Regulation of Sodium Balance and Blood Pressure by the AT<sub>1A</sub> Receptor for Angiotensin II

Michael I. Oliverio, Christopher F. Best, Oliver Smithies, Thomas M. Coffman

Abstract—To examine the role of the angiotensin II (AT)<sub>1A</sub> receptor in the regulation of blood pressure and sodium balance, we measured systolic blood pressure responses in AT<sub>1A</sub>- receptor–deficient (Agtr1a<sup>-/-</sup>) and wild-type (Agtr1a<sup>+/+</sup>) mice while dietary sodium content was systematically altered. On a 0.4% sodium diet, systolic blood pressures were significantly lower in Agtr1a<sup>-/-</sup> than in <sup>+/+</sup> mice. In Agtr1a<sup>+/+</sup> mice, changing dietary sodium content did not affect blood pressure. In contrast, when Agtr1a<sup>-/-</sup> mice were fed a high-salt diet (6% NaCl), their systolic blood pressures increased significantly from 79±4 to 94±4 mm Hg (P<0.006). The low blood pressures of Agtr1a<sup>-/-</sup> mice decreased further while on a low-salt diet from 82±3 to 69±3 mm Hg (P<0.03). On the high-salt diet, urinary sodium excretion increased to similar levels in Agtr1a<sup>+/+</sup> and <sup>-/-</sup> mice. Although urinary sodium excretion was substantially reduced in both groups during the low-salt diet, cumulative sodium balances became negative in Agtr1a<sup>-/-</sup> mice despite a 6-fold increase in urinary aldosterone. We infer, therefore, that the reduced blood pressures in Agtr1a<sup>-/-</sup> mice on a normal diet are caused by depletion of sodium and extracellular volume. Their “sodium sensitivity” suggests a critical role for renal AT<sub>1A</sub> receptors to modulate sodium handling. (Hypertension. 2000;35:550-554.)

Key Words: mice ■ aldosterone ■ receptors, angiotensin II ■ genes

The renin-angiotensin system (RAS) is a potent regulator of sodium and fluid balance. Dysregulation of the RAS causes chronic elevations in blood pressure in experimental models and in patients with hypertension. The efficacy of antagonists of the RAS as antihypertensive agents provides compelling evidence for the role of this system in the pathogenesis of hypertension in large numbers of affected individuals. The Guyton hypothesis proposes that the actions of the RAS to cause hypertension involve chronic alterations in renal sodium handling.

The role of the RAS to modulate sodium excretion by the kidney has been well documented. In animal models and in humans, suppression of angiotensin II production is required for normal excretion of a sodium load, whereas long-term administration of subpressor doses of angiotensin II increases blood pressure by altering sodium balance. Conversely, during sodium depletion, pharmacological inhibition of the RAS causes an inappropriate natriuresis and reductions in blood pressure. Angiotensin II may affect sodium excretion through several discrete mechanisms, including (a) effects on renal hemodynamics; (b) direct stimulation of renal tubular sodium reabsorption; and (c) stimulation of aldosterone production by the adrenal glands.

The physiological effects of angiotensin II are elicited through binding to specific cell surface receptors of 2 pharmacologically distinct types, designated AT<sub>1</sub> and AT<sub>2</sub>. The classically recognized functions of angiotensin II, including its effects to promote sodium retention by the kidney, are mediated by AT<sub>1</sub> receptors. Among the AT<sub>1</sub> receptors, 2 subtypes (AT<sub>1A</sub> and AT<sub>1B</sub>) have been identified in mouse and rat. These receptors are products of separate genes (Agtr1a and Agtr1b), and they share substantial sequence homology. Previous studies suggest that the AT<sub>1A</sub> receptors are the predominantly expressed AT<sub>1</sub> receptor in most tissues, including the kidney.

