Increased Cardiac Angiotensin II Levels Induce Right and Left Ventricular Hypertrophy in Normotensive Mice

Lucia Mazzolai, Thierry Pedrazzini, Françoise Nicoud, Giulio Gabbiani, Hans-R. Brunner, Jürg Nussberger

Abstract—Angiotensin II is a potent arterial vasoconstrictor and induces hypertension. Angiotensin II also exerts a trophic effect on cardiomyocytes in vitro. The goals of the present study were to document an in vivo increase in cardiac angiotensins in the absence of elevated plasma levels or hypertension and to investigate prevention or regression of ventricular hypertrophy by renin-angiotensin system blockade. We demonstrate that high cardiac angiotensin II is directly responsible for right and left ventricular hypertrophy. We used transgenic mice overexpressing angiotensinogen in cardiomyocytes characterized by cardiac hypertrophy without fibrosis and normal blood pressure. Angiotensin-converting enzyme inhibition and angiotensin II type 1 receptor blockade prevent or normalize ventricular hypertrophy. Surprisingly, in control mice, receptor blockade decreases tissue angiotensin II despite increased plasma levels. This suggests that angiotensin II may be protected from metabolization by binding to its receptor. Blocking of the angiotensin II type 1 receptor rather than enhanced stimulation of the angiotensin II type 2 receptor may prevent remodeling and account for the beneficial effects of angiotensin antagonists. (Hypertension. 2000;35:985-991.)

Key Words: angiotensin II ■ angiotensin-converting enzyme inhibitors ■ blood pressure ■ fibrosis ■ receptors, angiotensin II ■ angiotensin I ■ renin

Among the regulators of cardiac growth, the renin-angiotensin system (RAS) appears to play a prominent role. It is well known that an activated RAS with increased circulating angiotensin II (Ang II) levels can induce hypertension and that the increased pressure load provokes cardiac hypertrophy.1,2 However, for a long time, it has been debated whether Ang II, besides its effect on blood pressure, could also act directly on cardiomyocytes to trigger the hypertrophic response. With transgenic (TG) mice overexpressing angiotensinogen (Ang-N) exclusively in the heart, we have recently shown that a locally activated RAS can induce cardiac hypertrophy in the absence of blood pressure changes.3 Ang II itself may indeed directly stimulate myocardial growth independent of the mechanical stress caused by its blood pressure-raising effect. This hypothesis is supported by indirect evidence. In vitro studies have shown that the addition of Ang II to cultured cardiomyocytes induces hypertrophy.4,5 In animal models of hypertension/cardiac hypertrophy, treatment with blockers of the RAS induced regression of hypertrophy that was not evident when blood pressure was equally reduced by other classes of antihypertensive drugs.6–8 However, so far, no direct proof in vivo of the growth factor effect of Ang II in cardiac hypertrophy has been obtained. There are 2 main reasons for this shortcoming. First, it was almost impossible to generate an animal model in which manipulation of the RAS would not induce a concomitant change in blood pressure. Second, reliable methods to measure plasma and tissue Ang II and angiotensin I (Ang I) levels in mice were not available.

The present study tackles these shortcomings by specific measurement of Ang II and Ang I concentrations in plasma and tissue of TG mice that are characterized by cardiac hypertrophy in the presence of normal blood pressure. Right ventricular hypertrophy would exclude any undetected systemic blood pressure effect, and administration of an angiotensin-converting enzyme (ACE) inhibitor or an antagonist of the Ang II type 1 (AT1) receptor was hypothesized to prevent or reduce any Ang II–mediated cardiac hypertrophy.

Methods

Animals
TG mice used for the experiments were the recently produced TG1306 line (Transgenic mice should be requested directly from Dr. T. Pedrazzini, Division of Hypertension and Vascular Medicine, CHUV, 1011 Lausanne, Switzerland. E-mail Thierry.Pedrazzini@chuv.hospvd.ch).3 These are 1 renin gene C57BL/6 male TG mice with cardiac Ang-N gene overexpression. Mice were used at 8 and 12 weeks of age. Animals were handled in accordance with institutional guidelines.

