as percent increase over control.
Cardiac Angiotensinogen Overexpression

number and in normotensive TG153 mice with a lower copy number. In addition, the same degree of hypertrophy is observed in TG101 and in TG1306 mice, carrying the same transgene copy number, despite the fact that the latter have blood pressure values 40 mm Hg lower than those measured in TG101. In addition, statistical analysis demonstrated no dependence of hypertrophy on either blood pressure or PRA values in normotensive animals.

Taken together, the results demonstrate that cardiac hypertrophy does not appear to depend exclusively on increased load. Local renin synthesis is not a prerequisite for the stimulation of cardiac renin activity, since circulating renin has been shown to participate in intracardiac angiotensin formation from locally produced AGT. Moreover, because of the increased cardiac AGT concentrations, the local activation of the RAS can occur in the absence of stimulated renin release from the kidneys. Therefore, in a normal renin state as it occurs in one-renin gene mice, PRA remains constant and blood pressure is not affected. In contrast, increased renin secretion, either from the kidneys in hypertensive individuals or from the submaxillary glands in two-renin gene mice, induces high blood pressure and wall stress. Increased wall stress may in turn stimulate cardiac AGT expression that leads, in a context of high renin, to further activation of the RAS in cardiac tissues.

Acknowledgments

This study was supported by a grant from the Swiss National Science Foundation (Dr Pedrazzini, grant 32-43293.95) and the Sandoz Foundation. We thank P. Künstner and C. Munoz for technical assistance and M. Gomez for histological analysis.

References

Blood Pressure–Independent Cardiac Hypertrophy Induced by Locally Activated Renin-Angiotensin System
Lucia Mazzolai, Jürg Nussberger, Jean-François Aubert, Dorette B. Brunner, Giulio Gabbiani, Hans R. Brunner and Thierry Pedrazzini

*Hypertension*. 1998;31:1324-1330
doi: 10.1161/01.HYP.31.6.1324

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/31/6/1324

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Hypertension* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Hypertension* is online at:
http://hyper.ahajournals.org//subscriptions/