Aldosterone Synthase

Disturbed Homeostasis in Sodium-Restricted Mice Heterozygous and Homozygous for Aldosterone Synthase Gene Disruption

Natalia Makhanova, Maria L.S. Sequeira-Lopez, R. Ariel Gomez, Hyung-Suk Kim, Oliver Smithies

Abstract—We have determined that differences in expression of aldosterone synthase (AS) affect responses to a low-salt diet. In AS-null mice (AS<sup>−/−</sup>), but not in wild-type, low salt significantly decreased plasma sodium and increased potassium. The increased urine volume (1.5×wild-type) and decreased urine osmolality (0.7×wild-type), present in AS<sup>−/−</sup> mice on normal salt, became more severe (2.3×wild-type and 0.5×wild-type) on low salt, but neither changed in wild-type. In both genotypes, plasma vasopressin was similar on normal and low salt, and desmopressin injection significantly increased urine osmolality. Renal mRNA levels for aquaporin 1 and 3 were unchanged by genotype or diet and epithelial sodium channel and Na<sup>+</sup>-K<sup>+</sup>-2Cl<sup>−</sup>-cotransporter by genotype. In AS<sup>−/−</sup> mice, aquaporin 2 mRNA increased on normal salt, whereas Na<sup>+</sup>Cl<sup>−</sup>-cotransporter and cortex K<sup>+</sup>-channel mRNAs decreased on both diets. The low blood pressure of AS<sup>−/−</sup> mice was decreased further by low salt, despite additional increases in renin, intrarenal arterial wall thickness, and macula densa cyclogegenase-2 mRNA. In AS<sup>−/−</sup> mice on normal salt, adrenal AS mRNA was slightly decreased (0.7×wild-type), but blood pressure was normal. On low salt, their blood pressure was less than wild-type (101±2 mm Hg versus 106±2 mm Hg), even though renin mRNA increased to 2×wild-type. We conclude that aldosterone is critical for urine concentration and maintenance of blood pressure and even a mild reduction of AS expression makes blood pressure sensitive to low salt, suggesting that genetic differences of AS levels in humans may influence how blood pressure responds to dietary salt. (Hypertension. 2006;48:1151-1159.)

Key Words: aldosterone synthase ■ blood pressure ■ electrolytes ■ renin ■ COX-2

The mineralocorticoid aldosterone plays a central role in the control of blood pressure (BP) and fluid and electrolyte homeostasis. Aldosterone modulates BP through several mechanisms. Thus, aldosterone can affect BP by altering sodium excretion in the distal nephron of the kidney, where it increases reabsorption of sodium and potassium secretion by increasing the activities of the amiloride-sensitive epithelial sodium channel (ENaC), the Na<sup>+</sup>-K<sup>+</sup>-2Cl<sup>−</sup>-cotransporter by genotype. In AS<sup>−/−</sup> mice, aquaporin 2 mRNA increased on normal salt, whereas Na<sup>+</sup>Cl<sup>−</sup>-cotransporter and cortex K<sup>+</sup>-channel mRNAs decreased on both diets. The low blood pressure of AS<sup>−/−</sup> mice was decreased further by low salt, despite additional increases in renin, intrarenal arterial wall thickness, and macula densa cyclogegenase-2 mRNA. In AS<sup>−/−</sup> mice on normal salt, adrenal AS mRNA was slightly decreased (0.7×wild-type), but blood pressure was normal. On low salt, their blood pressure was less than wild-type (101±2 mm Hg versus 106±2 mm Hg), even though renin mRNA increased to 2×wild-type. We conclude that aldosterone is critical for urine concentration and maintenance of blood pressure and even a mild reduction of AS expression makes blood pressure sensitive to low salt, suggesting that genetic differences of AS levels in humans may influence how blood pressure responds to dietary salt.

