Aldosterone Synthase and Blood Pressure

Salt-Sensitive Blood Pressure in Mice With Increased Expression of Aldosterone Synthase

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Abstract—To study the effects of modestly increased expression of aldosterone synthase (AS), we generated mice (AS<sup>hi/hi</sup>) by replacing the 3’ untranslated region of AS mRNA with that from a stable mRNA. AS<sup>hi/hi</sup> mice on a normal-salt diet had 1.5 times the wild-type AS mRNA in adrenals, although their blood pressure and plasma aldosterone did not differ from wild-type mice. Changes in dietary salt did not affect the blood pressure of wild-type mice, but AS<sup>hi/hi</sup> mice had ~10 mm Hg higher blood pressure on a high-salt diet than on a low-salt diet and than wild-type mice on either diet. The AS<sup>hi/hi</sup> mice on a high-salt diet also had higher plasma aldosterone, lower plasma potassium, and greater renal expression of the α subunit of epithelial sodium channel compared with wild-type mice. The AS<sup>hi/hi</sup> mice on a high-salt diet also had more water intake and urine volume and less urine osmolality than wild-type mice. On a low-salt diet, AS<sup>hi/hi</sup> mice maintained normal blood pressure with less activation of the renin-angiotensin-aldosterone system than wild-type mice. The AS<sup>hi/hi</sup> mice also had less water intake and urine volume and higher urine osmolality than wild-type mice. On a medium high-salt diet, AS<sup>hi/hi</sup> mice were more susceptible than wild-type mice to infusion of angiotensin II, having a higher blood pressure, greater cardiac hypertrophy, and increased oxidative stress. Thus, a modest increase in AS expression makes blood pressure more sensitive to salt, suggesting that genetically increased AS expression in humans may contribute to hypertension and cardiovascular complications in societies with high-salt diets. (Hypertension. 2008;51:134-140.)

Key Words: aldosterone synthase ▪ blood pressure ▪ dietary salt ▪ renin-angiotensin-aldosterone system ▪ water metabolism

A n increasing body of data indicates that genetic susceptibility to essential hypertension is determined by small changes in many genes<sup>1</sup> rather than large changes in a few genes.<sup>2</sup> As a result, it is very difficult to establish a causative link between inherited differences in a candidate gene when studying human population, even when a strong correlation can be demonstrated. One approach to establish causation is to create differences in a candidate gene in mice and to ask whether blood pressure (BP) or other relevant parameters change. Here, we use this approach to determine the effect of modest increase in the expression of the gene cyp11b2, coding for aldosterone synthase (AS).

Recent studies show that ~10% of patients with essential hypertension have a high ratio of plasma aldosterone/plasma renin activity, suggesting some degree of inappropriately increased production of aldosterone.<sup>3,4</sup> Aldosterone plays an important role in controlling electrolyte homeostasis and BP, but it also has newly recognized effects in the remodeling of the heart, in abnormal vascular endothelial function, and in renal injury.<sup>5</sup> AS catalyzes the last step of aldosterone synthesis, and several investigators have published data suggesting that variations in the human AS gene (CYP11B2) may contribute to dysregulation of aldosterone synthesis.<sup>6,7</sup> However, the association of polymorphisms in the AS gene with differences in BP remains controversial. Thus, several investigators have reported that polymorphic variations in the AS gene (CYP11B2) are associated with differences in BP<sup>8–10</sup> although others have found no association.<sup>11–13</sup>

To determine the effects of increased expression of AS on BP, electrolyte homeostasis, and cardiovascular phenotype, we have generated mice (AS<sup>hi/hi</sup>) that have increased expression of the AS gene as a result of replacing the relatively unstable 3’ untranslated region (UTR) of its mRNA with the more stable 3’-UTR of bovine growth hormone (bGH). (This strategy stabilizes the corresponding mRNA<sup>14</sup> and increases the steady-state level of a gene product in all of the tissues where it is normally expressed but not in other tissues.) It is noteworthy that 1 of the polymorphisms in the human AS gene, A6547G, which has been shown to be associated with BP, is located in the 3’-UTR and may play a role in RNA stability.<sup>10</sup>

