Effect of Genetic Deficiency of Angiotensinogen on the Renin-Angiotensin System

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Abstract—This study examined expression of renin-angiotensin system (RAS) component mRNAs in angiotensinogen gene knockout (Atg−/−) mice. Wild-type (Atg+/+) and Atg−/− mice were fed a normal-salt (0.3% NaCl) or high-salt (4% NaCl) diet for 2 weeks. Angiotensinogen, renin, angiotensin-converting enzyme (ACE), angiotensin II type 1a receptor (AT1A), and angiotensin II type 2 receptor (AT2) mRNA levels were measured by Northern blot analysis. In Atg+/+ mice, activities of circulating RAS and renal angiotensinogen mRNA level were decreased by salt loading, whereas levels of renal and cardiac ACE; renal, brain, and cardiac AT1A; and brain and cardiac AT2 mRNA were increased by salt loading. Although activities of circulating RAS were not detected in Atg−/− mice, salt loading increased blood pressure in Atg−/− mice. In Atg−/− mice, renal renin mRNA level was decreased by salt loading; in contrast, salt loading increased renal AT1A and cardiac AT2 mRNA levels in Atg−/− mice, and these activated levels in Atg−/− mice were higher than those in Atg+/+ mice fed the high-salt diet. Thus, expression of each component of the RAS is regulated in a tissue-specific manner that is distinct from other components of systemic and local RAS and that appears to be mediated by a mechanism other than changes in the circulating or tissue levels of angiotensin peptides. (Hypertension. 1998;32:223-227.)

Key Words: renin-angiotensin system ■ angiotensinogen ■ angiotensin-converting enzyme ■ receptors, angiotensin ■ RNA, messenger ■ sodium, dietary

The RAS plays a critical role in maintaining blood pressure and fluid electrolyte balance. The RAS historically has been viewed as a circulatory system. The various components of the RAS are produced by different organs and are delivered to their site of action by the bloodstream. However, accumulated evidence derived from biochemical and molecular studies of the physiological properties of angiotensin suggests that distinct local RAS with different regulatory mechanisms exist and function in the brain, heart, adrenal gland, kidney, vessel wall, and adipose tissue. Although it is controversial whether all components of the RAS are physiologically relevant and the exact pathophysiological role of the local RAS remains elusive, it is interesting to speculate that the local RAS may enhance the actions of Ang II in a specific physiological process of a given tissue system. In addition, previous studies showed that a variety of stimuli, including blood pressure, sodium intake, inflammation, and sympathetic nerve activity, modulate the expression of the tissue RAS genes in physiological and pathophysiological states.14 Furthermore, several studies using antagonists of Ang II receptor subtypes suggest that Ang II exerts various effects on the expression of major component genes of the RAS by positive or negative feedback mechanisms.5-7

Recently, we and others generated angiotensinogen-deficient mice by gene targeting.8-10 Homozygous mutant (Atg−/−) mice have no detectable plasma angiotensinogen or angiotensin peptides; they therefore lack a functional RAS and exhibit chronic hyptension. The aim of the present study was to examine whether dietary salt loading modulates the expression of major component genes of the RAS in Atg−/− mice without affecting angiotensin formation, as a first step to analyze a feedback mechanism by which angiotensin peptides exert influences on expression of the RAS gene.

Methods

Animals

Atg−/− mice were generated by gene targeting as described previously.8 Atg−/− (n=12) and Atg+/+ (n=12) mice, aged 6 weeks, were used in this study. The mice were housed under a 12/12-hour day/night cycle at a temperature of 25°C and fed a normal-salt (0.3% NaCl) diet for 2 weeks. At 8 weeks of age, Atg−/− and Atg+/+ mice were divided into 2 groups, were placed on either a high-salt (4% NaCl) or normal-salt (0.3% NaCl) diet, and were kept in metabolic cages for determination of daily urine output and levels of urinary aldosterone and electrolytes. Tap water was provided ad libitum. At 10 weeks of age, SBP and BW were measured. SBP was measured by tail-cuff plethysmography. Mice were killed by decap-
Selected Abbreviations and Acronyms
ACE = angiotensin-converting enzyme
Ang = angiotensin
AT$_1$ = angiotensin II type 1A receptor
AT$_2$ = angiotensin II type 2 receptor
BW = body weight
HW = wet tissue heart weight
RAS = renin-angiotensin system
SBP = systolic blood pressure

Biochemical Assays
The concentrations of electrolytes were measured with an automated analyzer for routine laboratory tests (Hitachi-736). Plasma Ang I concentration was measured with a radioimmunoassay kit (Rinabead Ang I kit, Dainabot Co Ltd). Plasma Ang II concentration was measured with a radioimmunoassay kit (Renin analyzer for routine laboratory tests (Hitachi-736). Plasma Ang I concentration was measured with a radioimmunoassay kit (SPAC-S aldosterone kit, Daiichi Radio-isotope Co).