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Methods

Mice

AS-null mice were generated by gene targeting in strain 129-derived ES cells, as described previously.\textsuperscript{15} Wild-type mice (AS\textsuperscript{+/+}) and heterozygous (AS\textsuperscript{+-}) and homozygous null (AS\textsuperscript{---}) littermates, all on the inbred strain 129 Sv/Ev genetic background, were used in this study. Mice in the NS group were fed regular mouse chow (0.26% NaCl, Prolab, Isopro RMH 3000). Mice in the LS group were raised on an NS and then changed to an LS diet (0.05% NaCl, Harlan Teklad, TD 94025). These diets are comparable except for their NaCl content. After 3 to 4 weeks on the LS diet, BPs were measured (7 days), and urine collections were then made in metabolic cages (3 days). Individual blood samples were taken for plasma measurements 4 days after completing of the metabolic studies, except that aldosterone, angiotensin II (Ang II), and vasopressin measurements were collected during the terminal anesthesia with 2.5% avertin, which was after 6 to 8 weeks on the LS diet. The numbers of mice used in each experiment are shown in the tables and figures. Mice were maintained according to the National Institutes of Health Guide for the Care and the Use of Laboratory Animals. All of the experiments were conducted with male and female mice \textasciitilde3 to 5 months old and were approved by the Institutional Animal Care and Use Committee of the University of North Carolina, Chapel Hill.

BP Measurement

BPs were measured in conscious mice with a computerized tail-cuff system (Visitech Systems).\textsuperscript{17}

Blood Analysis

Blood from the retro-orbital sinus was collected under anesthesia with 2.5% avertin, and plasma electrolyte concentrations were measured using a VT250 Chemical Analyzer (Orthodiagnostic Clinical). Hematologic analyses were performed with an Animal Blood Counter (Heska). Plasma aldosterone was measured by radioimmunoassay (RIA) using the Coat-A-Count RIA procedure (Diagnostic Products). Plasma corticosterone was determined with an RIA kit (ICN Biomedicals). The concentration of Ang II was measured with a bioassay (RIA) using the Coat-A-Count RIA procedure (Diagnostic Products). Plasma aldosterone, angiotensin II (Ang II), and vasopressin measurements were collected during the terminal anesthesia with 2.5% avertin, which was after 6 to 8 weeks on the LS diet. The numbers of mice used in each experiment are shown in the tables and figures. Mice were maintained according to the National Institutes of Health Guide for the Care and the Use of Laboratory Animals. All of the experiments were conducted with male and female mice \textasciitilde3 to 5 months old and were approved by the Institutional Animal Care and Use Committee of the University of North Carolina, Chapel Hill.

Histological Analysis

Mice were euthanized with 2.5% avertin for harvesting organs. The organs were fixed in 4% buffered paraformaldehyde overnight, embedded in paraffin, and sectioned and stained with hematoxylin and eosin, periodic acid-Schiff, or Masson-Trichrome for light microscopy. The immunohistochemical detection of renin and cytochrome (COX)-2 was performed as described previously.\textsuperscript{16}

Real-Time RT-PCR

Expression in the kidney of genes responsible for salt and water transport was determined by quantitative real-time PCR with an Applied Biosystems 7700 Sequence Detection System (Perkin-Elmer), as described.\textsuperscript{18}

Analyses of Urine and Kidney Function

To determine 24-hour water and food intakes, urine volumes, and excretion of electrolytes, mice were housed in metabolic cages for 3 days. Urine osmolality was measured by freezing point depression. After 5 days of drinking 5% dextrose, the effect of vasopressin on urinary concentrating mechanisms was investigated by measuring urinary osmolality before and 4 hours after subcutaneous injection of 1-ng/g body weight of desmopressin (dDAVP, Sigma) as described previously.\textsuperscript{19} To eliminate the effects of increased water intake during assays of urinary concentrating ability, we restricted the water intake in the AS\textsuperscript{--} mice to be the same for 4 days as that consumed by wild-type mice with similar body weights and then measured their body weights and urine osmolality.

Statistical Analysis

All of the statistical analyses were performed using JMP Statistical Software (SAS Institute) and are presented as mean\textpm SEM. Statistical significances were assessed with ANOVA, and posthoc analyses were performed using the unpaired \textit{t} test.