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TABLE 1. Cardiovascular Characteristics of TG Mice and Control Littermates at 8 wk (Prevention) and 12 wk of Age (Regression)

<table>
<thead>
<tr>
<th>Type</th>
<th>Treatment</th>
<th>MBP, mm Hg</th>
<th>HR, bpm</th>
<th>BW, g</th>
<th>RVI, mg/g</th>
<th>LVI, mg/g</th>
<th>CWI, mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevention</td>
<td>Ctrl Tap water</td>
<td>116±2</td>
<td>643±20</td>
<td>22±0.3</td>
<td>0.62±0.1</td>
<td>3.15±0.1</td>
<td>4.01±0.1</td>
</tr>
<tr>
<td>TG</td>
<td>121±2</td>
<td>645±23</td>
<td>23±0.6</td>
<td>0.74±0.1</td>
<td>3.49±0.1</td>
<td>4.79±0.2***</td>
<td></td>
</tr>
<tr>
<td>Ctrl Ramipril</td>
<td>96±3++</td>
<td>634±39</td>
<td>23±0.6</td>
<td>0.65±0.1</td>
<td>2.99±0.1</td>
<td>3.50±0.1+</td>
<td></td>
</tr>
<tr>
<td>TG</td>
<td>96±4++</td>
<td>658±30</td>
<td>23±0.6</td>
<td>0.60±0.1</td>
<td>2.90±0.1</td>
<td>3.60±0.1++</td>
<td></td>
</tr>
<tr>
<td>Ctrl Losartan</td>
<td>104.7+</td>
<td>652±37</td>
<td>23±1.0</td>
<td>0.58±0.1</td>
<td>3.01±0.2</td>
<td>3.55±0.1</td>
<td></td>
</tr>
<tr>
<td>TG</td>
<td>98±3+++</td>
<td>667±22</td>
<td>24±0.7</td>
<td>0.52±0.1</td>
<td>2.95±0.2</td>
<td>3.42±0.1+++</td>
<td></td>
</tr>
<tr>
<td>Regression</td>
<td>Ctrl Tap water</td>
<td>121±3</td>
<td>658±21</td>
<td>26±0.3</td>
<td>0.57±0.1</td>
<td>3.14±0.1</td>
<td>3.70±0.1</td>
</tr>
<tr>
<td>TG</td>
<td>125±2</td>
<td>670±19</td>
<td>27±0.4</td>
<td>0.76±0.1**</td>
<td>4.12±0.5**</td>
<td>4.92±0.3***</td>
<td></td>
</tr>
<tr>
<td>Ctrl Ramipril</td>
<td>106.2++</td>
<td>642±21</td>
<td>28±0.3</td>
<td>0.49±0.1</td>
<td>3.07±0.2</td>
<td>3.60±0.1</td>
<td></td>
</tr>
<tr>
<td>TG</td>
<td>117±4*</td>
<td>637±48</td>
<td>28±0.5</td>
<td>0.57±0.1+</td>
<td>3.23±0.2+</td>
<td>4.08±0.2++</td>
<td></td>
</tr>
<tr>
<td>Ctrl Losartan</td>
<td>97.4+++</td>
<td>565±33+</td>
<td>26±0.3</td>
<td>0.71±0.1</td>
<td>3.07±0.1+</td>
<td>3.74±0.1</td>
<td></td>
</tr>
<tr>
<td>TG</td>
<td>104±2+++</td>
<td>576±19++</td>
<td>27±0.4</td>
<td>0.61±0.1</td>
<td>3.00±0.1+</td>
<td>3.69±0.1+++</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM. MBP indicates mean blood pressure; HR, heart rate; and BW, body weight.

*P<0.05, **P<0.01, ***P<0.001 vs control mice; +P<0.05, ++P<0.01, +++P<0.001 vs untreated mice (tap water).

Study Design
Untreated mice were studied at 8 and 12 weeks of age. No cardiac hypertrophy was found in the TG1306 mice at 4 weeks of age. For the prevention study, TG and control mice were treated at 4 weeks of age with either the Ang II receptor antagonist losartan (1 mg per milliliter of drinking water2) or the ACE inhibitor ramipril (0.05 mg per milliliter of drinking water10) for 4 weeks. In the regression study, mice were treated at 8 weeks of age for 4 weeks with either losartan or ramipril administered in drinking water at the same dosages as for the prevention group. In all mice, mean blood pressure, heart rate, and cardiac weight index (CWI) were obtained (n=30). Among these mice, subgroups were used for angiotensin measurements, determination of right ventricular index (RVI) and left ventricular index (LVI), and histology (n=5 to 10).