RNA Isolation and Analysis
Total RNA from tissues was extracted with the guanidinium thiocyanate–cesium chloride centrifugation method. Each RNA sample (20 µg) was denatured with 1 mol/L glyoxal and 50% DMSO, electrophoresed on 1.2% agarose gels, and transferred onto nylon membranes (GeneScreen Plus, DuPont-New England Nuclear). Filters were prehybridized for 30 minutes at 60°C in a solution consisting of 1% SDS, 1 mol/L NaCl, and 10% dextran sulfate. Hybridization proceeded for 18 hours at 60°C in the same solution containing 300 µg/mL denatured salmon sperm DNA and 1×10$^6$ cpm/mL of the $^{32}$P-labeled probes for angiotensinogen, renin, ACE, AT$_1$, or AT$_2$. Filters were washed twice with 2× SSC (1× SSC = 150 mol/L NaCl, 15 mol/L sodium citrate) for 5 minutes at room temperature, twice with 2× SSC and 1% SDS for 30 minutes at 60°C, and twice with 0.1× SSC for 15 minutes at room temperature. Dried filters were exposed to an imaging plate of Fujix Bio-Imaging Analyzer BAS2000 (Fuji Photo Film). Expression of mRNA was quantified with the BAS2000 computer analyzer and normalized to the signal generated by probing for the constitutively expressed 18S rRNA gene.

Table 1. Characteristics of Atg+/+ and Atg−/− Mice Fed a Normal- or High-Salt Diet

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Normal Salt (0.3% NaCl)</th>
<th>High Salt (4.0% NaCl)</th>
<th>Normal Salt (0.3% NaCl)</th>
<th>High Salt (4.0% NaCl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP, mm Hg</td>
<td>95±6.3</td>
<td>108±4.5</td>
<td>70±2.8†</td>
<td>95±5.3*</td>
</tr>
<tr>
<td>BW, g</td>
<td>36.4±1.1</td>
<td>37.9±0.9</td>
<td>31.2±0.8†</td>
<td>32.6±0.9†</td>
</tr>
<tr>
<td>HW, g</td>
<td>0.22±0.01</td>
<td>0.20±0.01</td>
<td>0.14±0.01†</td>
<td>0.13±0.01†</td>
</tr>
<tr>
<td>HW/BW ratio, ×10$^{-3}$</td>
<td>6.02±0.46</td>
<td>5.29±0.19</td>
<td>4.49±0.23†</td>
<td>4.09±1.08†</td>
</tr>
<tr>
<td>KW, g</td>
<td>0.30±0.02</td>
<td>0.34±0.02</td>
<td>0.29±0.03</td>
<td>0.34±0.03</td>
</tr>
<tr>
<td>KW/BW ratio, ×10$^{-3}$</td>
<td>8.12±0.25</td>
<td>8.95±0.39</td>
<td>9.34±0.81</td>
<td>10.49±0.83</td>
</tr>
<tr>
<td>p-Ang I, pmol/mL</td>
<td>1.85±0.19</td>
<td>1.20±0.12*</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>p-Ang II, fmol/mL</td>
<td>51.9±13.7</td>
<td>15.0±1.4*</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>U$_{Na}$, µmol/d</td>
<td>175.9±30.2</td>
<td>1168.4±48.5*</td>
<td>124.2±32.0</td>
<td>1052.9±93.6*</td>
</tr>
<tr>
<td>U$_{K}$, µmol/d</td>
<td>318.9±40.5</td>
<td>4586.6±221.1*</td>
<td>227.0±26.4</td>
<td>422.7±35.5*</td>
</tr>
</tbody>
</table>
Atg+/+ mice, urinary excretions of sodium and potassium were similar in these mice (Table 1).