Results

\textbf{AS\textsuperscript{--} Mice Survive on LS Diet}

We have shown previously\textsuperscript{15} that 30% of AS\textsuperscript{--} mice die before weaning between 7 and 28 days, probably from dehydration. However, after weaning, the homozygous mice survive on both an NS and an LS diet.

\textbf{AS\textsuperscript{--} Mice Show Decreases in BP on LS Diet}

The BP in AS-null mice on NS diet was 11 mm Hg lower (96\textpm2 mm Hg) compared with either wild-type (107\textpm2 mm Hg; \textit{P}<0.001) or heterozygous (108\textpm2 mm Hg; \textit{P}<0.0001) mice, in which BP did not differ significantly (Figure 1A). The LS diet had no effect on BP in wild-type mice (Figure 1A). In contrast, the LS diet in AS\textsuperscript{--} mice resulted in a further decrease in BP to 85\textpm3 mm Hg compared with their already reduced BP on NS diet (96\textpm2 mm Hg; \textit{P}<0.01).

\textbf{AS\textsuperscript{+/+} Mice Show Decreases in BP on LS Diet}

Although there were no significant differences in BP between AS\textsuperscript{++} and AS\textsuperscript{++} mice on the NS diet, the mean BP of the
heterozygous AS−/− mice on the LS diet was decreased to 101±2 mm Hg compared with the unchanged BP of wild-type mice (106±2 mm Hg; P<0.05) on the LS diet (Figure 1A).

Abnormalities in Electrolyte Homeostasis in the AS-Null Become More Severe on LS Diet

To determine the effects of LS diet on electrolyte homeostasis, we measured plasma Na+ and K+ concentrations in AS+/+, AS−/−, and AS−/− mice. There were no differences in plasma Na+ concentration on the NS diet among the 3 genotypes (Figure 1B). However, on the LS diet, plasma Na+ concentration was significantly lower in the AS−/− mice (139±2 mmol/L) compared with wild-type mice (145±2 mmol/L; P<0.05); and plasma K+ concentration in the AS−/− mice increased significantly from 6.6±0.1 mmol/L on the NS diet to 7.2±0.2 mmol/L (P<0.01) on the LS diet, which was also significantly higher than in AS−/− and AS+/+ mice on the LS diet (5.6±0.15 mmol/L; P<0.0001; Figure 1C). Dietary salt had no effect on these parameters in the AS+/+ mice.

Body and Kidney Weights, Urine Volume, and Osmolality in AS+/+ and AS−/− Mice on LS Diet

Table 1 shows that on the NS diet, homozygous AS−/− mice had significantly lower body weights compared with wild-type and heterozygous mice, and their kidney/body weight ratios were also decreased. The LS diet did not significantly affect the body weight or the kidney/body weight ratio in any of the 3 genotypes. Nor were there any differences in hematocrit on the NS and the LS diets among the 3 genotypes (Table 1). On the NS diet, the AS−/− mice showed higher urine outputs and decreased urine osmolalities than wild-type mice. On LS diet, the abnormalities in water handling in the homozygous mice became more severe. Thus, 24-hour urine volume in the AS−/− mice increased (P<0.01) and urine osmolality decreased (P<0.01) further on the LS diet compared with the NS diet. Urine volumes in the heterozygous AS−/− mice were also increased on the NS and the LS diets compared with AS+/+ mice; however, in AS−/− or AS+/+ mice, the dietary salt had no effect on any other of the parameters listed in Table 1, except plasma vasopressin.

Plasma Vasopressin in AS+/+ and AS−/− Mice

To determine whether changes in plasma vasopressin levels might be responsible for the abnormalities in water homeostasis seen in the AS null mice, the circulating levels of vasopressin of AS+/+, AS−/−, and AS−/− mice on NS and LS diets were measured by RIA. The plasma vasopressin levels were not different between the 3 genotypes (Table 1) on the NS diet. However, plasma vasopressin levels were markedly increased in all 3 of the genotypes (P<0.0001) when fed the LS diet, but this increase was unaffected by the AS genotype.