The binding signatures of the AT<sub>1A</sub> and AT<sub>1B</sub> receptors are identical, making it impossible to discriminate their in vivo functions with the use of pharmacological agents. Gene targeting experiments have established a physiological hierarchy for these receptors (AT<sub>1A</sub> > AT<sub>1B</sub>). Accordingly, AT<sub>1B</sub> receptor–deficient mice exhibit no abnormal phenotype. In contrast, the vasoconstrictor actions of angiotensin II are markedly diminished in AT<sub>1A</sub> receptor–deficient mice, and their resting blood pressures are 20 to 25 mm Hg below normal. Because this level of blood pressure reduction approaches that seen in mice completely lacking angiotensinogen, most of the effects of angiotensin II to regulate resting blood pressure in mice appear to be mediated through the AT<sub>1A</sub> receptor. Finally, AT<sub>1</sub>-specific binding is virtually absent in the kidneys of Agtr1a<sup>-/-</sup> mice, confirming that AT<sub>1A</sub> is the predominant isof orm at this site.

Because of the critical actions of AT<sub>1A</sub> receptors in blood pressure regulation and their position as the major renal AT<sub>1</sub>
receptor, \textit{Agtr1a}−/− mice provide a unique investigative tool for understanding the regulation of blood pressure and solute excretion by AT\textsubscript{1} receptors. The objective of the present study was to examine the role of the AT\textsubscript{1A} receptor in regulating blood pressure responses during alterations in dietary sodium content. We find that \textit{Agtr1a}−/− mice exhibit sodium-sensitive blood pressure changes despite an intact mineralocorticoid response.

**Methods**

**Animals**

Mice lacking AT\textsubscript{1A} receptors for angiotensin II were generated by homologous recombination in embryonic stem cells as previously described.\textsuperscript{18} Animals were bred and maintained in the animal facility of the Durham Veterans Administration Medical Center under National Institutes of Health guidelines. The experimental procedures described below were approved by the animal research committees of the Durham VA and Duke University Medical Centers. \textit{Agtr1a} genotypes, designated (+) for the wild-type allele and (−) for the targeted allele, were determined by Southern blot analysis of DNA isolated from tail biopsies.\textsuperscript{18} Mice were generated from crosses of (129xC57BL/6)\textsubscript{F1} mice. The F\textsubscript{2} generation \textit{Agtr1a}+/+ and −/− animals derived from these crosses were used in these experiments. We studied both male and female mice that were 2 to 4 months old.

**Systolic Blood Pressure Measurements in Conscious Mice**

Systolic blood pressures were measured in conscious mice with the use of a computerized tail-cuff system (Visitech Systems). Before the study was initiated, mice were adapted to the apparatus for 5 days. The validity of this system has been established previously.\textsuperscript{7,18,22}

**Effects of Altered Dietary Sodium on Systolic Blood Pressures**

To determine the effects of the \textit{Agtr1a} gene disruption on the adaptation to changes in dietary salt intake, we measured systolic blood pressures in mice that were sequentially fed diets of differing sodium chloride content. \textit{Agtr1a}+/+(n=7) and \textit{Agtr1a}−/− (n=6) mice were first fed a control diet containing 0.4% sodium chloride for 2 weeks (control period 1). This was followed by a 14-day period in which the animals were given a high-salt diet containing 6% sodium chloride (high salt). After the high salt feeding, the mice were reequilibrated on the control diet for 7 days (control period 2). The animals were then fed a low-salt diet containing <0.02% sodium chloride for the next 14 days (low salt). All diets were purchased from Harlan-Teklad. Mice were allowed free access to water. Systolic blood pressures were measured ≥5 times per week throughout the period of study.

**Measurement of Urinary Sodium and Aldosterone Excretion**

To estimate intake and urinary excretion of sodium, mice were individually housed in metabolic cages. Separate groups of \textit{Agtr1a}+/+ and −/− mice (n=6 for each genotype and diet) were fed a 0.4% sodium chloride diet for 7 days followed by either a 6% sodium chloride diet or a <0.02% sodium chloride diet for 7 days. Body weight, water and food intake, urine output, and urinary sodium excretion were measured daily. Urine sodium was measured with a Beckman E3 Na+/K+ autoanalyzer. Two 24-hour urine samples (days 5 and 6 of each dietary period) from each mouse were used for measurement of aldosterone by radioimmunoassay according to the manufacturer’s instructions (Diagnostic Labs).