Blood Pressure and Heart Rate Measurements
Mean blood pressure and heart rate were measured as previously described3 via an intra-arterial catheter connected to a pressure transducer in conscious mice.

Cardiac Indices
To evaluate cardiac mass, LVI, RVI, and CWI were measured. From harvested hearts, the atria were removed, and the ventricles were separated. The interventricular septum remained as part of the left ventricle. Ventricles were weighed, and indices were calculated as the ratio of ventricular weight (milligrams) to body weight (grams).

Blood and Tissue Sampling
After hemodynamic measurements, 300 μL blood was collected from conscious mice, through the arterial catheter, into chilled tubes containing 20 μL enzyme inhibitor cocktail (EDTA, α-phenanthroline, and renin inhibitor R-Pep156). Plasma was snap-frozen immediately, and samples were stored at −80°C. Mice were euthanized by neck dislocation under halothane anesthesia, and liver, kidney, and heart tissues were harvested and fixed in formalin and subsequently paraffin-embedded for histological analysis. For angiotensin measurements, tissue (10 to 100 mg) was rinsed with saline, blotted, and stored in ethanol at −80°C.

TABLE 2. Angiotensin Concentrations in Plasma and Heart of TG Mice and Control Littermates at 8 wk (Prevention) and 12 wk of Age (Regression)

<table>
<thead>
<tr>
<th>Type</th>
<th>Treatment</th>
<th>Angiotensin II, fmol/mL</th>
<th>Heart, fmol/g</th>
<th>Angiotensin I, fmol/mL</th>
<th>Heart, fmol/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevention</td>
<td>Ctrl Tap water</td>
<td>25.8 (16–48)</td>
<td>122 (49–201)</td>
<td>166 (102–328)</td>
<td>17.9 (5.3–36)</td>
</tr>
<tr>
<td>Ctrl Ramipril</td>
<td>6.0 (2.1–15)+</td>
<td>30 (11–63)+</td>
<td>20 (105–398)</td>
<td>32.1 (6.7–67)</td>
<td>51.9 (13–243)</td>
</tr>
<tr>
<td>TG</td>
<td>5.9 (3.6–11)+</td>
<td>39.8 (6.6–142)+</td>
<td>200 (105–398)</td>
<td>18.4 (5.8–101)</td>
<td>51.9 (13–243)</td>
</tr>
<tr>
<td>Ctrl Losartan</td>
<td>36.2 (15–80)</td>
<td>130 (32–399)</td>
<td>200 (105–398)</td>
<td>58.3 (20–104)</td>
<td>59.1 (13–243)</td>
</tr>
<tr>
<td>TG</td>
<td>74.4 (20–120)+</td>
<td>119 (17–355)+</td>
<td>200 (105–398)</td>
<td>58.3 (20–104)</td>
<td>59.1 (13–243)</td>
</tr>
<tr>
<td>Regression</td>
<td>Ctrl Tap water</td>
<td>26.0 (12–34)</td>
<td>145 (112–250)</td>
<td>135 (82–232)</td>
<td>8.89 (4.8–33)</td>
</tr>
<tr>
<td>Ctrl Ramipril</td>
<td>13.0 (5.0–43)+</td>
<td>119 (6.1–611)+</td>
<td>203 (65–1265)</td>
<td>32.2 (9.7–116)+</td>
<td>59.1 (13–243)</td>
</tr>
</tbody>
</table>

Values are expressed as geometric means (range).