Response to Desmopressin

To further investigate the underlying mechanism for the urinary concentrating defect in the AS−/− mice, we compared the responses of the AS+/+ and the AS−/− mice to the V2 receptor-specific vasopressin analog dDAVP. Figure 2A shows that 4 hours after subcutaneous injection of dDAVP,
urinary osmolality was significantly increased in both the AS+/− mice (from 1802±64 milliosmols [mosmol] to 2132 mosmol; n=6; P<0.05) and the AS+/- mice (from 2244±96 mosmol to 2910 mosmol; n=4; P<0.05), although urine osmolality was always lower in the AS-null mice than in wild-type.

Restriction of Water Intake Does Not Correct the Urinary Concentrating Deficit
To eliminate the effects of increased water intake on urinary concentrating capacity in the AS-null mice, we restricted their water consumption for 4 days to the same level as that consumed by wild-type mice with similar body weights. As shown in Figure 2B, there were no changes in the urine osmolality in the AS+/- mice during this period of water restriction, however, because body weights decreased markedly in the smaller mice, so that experiment was terminated. The average changes in body weight were from 19.0±1.3 g before water restriction to 15.6±1.6 on day 4 (n=5; P=0.1).

mRNAs in the Adrenal Gland
To characterize the effect of LS diet on gene expression in the adrenal gland of AS+/+, AS+/-, and AS-/- mice, we analyzed mRNA levels by quantitative RT-PCR. As expected, expression of the AS gene (Cyp11b2) was not detectable in the AS+/- mice on NS and LS diets. The AS mRNA in the adrenals of the heterozygous AS+/- mice on the NS diet was 70% of wild-type, although this difference did not reach significance (Figure 3A). The LS diet caused a significant increase in adrenal AS mRNA in both the wild-type and AS+/- mice (P<0.001 by ANOVA), with the heterozygotes AS+/- now showing a significant difference from wild-type mice (P<0.05).

There were no differences in the expression of Cyp11b1 mRNA, which codes for 11β-hydroxylase, among the 3 genotypes on the NS diet (Figure 3B). However, on the LS diet, expression of Cyp11b1 in AS+/- mice tended to increase (P=0.06) compared with that of wild-type mice.

Absence of aldosterone in the AS-/- mice resulted in a >100-fold increase in adrenal renin mRNA level on the NS diet compared with that of wild-type (P<0.0001) or heterozygous mice (P<0.0001), which did not differ from each other (Figure 3C). However, the LS diet has no detectable effect on renin expression in all 3 genotypes.

Histological Changes in the Adrenal Glands of AS-/- Mice
The significant histological differences in adrenal structure between AS+/- and AS-/- mice on a NS diet, described in our previous article,15 were exaggerated by the LS diet. Thus, under sodium restriction, the AS-/- mice showed an abnormal adrenal cortex with a marked increase in the zona glomerulosa and in the number of renin-producing cells (Figure 4C).

Renin–Angiotensin System Activation on LS Diet
Figure 5 compares the effects of the LS diet on the renin–angiotensin system (RAS) and plasma glucocorticoids of the AS+/-, AS+/-, and AS-/- mice. On the LS diet, the level of renin mRNA in the kidney in wild-type mice became 126% but did not reach significance (P=0.19; Figure 5A). However, the renin mRNA level in the AS+/- mice significantly increased (P<0.01) by the LS diet compared with the NS diet, reaching ≈2 times the level of wild-type mice on the LS diet (P<0.01). The increased renin mRNA level of the AS+/- mice on the NS diet was also further increased by the LS diet (P<0.01). The plasma concentration of Ang II in the AS-null mice on the NS diet was significantly higher than in wild-type mice (P<0.01) and was increased further by the LS diet (P<0.0001) (Figure 5B). The AS+/- heterozygous mice on the LS diet also showed a tendency toward an increased Ang II plasma concentration (P=0.09) compared with wild-type mice.