**Results**

**Absence of AT\textsubscript{1A} Receptors Results in Sodium-Sensitive Changes in Blood Pressure**

During the initial control period when both groups of mice were fed a normal 0.4% NaCl diet, systolic blood pressures were significantly lower in \textit{Agtr1a}−/− mice compared with \textit{Agtr1a}+/+ controls (79±4 vs 102±4 mm Hg; \(P<0.005\)). As demonstrated in Figure 1, dietary salt loading (6% NaCl) had no effect on the systolic blood pressures of \textit{Agtr1a}+/+ mice. However, blood pressures in the \textit{Agtr1a}−/− mice increased by 15 mm Hg on the high-salt diet from 79±4 to 94±4 mm Hg (\(P<0.006\)). When the control diet was reinstated, blood pressures in the \textit{Agtr1a}−/− mice returned to their initial reduced level (82±3 mm Hg). The low-salt diet (<0.02% NaCl) had no effect on blood pressures in the wild-type mice. In contrast, the \textit{Agtr1a}−/− mice had a further reduction in blood pressure on the low-salt diet, from 82±3 to 69±3 mm Hg (\(P<0.04\)).

**Sodium Balance Studies**

Because of the marked blood pressure change in \textit{Agtr1a}−/− mice with variation of dietary sodium content, we examined the sodium balance of separate groups of \textit{Agtr1a}−/− and wild-type mice during these dietary manipulations. While being fed the high-salt diet, all of the mice, regardless of genotype, lost a significant amount of body weight (from 36.3±1.5 to 33.4±1.4 g in \textit{Agtr1a}+/+ and from 30.8±2.3 to 27.6±2.6 g in \textit{Agtr1a}−/−; \(P<0.03\)). Figure 2 illustrates the daily sodium excretion in \textit{Agtr1a}+/+ and −/− mice during high salt feeding. Sodium excretion increased dramatically and almost immediately on initiation of high salt feeding, reaching near maximum levels by day 2. The levels of urine sodium excretion were not discernibly different between the groups.
When Agtr1a+/+ and −/− mice were fed the low-salt diet, their body weights remained stable (31.7±1.5 to 32.7±1.5 g in Agtr1a+/+ and 26.2±2.6 to 26.1±2.5 g in −/− mice). After introduction of the low-salt diet, urinary sodium excretion decreased markedly and rapidly in mice of both genotypes, as illustrated in Figure 3A. However, the sodium excreted was greater in Agtr1a−/− mice compared with +/+ mice at every time point during the 7 days of low salt feeding. Accordingly, as shown in Figure 3B, when cumulative sodium balances of the Agtr1a+/+ and −/− groups were calculated over the week of sodium restriction, the 7-day mean cumulative sodium balance was negative in the Agtr1a−/− mice (−0.077±0.018 mmol Na+) and significantly lower than wild-type controls, which remained in neutral balance (−0.003±0.022 mmol Na+; P=0.02 vs Agtr1a−/−).

Assessment of Mineralocorticoid Responses

Because angiotensin II is a major physiological regulator of aldosterone secretion, we examined levels of urinary aldosterone excretion in Agtr1a+/+ and −/− mice during each dietary regimen. The results from these experiments are shown in Figure 4. On the control diet, the levels of aldosterone were similar in the Agtr1a+/+ and −/− groups (12.4±2.8 vs 16.9±4.3 μg/d per 20 g body wt; P=0.4). The introduction of a high-salt diet caused similar suppression of aldosterone excretion in both Agtr1a+/+ (2.6±0.4 μg/d per 20 g body wt) and −/− groups (4.5±1.5 μg/d per 20 g body wt). Dietary sodium restriction caused marked stimulation of aldosterone excretion in both Agtr1a+/+ and −/− animals to levels that again were not significantly different between the groups (101.9±26.4 vs 103.5±28.3 μg/d per 20 g body wt; P=0.02 vs control for both groups).

Discussion

The type 1A receptor for angiotensin II plays an important role in the regulation of blood pressure, as demonstrated by the reduced blood pressures and altered vascular responses of Agtr1a−/− mice, which lack this receptor.18–20 We now show that Agtr1a−/− mice exhibit sodium-dependent changes in blood pressure. The low blood pressures of Agtr1a−/− mice are substantially increased by dietary salt loading, and they are reduced further during sodium restriction. These findings agree with prior studies in which pharmacological interruption of the RAS causes an inability to maintain arterial blood pressure during sodium depletion6–23 and the recent observation of Cervenka et al24 that acute volume expansion increases the blood pressure of Agtr1a−/− mice. Thus we infer that extracellular fluid volume depletion contributes significantly to the low blood pressure phenotype of the Agtr1a−/− mouse. These findings reflect the importance of the AT1A receptor in the regulation of blood pressure through its ability to affect sodium and extracellular fluid volume homeostasis.