*P<0.05, **P<0.01, ***P<0.001 vs control mice; +P<0.05, ++P<0.01, +++P<0.001 vs untreated mice (tap water).
TABLE 3. Angiotensin Concentrations in Liver and Kidney of TG Mice and Control Littermates at 8 wk (Prevention) and 12 wk of Age (Regression)

<table>
<thead>
<tr>
<th>Type</th>
<th>Treatment</th>
<th>Liver (Angiotensin II, fmol/g)</th>
<th>Kidney (Angiotensin I, fmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevention</td>
<td>Ctrl Tap water</td>
<td>88.1 (42–204)</td>
<td>234 (159–328)</td>
</tr>
<tr>
<td></td>
<td>TG</td>
<td>87.4 (28–152)</td>
<td>265 (134–488)</td>
</tr>
<tr>
<td></td>
<td>Ctrl Ramipril</td>
<td>11.5 (5.4–44)</td>
<td>+ + +</td>
</tr>
<tr>
<td></td>
<td>TG</td>
<td>9.03 (2.0–34)</td>
<td>+ + +</td>
</tr>
<tr>
<td></td>
<td>Ctrl Losartan</td>
<td>54.4 (4.1–234)</td>
<td>200 (72–366)</td>
</tr>
<tr>
<td></td>
<td>TG</td>
<td>24.8 (1.9–308)</td>
<td>135 (45–615)</td>
</tr>
<tr>
<td>Regression</td>
<td>Ctrl Tap water</td>
<td>87.1 (53–164)</td>
<td>361 (267–559)</td>
</tr>
<tr>
<td></td>
<td>TG</td>
<td>91 (73–104)</td>
<td>346 (231–484)</td>
</tr>
<tr>
<td></td>
<td>Ctrl Ramipril</td>
<td>72.0 (8.4–205)</td>
<td>181 (38–499)</td>
</tr>
<tr>
<td></td>
<td>TG</td>
<td>56.1 (21–93)</td>
<td>160 (66–325)</td>
</tr>
<tr>
<td></td>
<td>Ctrl Losartan</td>
<td>4.46 (1.2–25)</td>
<td>+ + +</td>
</tr>
<tr>
<td></td>
<td>TG</td>
<td>4.77 (1.2–15)</td>
<td>+ + +</td>
</tr>
</tbody>
</table>

Values are expressed as geometric means (range).

*P<0.05, **P<0.01 vs control mice; +P<0.05, ++P<0.01, +++P<0.001 vs untreated mice (tap water).

Angiotensin Measurements

Angiotensin concentrations were measured by radioimmunoassay after solid-phase extraction on phenylsilysilica and subsequent separation by reversed-phase isocratic high-performance liquid chromatography (HPLC). Samples from each mouse were analyzed individually. Tissue was immediately snap-frozen and homogenized in pure ethanol with a Polytron homogenizer (Kinematica), and the liquid phase after evaporation and reconstitution in Tris buffer was subjected to solid-phase extraction, HPLC, and radioimmunoassay in the same manner as that of the plasma samples. The detection limits were 2 fmol/mL for plasma and 2 fmol/mg for wet tissue. Within- and between-assay precision was consistently <15% for both plasma and tissue assays.

Histological Analysis

Tissue samples were fixed in 4% buffered formaldehyde and embedded in paraffin. Sections (5 μm thick) were stained by the following different histological methods: hematoxylin and eosin, Masson’s trichrome, and the Miller technique. Sections adjacent to the sections stained histologically were incubated with a mouse monoclonal IgG2a recognizing α-smooth muscle actin for 1 hour at room temperature. This was followed by incubation with goat anti-mouse biotinylated antibody (Jackson ImmunoResearch) for 1 hour at room temperature and treatment with streptavidin-biotin-peroxidase complex (Dako). The development of peroxidase activity was performed with diaminobenzidine (Serva). Slides were counterstained with hemalum and mounted in Aquatex (Dako). Samples were observed by use of a Zeiss Axiophot photomicroscope (Carl Zeiss) with an oil immersion Plan-Neofluar 40×1.3 objective. Images were acquired by use of a high-sensitivity Photonic Science Coolview camera (Carl Zeiss) with the software package Image Access 2.04 (Imagic). Images were processed with Adobe Photoshop 5.0 (Adobe System) and printed with a digital Fujifilm Pictography 4000 printer.

Statistical Analysis

Results are expressed as mean±SEM. Statistical analysis was performed by 2-way ANOVA followed by the Newman-Keuls multiple comparison test. Hormonal data were not normally distributed; therefore, a logarithmic transformation was performed before testing, and geometric means are reported in the tables. Significance was set at P<0.05.