**Effects of Salt Loading on mRNA Expression of RAS in Atg+/+ and Atg−/− Mice**

Because the level of renal renin expression in Atg−/− mice is reported to be much higher than in Atg+/+ mice,1,2,3 and the kidney plays a critical role in the maintenance of cardiovascular homeostasis, we examined expression of the RAS components in the kidney of Atg+/+ and Atg−/− mice and analyzed the effect of salt loading on expression of RAS as determined by Northern blot analysis (Figure 1). In the kidney, angiotensinogen mRNA is expressed in Atg+/+ mice but not in Atg−/− mice, and the high-salt diet decreased angiotensinogen mRNA levels in Atg+/+ mice. Atg−/− mice had higher levels of renin, ACE, and AT1A mRNA expression than Atg+/+ mice when fed the normal-salt diet; the high-salt diet significantly increased AT1A mRNA levels in both Atg−/− and Atg+/+ mice and ACE mRNA levels in Atg+/+ mice but decreased renin mRNA levels in Atg−/− mice. The ACE mRNA levels in Atg+/+ mice were comparable to those in Atg−/− mice fed the high-salt diet, and Atg−/− mice still had 5.3- and 2.0-fold higher levels of renin and AT1A mRNA expression than Atg+/+ mice, respectively, when fed the high-salt diet. The levels of ACE enzymatic activity showed the same trend with the ACE mRNA levels in the kidney of both Atg−/− and Atg+/+ mice (Table 2).

**TABLE 2. Renal ACE Activities of Atg+/+ and Atg−/− Mice Fed a Normal- or High-Salt Diet**

<table>
<thead>
<tr>
<th></th>
<th>Atg+/+</th>
<th>Atg−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Salt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(0.3% NaCl)</td>
<td>53.3±3.9</td>
<td>83.0±4.6</td>
</tr>
<tr>
<td>High Salt</td>
<td>67.7±3.7*</td>
<td>72.8±4.5</td>
</tr>
<tr>
<td>Normal Salt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(0.3% NaCl)</td>
<td>67.7±3.7*</td>
<td>72.8±4.5</td>
</tr>
<tr>
<td>High Salt</td>
<td>83.0±4.6</td>
<td>72.8±4.5</td>
</tr>
</tbody>
</table>

Renal ACE activities are expressed as units/mg tissue protein. Values are mean±SE; n=6 mice in each group.

*P<0.05 vs normal-salt diet; †P<0.05 vs Atg+/+ mice within the same salt group.

In the brain, angiotensinogen mRNA is expressed in Atg+/+ mice but not in Atg−/− mice, and the high-salt diet had no significant effect on levels of angiotensinogen mRNA (Figure 2). Both AT1A and AT2 mRNA levels in Atg+/+ mice were comparable to those in Atg−/− mice fed the normal-salt diet. In Atg+/+ mice, the high-salt diet significantly increased AT1A and AT2 mRNA levels, whereas in Atg−/− mice the high-salt diet had no effect on such levels. The levels of cardiac ACE, AT1A, and AT2 mRNA expression were similar in Atg+/+ and Atg−/− mice fed the normal-salt diet (Figure 3). The high-salt diet significantly increased ACE, AT1A, and AT2 mRNA levels in Atg+/+ mice. In Atg−/− mice, the high-salt diet did not affect cardiac ACE or AT1A mRNA levels, but it significantly increased cardiac AT2 mRNA expression to 1.4-fold higher than that in Atg+/+ mice fed the high-salt diet.

**Discussion**

Previous studies of regulation of the RAS components in genetically or experimentally hypertensive animals showed widespread abnormalities of RAS gene expression that were modulated in some tissues by the development of hypertension.4–7 However, little is known about regulation of the RAS genes in hypotensive animals. Because Atg−/− mice do not produce angiotensin peptides at all and thus are chronically hypotensive, Atg−/− mice may be a genetically suitable hypotension model for analysis of the regulation of RAS gene expression in vivo. In the present study, we showed that salt loading significantly increased SBP in Atg−/− mice and that expression of the RAS genes was regulated in a tissue-specific manner by salt loading in Atg−/− mice.