Our experiments show that a modest increase in AS expression does not affect BP in animals fed a normal-salt (NS) diet, but makes their BP sensitive to high-salt (HS) and to angiotensin II (Ang II) infusion on an increased salt diet. We also demonstrate that their Ang II/salt-dependent hypertension is accompanied by increased cardiac hypertrophy and oxidative stress. These data, together with our previous finding that a decreased level of AS decreases BP in mice on a low-salt (LS) diet,<sup>15</sup> indicate that genetic differences in AS levels in humans are likely to affect how BP responds to changes in dietary salt.
Methods

Gene Targeting

Mice with increased expression of the AS gene were generated by replacing the 3'-UTR of its relatively unstable mRNA with the 3'-UTR of the more stable mRNA of bGH, using the targeting strategy shown in Figure 1. Mouse strain 129–derived SvEv embryonic stem cells (TC-1) were cultured as described previously16 and electroporated with the targeting construct. Colonies surviving after selection with G418 and ganciclovir were screened with PCR and confirmed by Southern blotting analysis with a 700-bp probe external to the targeting construct. The modified ES cells were introduced into mouse blastocysts to generate chimeras. Male chimeras carrying the altered allele were mated with 129/SvEv females, and the inbred heterozygous progeny were used for breeding.

Mice

Wild-type (WT) mice (AS+/+) and homozygous (AS<sup>shi/hi</sup>) littermates on the inbred strain 129 SvEv genetic background were used to study effects of different diets on BP, electrolytes, and cardiac phenotype. The mice were fed NS chow (0.26% NaCl), an LS diet (0.01% NaCl, Harlan Teklad), a medium high-salt (MHS) diet (4% NaCl, Harlan Teklad), or an HS diet (8% NaCl, Harlan Teklad). Mice were maintained according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Experiments were conducted with male and female mice 3 to 5 months old and were approved by the institutional animal care and use committee of the University of North Carolina. Approximately 100 mice were used. BP measurements, blood analysis, echocardiographic assessment, histological analysis, real-time RT-PCR, and analysis of kidney function are described in the online supplement available at http://hyper.ahajournals.org.

Ang II Infusion and MHS Diet

These experiments were performed with 2- to 3-month-old male mice. After 4 days on the MHS diet, an osmotic minipump (Alzet, Durect) delivering Ang II (2 mg/kg per day) or PBS (control mice) was inserted subcutaneously for 14 days. BPs were measured in conscious mice with a computerized tail-cuff system (Visitech Systems) from day 7 until day 10. Urine collections were made in metabolic cages after 12 days of infusion of Ang II. Urinary 8-isoprostane, a marker of oxidative stress, was determined by enzyme immunoassay (Cayman Chemical). Echocardiography was then performed. The mice were euthanized on day 14, and their organs were weighed. Morphometric analysis and statistical analysis are described in online supplement.

Results

AS Expression in AS<sup>shi/hi</sup> and WT Mice on an NS Diet

To estimate AS gene expression in AS<sup>shi/hi</sup> and WT mice, we analyzed mRNA levels by quantitative RT-PCR. The AS mRNA in the adrenals of the AS<sup>shi/hi</sup> mice on the NS diet was ≈150% of WT (P<0.05; Table S1). The ratio of aldosterone/protein in the adrenals of mice on NS diet tended to be increased in AS<sup>shi/hi</sup> mice (P=0.07) compared with WT, although their plasma aldosterone did not differ significantly (Table S1). There
were also no significant changes in plasma corticosterone in AS<sup>hi/hi</sup> mice compared with WT mice (Table S1).

**General Characteristics of AS<sup>hi/hi</sup> and WT Mice on an NS Diet**
The increased expression of the AS gene in the AS<sup>hi/hi</sup> mice did not affect their survival, growth, or fertility. Histological examination of kidney, heart, and adrenal did not reveal detectable changes in structure of these organs. Although the BP of AS<sup>hi/hi</sup> mice on the NS diet was slightly increased, the difference did not reach significance ($P > 0.1$).

**General Characteristics of AS<sup>hi/hi</sup> and WT Mice on LS or HS Diets**
To determine whether the increased AS expression in AS<sup>hi/hi</sup> mice affects BP, electrolytes, and the activity of the renin-angiotensin-aldosterone system (RAAS) during various salt intakes, AS<sup>hi/hi</sup> and WT mice were placed on LS (0.01% NaCl) and HS (8% NaCl) diets for 3 weeks. The results showed that differences in the genotypes and the various salt intakes did not significantly affect body weight, heart/body weight ratio, kidney/body weight ratio, or hematocrit (data not shown).