Results

Cardiac Hypertrophy in Normotensive Untreated TG Mice (Tap Water)

At 8 weeks of age and in the absence of any drug treatment (tap water was used), control and TG mice had normal blood pressures, but CWI was 20% higher in TG mice; RVI and LVI tended to be increased in TG mice (Table 1). Plasma angiotensin concentrations did not differ significantly between TG and control mice, but tissue levels in the hearts of TG mice were increased 4-fold for Ang I and nearly doubled for Ang II (Table 2). In the livers of TG mice, Ang II and Ang I were unchanged, and in the kidneys of TG mice compared with control mice, Ang II was unchanged, and Ang I was decreased (Table 3). At 12 weeks of age, the findings were similar to those at 8 weeks, but the changes induced by the transgene were more marked: in TG mice, CWI was now increased by 33% (RVI 33% and LVI 31%, Table 1), and cardiac levels were increased 8-fold for Ang I and by 55% for Ang II (Table 2, Figure 1). Again, blood pressure, heart rate, and plasma angiotensin levels remained unchanged and so did liver and kidney angiotensin levels, with the exception of the again decreased kidney Ang I level in TG mice (Table 3).

Histological analysis of cardiac tissue, after staining with hematoxolin and eosin, revealed enlarged cardiomyocytes in TG mice compared with control mice (data not shown). Masson’s trichrome and Miller staining showed an absence of fibrotic changes in the myocardium of TG mice (Figure 2). When the myocardial sections were immunostained for α-smooth muscle actin, the staining appeared exclusively located in vessel walls, and no myofibroblasts were noted (data not shown).

Prevention of Cardiac Hypertrophy by RAS Blockade (Ramipril and Losartan)

At 8 weeks of age and after 4 weeks of ramipril administration, blood pressure was similarly lowered in TG and control
mice. In contrast to untreated mice, no increase in cardiac indices was found in TG mice (Table 1). In both TG and control mice, plasma Ang II was decreased and plasma Ang I was increased (Table 2). Cardiac tissue Ang II was decreased by 82% in TG and by 75% in control mice, respectively, compared with untreated mice. Cardiac Ang I was unchanged. Also, in liver and kidney, Ang II was similarly decreased by the ACE inhibitor in TG and control mice, and levels of Ang I were similar in both groups of mice and not significantly increased; the decrease in renal Ang I of TG mice was not found with preventive ramipril administration.

Losartan also prevented cardiac hypertrophy in TG mice: as with ramipril, in TG and control mice, blood pressure decreased slightly, and cardiac indices became normal (Table 1). Increases in plasma Ang II and Ang I did not reach significance in control mice, but in TG mice, these plasma peptides were increased (Table 2). In losartan-treated hearts of TG mice, Ang II levels were not increased, whereas Ang I concentration was 4-fold higher than in control mice. In the liver, the same unchanged Ang II and Ang I levels were found for TG and control mice. In TG mice, renal Ang II tended to be decreased, and like ramipril, losartan abolished the decrease in renal Ang I concentration found in TG mice.

Regression of Cardiac Hypertrophy by RAS Blockade (Ramipril and Losartan)

At 12 weeks of age and after 4 weeks of ramipril treatment, blood pressure was decreased, particularly in control mice but also in TG mice. This decrease in blood pressure did not reduce cardiac indices in control mice. In contrast to untreated TG mice, ramipril-treated TG mice no longer exhibited increased cardiac indices (Table 1). Plasma angiotensin levels were similarly affected by ramipril treatment in TG and control mice: compared with levels in untreated mice, Ang II levels were decreased by 40% to 50%, and Ang I levels tended to be increased. In the heart, Ang I was still increased in TG mice, but cardiac Ang II was not significantly different in TG and control mice. In the liver and kidney of TG compared with control mice, Ang I was reduced by half, whereas Ang II was unchanged.

Losartan, much like ramipril, slightly reduced blood pressure in control and TG mice and completely abolished cardiac hypertrophy in TG mice (Table 1). Plasma angiotensin levels were the same in control and TG mice, but both were more than doubled compared with levels in untreated mice (Table 2). Cardiac Ang I was not changed by losartan treatment in control mice, whereas in TG mice, cardiac Ang I was increased 3-fold. In TG mice, in contrast to plasma Ang II, cardiac Ang II was not significantly increased by losartan; surprisingly, losartan treatment reduced cardiac Ang II levels in control mice by 86%. As a consequence of the losartan treatment, renal tissue of control mice contained only 1/10 of normal Ang I concentrations and 1/5 of normal Ang II concentrations (Table 3). In TG mice, renal Ang II was comparably reduced by 75%, but Ang I levels remained unchanged. In TG and control mice, losartan reduced liver Ang II concentration by 95%, but liver Ang I remained unchanged at very low levels.