Plasma aldosterone was not detectable in the AS-/- mice on any diet. Plasma aldosterone concentration in heterozygous AS+/- mice on the NS diet did not differ significantly from that of wild-type mice, although the plasma aldosterone concentration in these mice on the LS diet tended to be lower (P=0.08) compared with wild-type mice (Figure 5C).

Absence of aldosterone in the AS+/- mice on the NS diet resulted in an increased level of corticosterone compared with wild-type mice (Figure 5D). On the LS diet, the level of glucocorticoids in the AS+/- mice further increased compared with AS+/- mice on normal chow (P<0.01). However, the LS diet had no effects on the level of glucocorticoids in wild-type and heterozygous mice.
Abnormalities in Kidney Structure in AS-Null Mice Became More Severe on LS Diet

Histological study revealed that the abnormalities in kidney structure already apparent in AS/+/+ mice on NS became more severe on the LS diet. Thus, as shown in Figure 4E and 4F, the marked hypertrophy of the juxtaglomerular apparatus and expansion of renin-producing cells along afferent arterioles seen in the AS/+/+ mice on NS (Figure 4E) were markedly exaggerated when they were fed the LS diet (Figure 4F).

On the NS diet, AS-null mice showed strongly positive immunohistochemical COX-2 staining in macula densa cells as reported previously16 (Figure 4H). The LS diet resulted in a very strong positive signal for COX-2, as shown in Figure 4I, in agreement with the increased COX-2 mRNA in the kidney cortex of these mice when fed LS compared with NS (Table 2). However, no immunoreactive COX-2 was detectable in the macula densa region of the kidney cortex in wild-type (Figure 4J) and AS-/- (K) mice. Thickening of intrarenal artery walls in the AS/+/+ mice on LS diet (L).

RNA Studies in the Kidney

To determine how the AS genotypes affect renal responses to the LS diet, we measured the expression levels of several genes potentially important for the control of Na+ and water reabsorption by the kidney. These included genes responsible for renal Na+ and K+ transport (NCC, Na+-K+-2Cl- cotransporter [NKCC2], ENaC, and ROMK) and for water reabsorption (aquaporin water channels [AQPs] 1, 2, and 3). We also measured changes in renin and COX-2 expression. The level of mRNA expression for the α, β, and γ subunits of ENaC and NKCC2 did not differ significantly between the genotypes on either the NS or the LS diets (Table 2). NCC expression was decreased in the AS-/- mice on the NS diet compared with AS+/+ mice, but the LS diet had no effect on this difference. The LS diet resulted in a significant increase in ROMK expression in all 3 of the genotypes (P<0.0001×2-way ANOVA), but the AS-/- mice still had lower expression of the ROMK gene on the LS diet than wild-type mice (P<0.05). Of the 3 water channels tested, only the level of mRNA for AQP2 was affected by the AS genotype; it was 70% greater in the AS-/- mice than in wild-type mice when the mice were fed the NS diet but was not significantly affected by the AS genotype in mice fed the LS diet (Table 2).
COX-2–derived prostaglandins in the renal cortex have been shown to play a role in the adjustment of the renin system to sodium restriction. The COX-2 mRNA in renal cortex in the AS-null mice was 42%/H11006/56% of wild-type and was even further increased on the LS diet to 72%/H11006/132% (P/0.05; Table 2). However, the level of COX-2 expression in the renal cortex of the AS/HS/HT mice did not differ significantly from wild-type mice and was not affected by the LS diet. The expression of the genes listed in Table 2 did not differ between the AS/HS/HLT heterozygotes and wild-type mice on either the NS or the LS diets.

Discussion
To study the effects of decreased amounts or absence of aldosterone, we have disrupted the gene coding for AS in mice. We described previously the changes in kidney function in AS/HS/HT mice on an NS diet that occur in response to their inability to synthesize aldosterone. Here we investigate the role that aldosterone plays in the responses of mice to a sodium restriction by examining the BP, electrolyte homeostasis, water handling, and kidney structure in mice with complete aldosterone deficiency. We also show how the loss of 1 copy of the AS gene in heterozygous AS/HS/HLT mice affects their response to an LS diet.