**BPs**
The altered salt diets (LS, NS, and HS) did not significantly affect the BPs of WT mice (Figure 2A). In contrast, dietary salt affected the BP of the AS<sup>hi/hi</sup> mice. The BP of AS<sup>hi/hi</sup> mice on an LS diet was not significantly different from WT mice (107±3 mm Hg [n=9] versus 104±3 mm Hg [n=8]). However, on the HS diet, BP of AS<sup>hi/hi</sup> mice was significantly increased compared with WT mice on the HS diet (117±3 mm Hg [n=8] versus 107±3 mm Hg [n=9]; $P < 0.05$; Figure 2A).

**RAAS**
Kidney renin expression did not differ significantly between the genotypes on the NS diet (NS in Figure 2B). However, on an LS diet, renal renin mRNA level increased markedly (~2-fold) in the AS<sup>hi/hi</sup> mice, although the increase was much less than the 5-fold increase developed in WT mice ($P < 0.05$; LS in Figure 2B). The HS diet resulted in a significant reduction of renin expression in both genotypes, although these renin mRNA levels were not significantly affected by the AS genotypes (HS in Figure 2B).

Plasma renin activity and Ang II were markedly increased in both WT and AS<sup>hi/hi</sup> mice on the LS diet and were decreased by the HS diet, although the differences between the genotypes did not reach significance (Figure S1).

Adrenal AS mRNA levels on the NS diet were 50% higher in AS<sup>hi/hi</sup> mice than in WT ($P < 0.05$; NS in Figure 2C). The LS diet caused a marked increase in AS mRNA in AS<sup>hi/hi</sup> mice (7-fold; $P < 0.0001$), but again the increase was significantly less than the 25-fold increase in adrenal AS mRNA in the WT mice ($P < 0.01$; LS in Figure 2C). The HS diet caused a significant decrease ($P < 0.01$) in AS expression to approximately one quarter that on the NS diet in the adrenals in both genotypes, with expression of AS being greater in AS<sup>hi/hi</sup> mice compared with WT mice on the HS diet (NS and HS in Figure 2C), although the difference did not reach significance ($P = 0.09$; Figure 2C).

The marked stimulation of the RAAS system induced by the LS diet resulted in a comparable augmentation of aldosterone secretion in mice of both genotypes, but again this augmentation was less in AS<sup>hi/hi</sup> mice compared with WT mice ($P < 0.01$; LS in Figure 2D). Plasma aldosterone was reduced in both genotypes in response to the HS diet, but the reduction was proportionately less pronounced in the AS<sup>hi/hi</sup> mice ($P < 0.05$; HS in Figure 2D).
In sum, the AS_{hi} mice adjusted to decreased dietary salt with less activation of the RAAS system than WT mice but they less deactivated the RAAS when fed increased salt.

**Metabolic Studies**

To determine the effects of the AS genotype on water metabolism and food intake on various salt diets, mice were housed in metabolic cages. As shown in Figure 3A, AS_{hi} mice consumed the same amount of food as WT mice on all of the diets. On the NS diet, there were no significant differences in water metabolism between the genotypes (NS in Figure 3B through 3D). However, on the LS and HS diets, the AS genotype affected water metabolism. Thus, daily water intake was significantly decreased in the AS_{hi} mice on the LS diet compared with WT mice (P<0.05) but was increased in these mice on the HS diet compared with WT mice on the same diet (P<0.05; Figure 3B). Likewise, urine volume was lower in AS_{hi} mice on the LS diet than in WT mice (P<0.01) and was higher in these mice on the HS diet than in WT mice on the same diet (P<0.05; Figure 3C). The AS genotype had a reverse effect on urine osmolality. Thus, urine osmolality was increased in AS_{hi} mice on the LS diet compared with WT mice (P<0.01) but was decreased in these mice on the HS diet compared with WT mice (P<0.05; Figure 3D). In sum, the AS_{hi} mice were significantly better than WT at reducing water intake in adjusting to the LS diet, but they required more water intake to adjust to the HS diet.