Only in 12-week-old animals did losartan treatment reduce heart rate. Heart rate did not change in any other experimental condition (Table 1).
Discussion

TG mice overexpressing Ang-N in cardiomyocytes develop cardiac hypertrophy even in the absence of hypertension. The present study demonstrates that cardiac, but not plasma, angiotensin levels are increased in these TG mice. Left and right ventricles are hypertrophic, reflecting the trophic effect of Ang II independent of an enhanced load. Left and right ventricular hypertrophy can be prevented/reversed by ACE inhibition or by Ang II receptor antagonism.

Angiotensin Concentrations and Cardiac Hypertrophy

Sensitive micromethods for the specific measurement of plasma and tissue Ang II and Ang I concentrations allowed us to demonstrate for the first time in the living organism that enhancement of local angiotensin production in the heart induces myocardial hypertrophy. Angiotensin levels were not increased in plasma, liver, and kidneys; thus, enhancement of angiotensin generation was successfully limited to cardiac tissue. Blood pressure and heart rate changes did not account for the cardiac hypertrophy. The enhanced RVI, particularly in 12-week-old mice, could hardly be explained by an Ang II–mediated increased pressure load. Furthermore, 12-week-old mice treated with the Ang II antagonist losartan showed more of a blood pressure decrease (–24 mm Hg) than did the TG peers (–21 mm Hg), but only the TG mice showed reductions in cardiac mass; the control mice did not. That Ang II indeed caused the cardiac hypertrophy was demonstrated by the administration of the Ang II receptor antagonist losartan and the ACE inhibitor ramipril, both of which prevented or fully reversed cardiac hypertrophy. The TG animals never became hypertensive, and the drug-induced decrease in blood pressure from normal to low-normal values was comparable in control and TG mice. Drug treatment changed cardiac weight indices only in TG mice; the hearts of control animals remained unchanged.

The hormonal measurement of Ang I and Ang II in plasma and tissues of mice has been achieved by combining highly specific HPLC and very sensitive radioimmunoassay. This allowed specific quantification of angiotensin peptides in 100 μL plasma and 100 mg tissue with detection limits of 2 fmol/mL and 2 fmol/g, respectively. We have previously shown that Ang-N from TG hearts spills over in the circulation and tends to increase circulating Ang-N levels. Consequently, plasma Ang II would be expected to rise unless renal renin secretion is successfully reduced by the Ang II–mediated negative feedback. The success of this feedback control can be seen by the almost unchanged plasma Ang II levels in our TG mice (Table 2). Circulating renin concentrations in TG mice were actually 95% to 98% lower than those in control mice (data not shown). Indeed, in the kidney, where Ang-N levels usually limit the production of Ang I by the abundant renal renin, our untreated TG mice exhibit, despite the increased circulating Ang-N, suppressed Ang I levels (but unchanged Ang II levels), reflecting reduced renal active renin. Thus, the kidneys have shut down renin secretion and have succeeded in preventing renal Ang II levels from rising.

As anticipated, cardiac angiotensin production was increased in TG mice: the genetically induced overproduction of cardiac Ang-N could not be offset by reduced circulating renin. Cardiac angiotensin levels rose. In the presence of high cardiac Ang-N concentrations in TG mice, the actual plasma renin concentration is not sufficiently suppressed to avoid enhanced production of angiotensin in the heart, although this suppression of renin appears to sufficiently compensate increased Ang-N levels in the kidneys, liver, and plasma, as can be seen from unchanged Ang I and Ang II levels in these organs of TG mice. In the liver (the ordinary factory of mouse Ang-N), Ang I and Ang II concentrations are unaffected by the cardiac TG production of (rat) Ang-N.

