Effects of AT1A receptor deletion on blood pressure and sodium excretion during altered dietary salt intake

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Mangrum, Amy J., R. Ariel Gomez, and Victoria F. Norwood. Effects of AT1A receptor deletion on blood pressure and sodium excretion during altered dietary salt intake. Am J Physiol Renal Physiol 283: F447–F453, 2002.—The present study was performed to investigate the role of type 1A ANG II (AT1A) receptors in regulating sodium balance and blood pressure maintenance during chronic dietary sodium variations in AT1A receptor-deficient (−/−) mice. Groups of AT1A (−/−) and wild-type mice were placed on a low (LS), normal (NS), or high-salt (HS) diet for 3 wk. AT1A (−/−) mice on an LS diet had high urinary volume and low blood pressure despite increased renin and aldosterone levels. On an HS diet, (−/−) mice demonstrated significant diuresis, yet blood pressure increased to levels greater than control littermates. There was no effect of dietary sodium intake on systolic blood pressures in wild-type animals. The pressure-natriuresis relationship in AT1A (−/−) mice demonstrated a shift to the left and a decreased slope compared with wild-type littermates. These studies demonstrate that mice lacking the AT1A receptor have blood pressures sensitive to changes in dietary sodium, marked alterations of the pressure-natriuresis relationship, and compensatory mechanisms capable of maintaining normal sodium balance across a wide range of sodium intakes.

EXTRACELLULAR FLUID VOLUME is normally maintained within narrow limits despite considerable variations in daily salt and water intake. Therefore, renal sodium excretion is generally considered a crucial variable in the control of extracellular fluid volume and blood pressure. The kidneys determine the long-term blood pressure response by altering sodium excretion and maintaining extracellular volume. In normal conditions, small elevations in blood pressure result in increases in urinary excretion of sodium and water, reducing the blood pressure to baseline levels. The relationship between sodium excretion and blood pressure is called pressure-natriuresis (5, 8–11). The renin-angiotensin system (RAS) and pressure-natriuresis relationship are closely coordinated mechanisms that are crucial for maintaining sodium balance and systemic blood pressure (8).

The RAS is the major hormone system regulating sodium balance. At physiological concentrations, ANG II stimulates proximal tubular sodium reabsorption. ANG II may also act to decrease sodium excretion and increase urinary concentrating ability by reducing renal medullary blood flow. Through indirect effects, ANG II enhances sodium reabsorption through stimulation of aldosterone release from the adrenal gland.

On the basis of pharmacological criteria, ANG II exerts its actions via two subtypes of receptors, AT1 and AT2. Most of the classic functions of ANG II are mediated through the AT1 receptor. In rodents, the AT1 receptor is divided into two subtypes, AT1A and AT1B. With the use of gene-targeting techniques, the AT1A gene has been inactivated in mice, resulting in the functional deletion of the AT1A receptor (4). Previous studies using these mice demonstrated that the AT1A receptor has a critical role in regulating blood pressure (2, 4, 13, 15, 19, 20). In these mice, loss of AT1A resulted in lower blood pressure, decreased ability to conserve sodium, and an inability to appropriately concentrate the urine. However, the interaction between the AT1A receptor deletion and the pressure-natriuresis relationship has not been defined. The present study was designed to assess the importance of the AT1A receptor in chronically regulating blood pressure and sodium balance across a spectrum of varying dietary sodium intakes.

MATERIALS AND METHODS

Animals. Mice heterozygous at the AT1A locus, originally derived from the line described by Coffman et al. (13) and backcrossed more than five generations into the C57/B6 strain, were bred to generate wild-type (+/+), heterozygous (+/-), and homozygous null (−/−) littermates. The genotype at the AT1A locus was determined by PCR analysis of genomic DNA isolated from tail biopsies. The chosen primers [5’-ACCAACTCAACCCAGAAAAGC-3’ (upstream) and 5’-CCAGATGTCTTGGTGGTAGG-3’ (downstream)] amplify both the wild-type (620-bp) and mutant (1.2-kb) sequences, using an annealing temperature of 55°C for 1 min and an extension temperature of 72°C for 1.2 min. Genotyping was accom-
Table 1. Effect of dietary sodium content on food intake

<table>
<thead>
<tr>
<th></th>
<th>Food intake, g/day</th>
<th>Sodium intake, mmol/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>LS</td>
<td>5.9 ± 0.6†</td>
<td>0.07 ± 0.006*</td>
</tr>
<tr>
<td>NS</td>
<td>5.5 ± 0.6</td>
<td>0.08 ± 0.007*</td>
</tr>
<tr>
<td>HS</td>
<td>8.1 ± 0.8*</td>
<td>11.2 ± 1.030*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 mice/group. LS, NS, and HS: low-, normal-, and high-salt diet, respectively. *P < 0.05 vs. NS of the same genotype. †P < 0.05 vs. HS in the same genotype.