Our data demonstrate that the abnormalities already present in AS/HS/HT mice become more severe on the LS diet, but they are still compatible with survival. Thus, the BP of AS/HS/HT mice was further reduced by the sodium restriction and was now accompanied by a detectable decrease in plasma sodium concentration. However, despite this lower plasma Na⁺ concentration, the levels of renal α, β, and γ ENaC mRNAs on the LS diet in the AS/HS/HT mice were not significantly different from wild-type mice, although in the AS/HS/HT mice the LS diet increased α ENaC. Berger et al. have similarly reported that mRNA levels for ENaC were not changed in MR-null mice on an NS diet. It has been shown that Ang II and glucocorticoids can increase the abundance of α ENaC mRNA. Therefore, it is possible that the increase in the plasma concentration of glucocorticoids and of Ang II that occurs in the AS/HS/HT mice on an LS diet is sufficient to maintain normal RNA levels of sodium channels. Circulating plasma vasopressin levels were markedly increased in both the AS/HS/HT and AS/HS/HT mice when fed the LS diet, but this increase was not affected by absence of aldosterone. This suggests that the decrease in plasma Na⁺ observed in the AS/HS/HT mice on the LS diet is because of urinary sodium wasting caused by absence of aldosterone action in the kidney epithelium rather than because of abnormal regulation of vasopressin secretion in the central nervous system.

One of the most striking findings of our study is how critical aldosterone is for water retention, for urine concentration, and for adjustments to an LS diet. Thus, the abnormalities in water handling (increased urine volume and decreased urine osmolality) present in the AS/HS/HT mice on an NS diet become markedly more dramatic on the LS diet. These findings have allowed us to use the AS/HS/HT mice to investigate how aldosterone contributes to the urinary concentrating mechanism. To test the possibility that the decrease in urinary osmolality is caused by changes in the central control of drinking behavior, we restricted the water consumption (increased urine volume and decreased urine osmolality) present in the AS/HS/HT mice on an NS diet to become markedly more dramatic on the LS diet. These findings have allowed us to use the AS/HS/HT mice to investigate how aldosterone contributes to the urinary concentrating mechanism. To test the possibility that the decrease in urinary osmolality is caused by changes in the central control of drinking behavior, we restricted the water consumption of the AS/HS/HT mice to the level of wild-type mice having similar body weights. This caused the AS/HS/HT mice to lose weight but did not significantly change the osmolality of their urine; we conclude that the increased water consumption of the AS/HS/HT is not the cause of their inability to concentrate urine.

**Figure 5.** Renin mRNA level (values are proportional to the level of wild-type mice (100%) on NS diet; A), plasma Ang II (B), plasma aldosterone (C), and plasma corticosterone (D) in AS/HS/HT and AS/HS/HT mice on NS and LS diets. Boxed numbers indicate the numbers of animals.
Vasopressin and aldosterone have interacting effects. Thus, it has been demonstrated that mineralocorticoids affect the vasopressin-induced transport of water in rabbit collecting ducts in vitro. We, therefore, evaluated the responses of the AS\textsuperscript{−/−} and AS\textsuperscript{−/+} to the V2 receptor–specific agonist dDAVP. Injection of dDAVP resulted in significant increases in urinary osmolality in both the AS\textsuperscript{−/−} and AS\textsuperscript{−/+} mice, although maximal urinary osmolality was not achieved in AS\textsuperscript{−/−} mice; we conclude that absence of aldosterone does not prevent vasopressin from affecting water transport in the kidney.