Cardiac tissue Ang I levels are normally lower than Ang II levels, probably because Ang I is rapidly metabolized, whereas Ang II is partially protected from metabolism by binding to receptors. Generation of Ang I in the heart of untreated TG mice is increased 5- to 8-fold; Ang II levels at the same time are almost doubled in TG mice. The absolute increase in Ang II of 80 to 100 fmol/g in TG mice is greater than the increase in Ang I and may reflect stimulated production as well as more receptor-bound peptide (Table 2).

RAS Blockade by ACE Inhibition

The administered dose of ramipril of 200 μg/d is higher than the dose used by other investigators, but full blockade of Ang II production was not obtained. In the heavier 12-week-old mice, plasma Ang II levels were decreased by 50%, and Ang I levels were not significantly increased. Cardiac tissue Ang II was decreased by 82% in TG and by 75% in control mice when compared with untreated mice. Whether a relative higher dose of ramipril or eventually smaller Ang-N production in the younger mice accounts for the enhanced effects of ramipril cannot be decided from the present data. Also, the blood pressure–lowering effect of ramipril was more marked in the prevention than in the regression study (–20/–25 mm Hg versus –15/–8 mm Hg, respectively). In contrast, the losartan dose of 4 mg/d appeared to be equally effective in all mice.

Ramipril reduced renal Ang II production in the prevention study by 82% in control mice and by 86% in TG mice. At the same time, the limited supply of Ang-N in control mice does not allow higher renal Ang I production by the stimulated renin secretion in response to the decrease in Ang II. In contrast, in TG mice, the increased Ang-N supply from the heart allows us to visualize, by the 3-fold increase in renal Ang I, the effort of the RAS to overcome the shortage of renal Ang II. During tap water administration, renin secretion is turned off in TG kidneys (decreased Ang I) because much Ang II is generated from increased plasma Ang-N and reaches the receptors. In the kidneys of the regression study, very similar, though less striking, results were obtained with ramipril: Ang II levels were decreased by half, and renal Ang I levels in TG mice treated with ramipril were twice those found in tap water–fed TG mice (not significant). In both studies, mean liver Ang II levels were decreased with ramipril, and mean liver Ang I levels were increased in TG and control mice, but these changes were only partially significant (Table 3).

In the heart, efficient ACE inhibition as seen in the prevention study brings Ang I and Ang II concentrations to equivalent levels. The low cardiac Ang II levels after preventive ramipril
treatment may well explain the perfectly normal cardiac weight indices, whereas high cardiac Ang II levels in untreated TG mice caused significant hypertrophy. In the regression study, cardiac hypertrophy was established at 8 weeks of age in TG mice, and a 4-week subsequent treatment with ramipril completely abolished the hypertrophy. Untreated mice at 12 weeks of age had virtually the same cardiac Ang II levels as they had at 8 weeks of age; also, Ang I levels were comparable at the 2 ages. At 12 weeks, cardiac Ang II levels were again reflected in the CWI. At the same time, in the ramipril-treated TG mice, a 20% decrease in mean cardiac Ang II was accompanied by a 20% decrease in CWI. Cardiac Ang I levels of these older (and heavier) ramipril-treated mice were below Ang II levels, which were no longer suppressed as they had been at 8 weeks after preventive ramipril treatment. This may indicate that these 12-week-old “regression” mice successfully counterbalance ACE inhibition to achieve normal cardiac Ang II receptor occupancy. Interestingly, in these TG mice at 12 weeks, the slightly higher (than control mice) though normalized (by ramipril) cardiac indices occurred concurrently with slightly higher, though normalized, cardiac Ang II levels. The observations made in the present study with ramipril in control and TG mice would be compatible with an Ang II–mediated pathogenesis of cardiac hypertrophy on the basis of 2 assumptions: (1) hypertrophic response requires stimulation of myocardial Ang II receptors by >140 fmol/g receptor-bound Ang II, and (2) cardiac tissue levels of free (ie, not receptor-bound) Ang I and Ang II are approximately equal, and receptor bound Ang II can therefore be calculated by subtraction of Ang I from total Ang II concentrations.