Table 2. Effect of chronic dietary sodium manipulation on body weight and organ weight

<table>
<thead>
<tr>
<th>Diet</th>
<th>BW, g</th>
<th>KW/BW, g</th>
<th>HT/BW, g</th>
<th>LV/BW, g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+/+</td>
<td>−/−</td>
<td>+/+</td>
<td>−/−</td>
</tr>
<tr>
<td>LS</td>
<td>15.9 ± 0.9</td>
<td>13.8 ± 0.5†</td>
<td>0.722 ± 0.02</td>
<td>0.654 ± 0.07</td>
</tr>
<tr>
<td>NS</td>
<td>17.8 ± 1.3</td>
<td>14.3 ± 1.0</td>
<td>0.731 ± 0.02</td>
<td>0.625 ± 0.02</td>
</tr>
<tr>
<td>HS</td>
<td>16.4 ± 0.8</td>
<td>14.3 ± 1.3</td>
<td>0.728 ± 0.02</td>
<td>0.810 ± 0.07</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 mice/group. BW, body wt; KW, kidney wt; HT, heart wt; LV, liver wt. *P < 0.05 vs. NS (−/−) genotype. †P < 0.05 vs. (+/+) on the same diet.
Sodium excretion. Table 3 illustrates 24-h urinary sodium excretion rates obtained after 3 wk of dietary sodium manipulation. To ensure adequacy of urine collections, daily urine creatinine excretion was checked in all mice placed in the metabolic cage. No significant differences between urine collection accuracy were found among the groups.

No significant differences in daily sodium excretion were found between (+/+) and (-/-) littermates on any diet. As expected, animals on an HS diet excreted significantly more sodium than animals on the NS or LS diet (Table 3).

Serum sodium and potassium concentrations. Serum sodium and potassium concentrations were unaffected by genotype or dietary sodium intake (Table 4).

Table 4. Effect of chronic dietary sodium manipulation on serum sodium, net sodium balance, and serum potassium

<table>
<thead>
<tr>
<th></th>
<th>Serum Sodium, mmol/l</th>
<th>Net Sodium Balance, mmol·g⁻¹·day⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(+/)</td>
<td>(-/−)</td>
</tr>
<tr>
<td>LS</td>
<td>139 ± 2</td>
<td>143 ± 1</td>
</tr>
<tr>
<td></td>
<td>0.005 ± 0.0005</td>
<td>0.005 ± 0.0006</td>
</tr>
<tr>
<td>NS</td>
<td>143 ± 3</td>
<td>140 ± 4</td>
</tr>
<tr>
<td></td>
<td>0.056 ± 0.005</td>
<td>0.062 ± 0.003</td>
</tr>
<tr>
<td>HS</td>
<td>140 ± 2</td>
<td>142 ± 3</td>
</tr>
<tr>
<td></td>
<td>0.652 ± 0.104</td>
<td>0.692 ± 0.028</td>
</tr>
</tbody>
</table>

Table 3. Effect of chronic dietary sodium manipulation on urinary sodium excretion

<table>
<thead>
<tr>
<th>Urinary Sodium Excretion, mmol Na⁺·mg creatinine⁻¹·g BW⁻¹·day⁻¹</th>
<th>(+/)</th>
<th>(-/−)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LS</td>
<td>0.25 ± 0.05</td>
<td>0.40 ± 0.11</td>
</tr>
<tr>
<td>NS</td>
<td>2.60 ± 0.66</td>
<td>3.42 ± 1.39</td>
</tr>
<tr>
<td>HS</td>
<td>36.8 ± 6.20</td>
<td>31.4 ± 2.50</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 mice/group.

Sodium balance. Sodium balance was calculated as sodium intake minus sodium excretion and expressed as a function of body weight. All animals remained in positive sodium balance regardless of genotype (Table 4). Positive sodium balance increased as dietary intake of sodium increased. There were no differences in net sodium balance between (+/+) and (-/-) mice.