Consistent with the results of previous studies,8,9 we showed an increase in the levels of renin mRNA in the kidney of Atg−/− mice compared with Atg+/+ mice. The mRNA levels of ACE and AT1A were also increased in the kidney of Atg−/− mice. Upregulation of ACE and AT1A mRNA levels in Atg−/− mice was observed in the kidney but not in either the brain or heart when these mice were fed the normal-salt diet. These results suggest that expression of the renal ACE and AT1A genes is specifically activated in Atg−/− mice. Previous studies...
revealed that the kidney in Atg−/− mice was undergoing numerous pathological changes, including marked vascular hypertrophy, interstitial inflammation, atrophic changes in the tubules and papilla, and increased expression of growth factors and neuronal nitric oxide synthase genes, as well as increased urine output and decreased urine osmolality compared with Atg+/+ mice. In contrast to these remarkable abnormalities in the kidney, no appreciable abnormality was found in the brain or heart of Atg−/− mice. Thus, these pathological changes may be involved in increases in the mRNAs levels of ACE and AT1A in the kidney in Atg−/− mice fed the normal-salt diet. In particular, Atg−/− mice have reduced renal medullas; thus, their renal RNA is enriched in RNA from the renal cortex where ACE is produced. This could explain the elevations of ACE mRNA levels observed in Atg−/− mice. Another possibility is that the higher mRNA levels of ACE and AT1A in the kidney of Atg−/− mice than of Atg+/+ mice may be due to complete disruption of the negative feedback of Ang II on ACE- and AT1A-expressing cells in the kidney of Atg−/− mice. Further study is needed to clarify the mechanisms responsible for these increases in the kidney of Atg−/− mice.

Tissue ACE in the heart may play a role in the pathogenesis of cardiac hypertrophy and remodeling. However, little is known regarding the regulation of the cardiac ACE gene by altered sodium intake. In this study, salt loading increased the cardiac ACE mRNA levels in Atg+/+ mice but not in Atg−/− mice. Because a previous study showed a significant increase in cardiac ACE mRNA in response to a high-sodium diet in both Wistar-Kyoto and stroke-prone spontaneously hypertensive rats, the findings in the present study suggest that angiotensin peptides are necessary for a salt-mediated increase in the cardiac ACE mRNA levels.

Similar to the regulation of cardiac ACE gene, salt loading increased the brain and cardiac AT1A mRNA levels in Atg+/+ mice but not in Atg−/− mice. Previous studies showed that high sodium intake increased expression of the AT1A mRNA in the brain and kidney. Dietary sodium loading is known to suppress the circulating RAS and to decrease circulating levels of Ang II. Because Ang II downregulates expression of the AT1A gene, the decrease in circulating Ang II levels by salt loading may upregulate AT1A gene expression. In the present study, there was no significant difference in AT1A mRNA expression in the brain and heart in Atg+/+ and Atg−/− mice when fed the normal salt diet, and salt loading increased the AT1A mRNA levels in Atg+/+ mice but not in Atg−/− mice. Thus, complete lack of angiotensin peptides may make the AT1A gene unable to respond to salt loading in the brain and heart.
In contrast to the AT1A gene, much less is known about the regulation of the AT2 gene. Abundant expression of AT2 has been found in the mesenchymal tissues of a developing rat fetus, indicating an important role of AT2 in growth, development, and apoptosis. Previous studies reported an increase in AT2 expression in the hypertrophic myocardiun of experimental hypertensive rats and showed that the process of cardiac remodeling after myocardial infarction induced not only AT1A, but also AT2 expression. In the present study, salt loading increased the brain AT2 mRNA levels in both AT2+/+ mice and AT2−/− mice, and the activated levels of cardiac AT2 mRNA were higher in AT2+/+ mice than in AT2+/− mice. Therefore, these results suggest that the complete lack of angiotensin peptide activates the salt-mediated expression of cardiac AT2 mRNA but inhibits the salt-mediated expression of brain AT2 mRNA.

In conclusion, the findings obtained in the present study suggest that dietary salt loading exerts a systemic influence on the expression of AT2 in cardiac tissues of both AT2+/+ and AT2−/− mice. Future research must elucidate the systemic and cellular mechanisms by which salt intake modulates the expression of AT2 genes and the possible roles of angiotensin peptides in these mechanisms.

Acknowledgments

This work was supported in part by grants from the Ministry of Education, Science, and Culture of Japan; the Ichiro Kanehara Foundation; the Uehara Memorial Foundation; the Yokohama Foundation for Advancement of Medical Science; and the Naito Memorial Foundation.

References

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Hypertension. 1998;32:223-227
doi: 10.1161/01.HYP.32.2.223

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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