Decreased responsiveness to vasopressin in AS\textsuperscript{−/−} mice may be partly because of structural changes in their kidneys (some AS\textsuperscript{−/−} mice are hydronephrotic) or to changes in reabsorption of sodium in the thick-ascending limb of Henle (TAL) and in the distal convoluted tubule (DCT), which is critical for urinary concentration. Reabsorption of Na\textsuperscript{+} in TAL and in DCT is mediated primarily by the NKCC2 cotransporter expressed in TAL, which requires K recycling through K channels (ROMK), and by the NCC transporter expressed in DCT. We, therefore, examined the effects of a decreased amount or absence of AS on the renal mRNA levels of these transporters. We found no differences in NKCC2 mRNA expression among genotypes. However, absence of aldosterone caused decreases in NCC expression in the whole kidney and in cortical ROMK in the AS\textsuperscript{−/−} mice on NS and LS, both of which could contribute to the defect in urinary concentration that we observe. In support of this, we note that Wald et al\textsuperscript{28} have already demonstrated that cortical ROMK expression is regulated by aldosterone and K\textsuperscript{+}. Thus, our data show that absence of aldosterone leads to urinary concentrating abnormalities mediated in part by decreases in the expression of NCC and ROMK.

We also investigated the expression of 3 major water channels, the AQPs, in AS\textsuperscript{−/−}, AS\textsuperscript{−/+}, and AS\textsuperscript{−/−} mice. The results showed an increase in AQP2 mRNA in the AS\textsuperscript{−/−} mice fed the NS diet relative to wild-type mice, despite the absence of any differences in their vasopressin levels. Previous studies have shown that several pathways in addition to the vasopressin pathway\textsuperscript{29} can affect the expression of AQP2. It is, therefore, likely that the increased level of AQP-2 mRNA in the AS\textsuperscript{−/−} mice is because of a compensatory mechanism other than via vasopressin. Mineralocorticoid deficiency in rats was also found to cause increased expression of the renal AQP-2 water channel,\textsuperscript{30} although in this case vasopressin was also increased.

Despite their failure to completely adjust to sodium restriction, the LS diet clearly caused the AS-null mice to increase the activity of their compensatory mechanisms, including more extensive RAS activation. Thus, the marked hypertrophy of the juxtaglomerular apparatus and expansion of renin-producing cells along afferent arterioles in the AS\textsuperscript{−/−} mice on an NS diet were further exaggerated under sodium restriction in parallel with the increased level of renin mRNA in the kidneys of these mice. The increase in renin production likely occurs by recruitment of additional renin-producing cells from vascular smooth muscle cells as suggested previously by Gomez et al.\textsuperscript{31} Recruitment of renin-producing cells was also observed in the adrenal cortex of the AS\textsuperscript{−/−} mice on the LS diet. Several studies have suggested a role for prostanooids produced by COX-2 in stimulating the renin system when salt is restricted.\textsuperscript{20,21} Our data are consistent with these studies and demonstrate a further increase of COX-2 expression in the kidney cortex of AS\textsuperscript{−/−} mice when they are fed the LS diet. On the LS diet, AS\textsuperscript{−/−} mice also developed thickening of walls of the intrarenal arteries. The same arterial phenotype has also been seen in mice lacking MR,\textsuperscript{32} angiotensinogen,\textsuperscript{33–35} angiotensin-converting enzyme,\textsuperscript{36,37} Ang II type 1a receptors,\textsuperscript{38} and Ang II type 1a and type 1b receptors\textsuperscript{39} on NS diets. Although an increase in Ang II concentration may be partly responsible for this vessel wall thickening, it cannot account for its occurrence in animals lacking angiotensinogen or angiotensin-converting enzyme. An additional possibility, suggested by Pentz et al\textsuperscript{40} to account for their observation that mice with ablation of renin cells do not have arteriolar hypertrophy, is that the cells that normally produce renin during development may also secrete factors that stimulate vessel thickening.

In our previous work, we have shown that the heterozygous AS\textsuperscript{−/+} mice are able to maintain normal BPs and plasma electrolyte concentrations on NS diets.\textsuperscript{16} However, our present work demonstrates that these mice are unable to maintain either normal BP or their AS mRNA level when fed an LS diet, despite an ∼2-fold increase in their expression of renin.