Blood pressure. Figure 2 shows that (-/-) mice on the LS diet had significantly lower systolic blood pressures compared with their (+/+) littermates (P = 0.019). On the HS diet, AT₁A (-/-) mice had systolic pressures similar to (+/+) littermates. Interestingly, the systolic blood pressures of the null mice on the HS diet were higher than the null mice on both LS and NS diets. In contrast, there was no effect of dietary sodium intake on systolic blood pressure in wild-type animals. Similarly, there was no significant difference in systolic blood pressure between (+/+) and (-/-) mice on the NS diet.
Pressure natriuresis. To evaluate the effect of the AT₁A receptor on the pressure-natriuresis relationship, the urinary sodium excretion rate (y-axis) was plotted against the average systolic blood pressure (x-axis). Figure 3 shows that AT₁A-deficient mice had a marked decrease in the slope of the pressure-natriuresis curve compared with (+/+ ) littermates. Furthermore, the pressure-natriuresis curve was significantly shifted to the left in the AT₁A-deficient mice compared with wild-type littermates.

PRC. AT₁A (−/−) mice on the LS diet had PRCs that were significantly higher than (+/+ ) controls. This difference between genotypes was not apparent in mice on the NS or HS diet. As expected, the PRC was inversely related to sodium intake in all animals (Fig. 4).

Plasma aldosterone concentrations. Aldosterone concentrations were not significantly different between the (+/+ ) and (−/−) mice on any diet (Fig. 5). However, plasma aldosterone concentration in the (−/−) mice decreased significantly as the sodium content in the diet was increased. Serum and urine potassium levels were not significantly different between the genotypes.

Urine osmolality. On normal sodium intake, urine osmolality was not different between the null and wild-type littermates. However, in animals on either the LS or HS diet, urine osmolalities were lower in AT₁A null mice compared with wild-type littermates (Fig. 6).

Histology. Renal histopathology was significantly altered by the absence of AT₁A but was not affected by diet. Macroscopically, the AT₁A receptor-deficient kidneys showed an irregular lobulated surface compared with the smooth surface of control kidneys. Figure 7, A and B, shows periodic acid-Schiff-stained kidney sections from (+/+ ) and (−/−) kidneys, respectively. Atrophic tubules with flattened tubular epithelia were present in all (−/−) mice. Thickened basement glomerular basement membranes were also apparent. Trichrome staining also revealed abnormal renal architecture [Fig. 7, C (+/+ ) and D (−/−)]. Focal areas of increased hypercellular interstitial tissue (Fig. 7D, arrow) in the (−/−) kidneys were not present in the wild-type controls. Cortical glomeruli in (−/−) mice were variable in size and appeared immature (arrow-
head). Juxtamedullary glomeruli appeared normal. In stark contrast to (+/+ ) kidneys in which glomeruli were buried within a layer of proximal and distal tubules located just below the capsule (Fig. 7C), the glomeruli of the (−/−) kidneys were found adjacent to the renal capsule, possibly suggesting loss of tubular mass (Fig. 7D, arrowhead).

DISCUSSION

The RAS is one of the most powerful hormone systems for regulating blood pressure and body fluid volumes. Most RAS functions are exerted through ANG II actions on AT1 receptors. The direct vasoconstrictor effects of ANG II on blood pressure are closely intertwined with indirect control of volume homeostasis through effects on renal excretion of salt and water (7). The major objectives of this study were to examine the role of the AT1A receptor on sodium excretion, blood pressure, and the pressure-natriuresis relationship during chronically high and low sodium intakes in a mouse line that lacks the AT1A receptor. The results provide evidence supporting the notions that 1) blood pressure is regulated in a salt-sensitive manner in the absence of the AT1A receptor; 2) this regulation is dependent on changes mediated through the pressure-natriuresis relationship; and 3) the AT1A receptor is not required to maintain normal sodium homeostasis.