RAS Blockade by Ang II Receptor Antagonism

The clear abolishment of cardiac hypertrophy by losartan treatment in the prevention and in the regression studies also demonstrates that Ang II most likely caused the myocardial hypertrophy in our TG mice. As expected, Ang II receptor blockade by losartan increased plasma Ang I and Ang II levels, because Ang II no longer restrained renal renin secretion. High renin levels in plasma and kidneys necessarily reduce circulating Ang-N concentrations (substrate consumption). In the kidneys, in the presence of efficient angiotensinases and with losartan-blocked protecting receptors, Ang I levels may fall as soon as the shortage in Ang-N no longer allows for the maintenance of Ang II levels at a set point of \( \approx 100 \) fmol/g at 8 weeks and 230 fmol/g at 12 weeks. All our results suggest that the RAS primarily regulates renal Ang II concentration. In the heart, preventive losartan did not change angiotensin levels in control mice, but in TG mice, losartan normalized Ang II levels together with the normalization of the hypertrophy. Losartan may have prevented excessive Ang II from being protected against angiotensinases. The most striking finding of the regression study is the very low cardiac level of Ang II in the losartan-treated control mice, whereas the very high corresponding levels in the TG mice are easily explained by the high cardiac Ang-N and the high renal renin secretion (these “free” angiotensin concentrations are not trophic, in view of the fact that the corresponding Ang II receptor is blocked by losartan). The low cardiac angiotensin concentrations of the control mice are most likely the consequence of considerable renal consumption of the only available hepatogenic Ang-N. Indeed, in the kidney, high renin output cannot successfully overcome the Ang II receptor blockade, as suggested from decreased renal angiotensin levels: very little Ang-N may reach the heart, and virtually no angiotensin is formed or protected from degradation because protecting receptors are blocked by losartan. Losartan, like all commercially available Ang II antagonists, blocks only the subtype AT1 of the Ang II receptors and leaves subtype AT2 receptors unopposed. Because AT1 blockade induces high circulating Ang II levels, it has been postulated that enhanced AT2 stimulation mediates the antigrowth effect of AT1 antagonists. Indeed, Campbell et al have reported increased cardiac Ang II levels with losartan, but experimental conditions were different (Sprague-Dawley rats, 8 days, and intraperitoneal administration). In our TG mice, losartan tended to increase cardiac Ang II (17%), but in control mice, losartan decreased it by 86%. This points to the importance of the availability of Ang-N. Consistent with the importance of Ang-N is a previous observation involving the Langendorff rat heart, in which losartan abolished tissue Ang II when angiotensin but not when Ang-N/renin was perfused. Low cardiac Ang II levels that we actually measured under AT1 blockade provide a rationale for an antigrowth effect of AT1 antagonists in the absence of enhanced AT2. The explanation for the decreased Ang II in the liver after losartan treatment may be similar to the explanation for the decreased Ang II in the heart. Therefore, it would be expected that liver Ang II, unlike cardiac Ang II, is low also in TG mice, in view of the fact that only cardiac but not liver Ang-N is increased by the transgene. The substrate production of the liver does not appear to be enhanced in this situation. It remains to be seen whether the intermediate liver Ang II concentrations in losartan-treated 8-week-old mice can be explained by less Ang-N consumption by renal renin or by less efficient angiotensinases.

Histology

Histological examinations confirmed the previous observation that no fibrosis occurs in the hypertrophic heart of our TG mice. This could be of interest, because circulating Ang II concentrations are not increased, and aldosterone levels may also remain normal. Aldosterone has been postulated to be involved in the pathogenesis of cardiac fibrosis. The absence of increased aldosterone levels could explain the absence of fibrosis in our TG mice. Our results are in agreement with those of others, who reported no fibrosis in hypertrophied hearts of hypertensive rats without signs of heart failure. On the other hand, cardiac fibrosis was shown to be present in mouse and rats models in which concomitant heart failure developed. Therefore, fibrosis may characterize a failing heart, and no fibrosis may be found in compensated cardiac hypertrophy.

In conclusion, the local increase in angiotensin concentrations in the hearts of normotensive TG mice induces left and right ventricular myocardial hypertrophy without fibrosis. A causal relation between Ang II levels and myocardial hypertrophy is suggested because hypertrophy can be prevented by early treatment or reversed by late treatment with ramipril, which decreases the generation of Ang II, and with losartan, which
antagonizes Ang II at its receptor. Low tissue concentrations of Ang II during AT<sub>1</sub> receptor blockade challenge the concept of enhanced AT<sub>2</sub>-mediated growth inhibition in vivo.

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