### Table 2. mRNA Levels (% Expression of Wild-Type on NS Diet) in Kidney AS\textsuperscript{−/−}, AS\textsuperscript{−/+}, and AS\textsuperscript{−/−} Mice on NS and LS Diets

<table>
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<th>Gene</th>
<th>Diet</th>
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<th>AS\textsuperscript{−/−}</th>
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<td></td>
<td>LS</td>
<td>136 ± 19 (8)</td>
<td>131 ± 18 (8)</td>
<td>152 ± 19 (8)</td>
</tr>
<tr>
<td>AQP3</td>
<td>NS</td>
<td>100 ± 7 (8)</td>
<td>106 ± 19 (8)</td>
<td>99 ± 7 (8)</td>
</tr>
<tr>
<td></td>
<td>LS</td>
<td>115 ± 8 (8)</td>
<td>89 ± 12 (8)</td>
<td>117 ± 19 (8)</td>
</tr>
<tr>
<td>ROMK</td>
<td>NS</td>
<td>100 ± 12 (7)</td>
<td>102 ± 10 (7)</td>
<td>77 ± 6 (9)</td>
</tr>
<tr>
<td>( cortex)</td>
<td>LS</td>
<td>220 ± 32 (6)††</td>
<td>182 ± 24 (6)††</td>
<td>136 ± 8 (6)‡‡</td>
</tr>
<tr>
<td>COX-2</td>
<td>NS</td>
<td>100 ± 15 (5)</td>
<td>107 ± 18 (7)</td>
<td>422 ± 56 (6)††</td>
</tr>
<tr>
<td>( cortex)</td>
<td>LS</td>
<td>86 ± 9 (5)</td>
<td>89 ± 12 (6)</td>
<td>723 ± 132 (6)§‡‡‡**</td>
</tr>
</tbody>
</table>

Values are mean ± SE and parentheses are number of animals.

\*P < 0.05 vs AS\textsuperscript{−/+} mice on same diet; \#P < 0.01 vs AS\textsuperscript{−/+} mice on same diet; \$P < 0.001 vs AS\textsuperscript{−/+} mice on same diet; |P < 0.05 vs AS\textsuperscript{−/+} mice on same diet; ¥P < 0.001 vs AS\textsuperscript{−/+} mice on same diet; **P < 0.05 vs AS\textsuperscript{−/+} mice on same diet; ***P < 0.001 vs AS\textsuperscript{−/+} mice on same diet; §P < 0.05 vs AS\textsuperscript{−/−} mice on same diet; ||P < 0.001 vs AS\textsuperscript{−/−} mice on same diet; **P < 0.05 vs same genotype on NS diet; |||P < 0.01 vs same genotype on NS diet; ††P < 0.001 vs same genotype on NS diet.
mRNA. Several investigators have published data suggesting that variations in the human AS gene (CYP11B2) contribute to dysregulation of aldosterone synthesis and can result in cardiovascular abnormalities.\(^6\)\(^6\)\(^4\)\(^2\)\(^1\)\(^0\) One of these polymorphisms, T-344C, is located in the promoter region and may affect the binding of transcriptional protein SF-1.\(^4\)Another polymorphism, A6547G, is located in the 3 untranslated region and may play a role in RNA stability.\(^10\) Gene conversion in intron 2 (Int2 W/C) is the result of mispairing between AS and the 11β hydroxylase genes; therefore, intron 2 may contain regulatory elements of the 11β hydroxylase gene.\(^4\)\(^4\) However, the association of CYP11B2 polymorphisms with BP remains unexplained. Thus, in some studies,\(^10\)\(^4\)\(^5\) there was no significant correlation of plasma aldosterone with the genotype. Our study demonstrates that a modest reduction in the level of AS mRNA affects BP during sodium restriction, although we also are unable to detect a significant reduction in plasma aldosterone concentration. Thus, our data suggest that genetic variations that reduce AS gene expression in humans could affect BP when sodium is limited.

Perspectives

Our study demonstrates that on an LS diet the abnormalities in homozygous AS\(^{-/-}\) mice become more severe, including low BP, decreased plasma Na\(^+\), increased plasma K\(^+\), strong RAS activation, and increased plasma glucocorticoid concentration, but these changes are still compatible with survival. The most striking finding of this study is that the aldosterone concentration, but these changes are still compatible with survival.

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Disclosures

None.

References


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