Our data show that (−/−) mice on a LS diet had a mean decrease in blood pressure of 11 mmHg compared with (−/−) mice on a NS diet whereas there were no differences in blood pressure in (+/+ ) mice on a LS diet. To determine whether the drop in blood pressure in AT1A (−/−) mice was due to differences in sodium balance, we compared the sodium excretion rates for these animals on a LS diet. When challenged, the (−/−) mice were able to reduce urinary sodium excretion about ninefold, from 3.42 μmol Na/mg creatinine on an NS diet to 0.40 μmol Na/mg creatinine on a LS diet. Sodium balance studies show that AT1A (−/−) mice can maintain a minimally positive sodium balance and maintain normal serum sodium when faced with sodium restriction. However, the sodium-restricted (−/−) mice had lower body weights at the end of the study, suggesting that the minimally positive sodium accretion rate may not have been sufficient to allow for normal growth. Alternatively, extracellular fluid volume in (−/−) mice could be decreased in the presence of sodium restriction. Lower body weights cannot be attributed to low caloric intake because there were no differences in the daily food consumption in (−/−) mice compared with their wild-type controls.

On a HS diet, the AT1A receptor-deficient mice excreted the sodium load similarly to their (+/+ ) littermates. Despite similar sodium excretions, blood pressure increased in the (−/−) mice from 87 mmHg (NS) to 103 mmHg (HS). It is possible that even in the face of elevated water excretion, the concomitant elevation of water intake in salt-loaded (−/−) mice resulted in net volume expansion and increased blood pressure. In our study, the body weights of (−/−) mice did not differ from either the (+/+ ) controls on the HS diet or the (−/−) mice on NS intake, suggesting that body water was not significantly altered. However, Cervenka et al. (2) showed that 7–8% body weight volume expansion elicited marked increases in sodium excretion and increases in blood pressure without changing body weight. Our animals could also have experienced clinically significant volume expansion without statistically
In contrast to previous studies, the blood pressures of our AT1A receptor-deficient mice on NS intakes were not lower than in the wild-type controls. The earlier reports utilized F2 generations of AT1A receptor-deficient mice that demonstrated blood pressures significantly lower than wild-type controls. The mice utilized in the present study have been backcrossed more than six generations into a C57BL/6 background, thereby reducing the genetic heterogeneity in the originally described mice. In the first descriptions of the AT1A knockouts, only minimal renal abnormalities were found. These changes included hypertrophy of the juxtaglomerular apparatus and proximal expansion of renin-producing cells along the afferent arterioles (20). In an attempt to ascertain the reasons behind the differences between our baseline blood pressure data and the previous reports, we evaluated the renal histology in the inbred AT1A line. Our inbred AT1A receptor-deficient mice exhibit more significant renal pathology, consisting of delayed glomerular maturity and tubular atrophy. These findings are similar in many aspects to the studies in which angiotensin production was inhibited by the angiotensin-converting enzyme or the deletion of the angiotensinogen gene (6, 15, 23, 25, 26). Hence, background genetic variability clearly affects the renal phenotype of these animals and may manifest physiologically as differences in blood pressure.

In contrast to previous studies that suggest AT1A deficiency results in renal salt wasting (19), our experiments revealed no differences between (+/+) and (−/−) mice with respect to sodium excretion. These results may differ due to the chronic nature of the dietary sodium manipulation in our study compared with the shorter time period in other studies. Careful correction of urinary sodium excretion for creatinine excretion, and/or the previously mentioned differences in renal histopathology, may also account for these differences. Clearly, our results indicate an ability to...
maintain normal sodium excretion in the absence of the AT1A receptor.

In summary, our results indicate that AT1A receptor-deficient mice are able to reduce urinary sodium excretion under conditions of sodium restriction and excrete a sodium load on an HS diet. However, AT1A receptor-deficient mice require a larger change in blood pressure to achieve the same degree of sodium excretion than their wild-type littermates. Maintenance of equivalent sodium excretion between (+/+ ) and (-/-) animals on LS, NS, and HS diets suggests that the AT1A receptor is not the primary mechanism by which chronic renal sodium balance is controlled. Compensatory mechanisms such as aldosterone and AT1B receptors are likely to be involved. Importantly, however, blood pressure directly correlates with salt intake in AT1A receptor-deficient mice, while blood pressure is unaffected by dietary sodium manipulation in wild-type mice. This is illustrated by a leftward shift in the pressure-natriuresis curve compared with that for the (+/+ ) control mice. Although, these hemodynamic effects may be the direct result of deletion of the AT1A receptor, the architectural abnormalities seen in these mice cannot be ruled out as contributory processes. This study confirms the significant role of the AT1A receptor in the pressure-natriuresis relationship.

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