Agtr1a−/− and wild-type mice respond differently to dietary sodium restriction, as demonstrated by the higher levels of sodium excretion and the negative sodium balance observed in Agtr1a−/− mice. The physiological consequence of this inappropriate natriuresis is a further lowering of their blood pressures despite normal stimulation of aldosterone release. Thus the failure of renal sodium conservation...
in the Agtr1a−/− mice is due to the absence of AT1A receptors in the kidney rather than abnormal regulation of mineralocorticoid release. The dominant effect of intrarenal actions of angiotensin II in regulating sodium handling by the kidney has been suggested previously. For example, Hall et al23 have observed that chronic blockade of angiotensin II formation in sodium-depleted dogs lowered blood pressure and caused sodium wasting independent of changes in circulating aldosterone levels. Similarly, Lohmeier et al25 found that adrenalectomized dogs conserve sodium normally during sodium restriction, but this response can be inhibited by the administration of an angiotensin II peptide antagonist. Our studies confirm this previous work and demonstrate a critical role for direct actions of AT1A receptors in regulating blood pressure and sodium balance.

The specific cellular actions of AT1A receptors that mediate these dominant effects on renal sodium handling cannot be determined from our studies. However, AT1 receptors are expressed in discrete cellular compartments in the kidney, where they may have potent effects on renal hemodynamics and excretory functions.5 AT1 receptor stimulation causes distinct changes in the glomerular circulation that act to promote proximal tubular reabsorption of sodium. Activation of AT1 receptors in the renal vasculature also reduces blood flow to the medulla, promoting antinatriuresis. In addition, AT1 receptors are expressed at high levels in epithelial cells in the proximal tubule, in the thick ascending limb of the loop of Henle, in the distal tubule, and in the collecting duct.26,27 Stimulation of AT1 receptors in these nephron segments causes solute and fluid reabsorption.7,28 We speculate that the absence of AT1A receptor–mediated functions in renal vasculature and epithelia produce abnormal renal sodium handling.

Angiotensin II is a physiological regulator of aldosterone release, and this action is mediated by AT1 receptors in the adrenal zona glomerulosa.30 Despite the absence of the major murine AT1 receptor isofrom, Agtr1a−/− mice appropriately increase their levels of aldosterone in response to sodium depletion. The similar basal aldosterone levels in Agtr1a−/− and wild-type mice, however, could be interpreted as an inappropriate response in the Agtr1a−/− mice in the setting of low blood pressures caused by reduced extracellular volume. The preservation of aldosterone regulation in response to changes in dietary sodium in Agtr1a−/− mice may be explained by residual AT1A receptors, which are prominently expressed in the adrenal glands of rats and mice.12,13 Alternatively, Okubo and associates31 found intact aldosterone responses in angiotensinogen-deficient mice, which suggests that substantive regulation of aldosterone release can occur without a functional RAS. Thus, although AT1A receptors are essential for normal sodium handling by the kidney, they are not required for enhancement of aldosterone release during sodium depletion.

In summary, the absence of AT1A receptors for angiotensin II produces a state of sodium sensitivity in which alterations in sodium intake cause marked fluctuations in blood pressure. These sodium-dependent blood pressure changes occur despite an intact mineralocorticoid response. Our findings suggest that AT1A receptors expressed on the renal vasculature and/or renal epithelia play a critical role in sodium and volume homeostasis.

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References


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Figure 4. Effect of dietary sodium intake on urinary aldosterone excretion measured in 24-hour urine collections from Agtr1a+/+ and Agtr1a−/− mice. Dietary regimens are depicted as in Figure 1. Agtr1a−/− values are depicted by black bars and those of Agtr1a−/− mice by white bars [P<0.02 vs Agtr1a+/+ (C); P=0.02 vs Agtr1a−/− (C)].
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