**Electrolytes**

To determine the effect of various salt diets on electrolyte homeostasis, we measured plasma Na\(^+\) and K\(^+\) concentrations and urinary excretion of these electrolytes in WT and AS_{hi} mice. Dietary salt had no effect on plasma electrolytes of WT mice. However, AS_{hi} mice show differences in adjusting to the HS diet and became hypokalemic; thus, their plasma K\(^+\) concentration on the HS diet was significantly lower than in WT mice (5.0±0.1 mmol/L [n=6] versus 5.4±0.1 mmol/L [n=8] P<0.05; Table S2). There were no significant differences in the daily excretion of electrolytes on any diet between the 2 genotypes (Table S2).

**NO Excretion**

NO is an important factor in the regulation of sodium handling by the kidney. We, therefore, examined the urinary excretion of nitrate+nitrite (NO\(_x\)=NO\(_2\)-+NO\(_3\)), which are related to the renal production of NO, in AS_{hi} and WT mice. Our data show that the excretion of NO\(_x\) was significantly increased in mice of both genotypes on the HS diet compared with mice on the LS diet. However, there were no differences between the 2 genotypes (Table S2).

**Kidney Gene Expression**

To define the differences in the responses of AS_{hi} and WT mice in handling changes in salt intake, we measured the expression levels of several genes potentially important for the control of Na\(^+\) and water reabsorption by the kidney. These included genes affecting renal Na\(^+\) and K\(^+\) transport (sodium hydrogen exchanger-3, Na\(^+\)-Cl\(^-\) cotransporter, Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter, and \(\alpha\)-subunit of the amiloride-sensitive epithelial sodium channel [\(\alpha\)ENaC]), genes affecting water reabsorption (aquaporin-2 water channel), and genes affecting NO production (endothelial NO synthase; eNOS; Table S3). The expression the \(\alpha\)-subunit of ENaC was not significantly different between genotypes on NS and LS diets; however, on the HS diet, the level of \(\alpha\)ENaC was significantly higher in AS_{hi} mice than in WT mice (Table S3). Na\(^+\)-Cl\(^-\) cotransporter and sodium hydrogen exchanger-3 mRNA levels were not significantly different between genotypes on any of the diets. The levels of mRNA for the aquaporin-2 water channel and for the Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporter were not affected by the AS genotype on the LS, NS, or the HS diets but were significantly affected by diet (Table S3). The level of kidney eNOS mRNA was unaffected by dietary salt or genotype except that eNOS was significantly decreased in the
AS<sup>hihi</sup> mice on the LS diet compared with AS<sup>shsh</sup> mice on the NS diet and WT mice on the LS diet (Table S3).

**BP Response to Ang II Infusion in AS<sup>hihi</sup> and WT Mice Fed an MHS Diet**

Because of our finding that the AS<sup>hihi</sup> mice showed an increase in BP on the HS diet that was not seen in WT mice, we next asked whether the AS genotype influences the responses of the mice to Ang II when fed a diet with MHS (4% NaCl). The results presented in Figure 4A show that Ang II infusion significantly increased BP in both AS<sup>hihi</sup> and WT mice fed MHS (P<0.0001) compared with PBS-infused control mice. Strikingly, the BP response to Ang II infusion was 17 mm Hg greater in the AS<sup>hihi</sup> mice than in WT mice (an increase of 57 mm Hg versus 41 mm Hg; P<0.05).

**Cardiac Hypertrophy in AS<sup>hihi</sup> and WT Mice After Ang II Infusion on the MHS Diet**

We also determined the effects of differences in ANP expression on the remodeling of the heart that occurs after Ang II infusion. After 2 weeks of Ang II infusion into mice fed the MHS diet, the heart weight/body weight ratio was significantly increased in both the WT and the AS<sup>hihi</sup> mice (P<0.0001), with development of cardiac hypertrophy being greater in the AS<sup>hihi</sup> mice (P<0.01; Figure 4B). In agreement with these results, we found that the cross-sectional areas of cardiac myocytes after the Ang II infusion were enlarged significantly in mice of both genotypes, again with the increase in the myocyte cross-sectional area being greater in the AS<sup>hihi</sup> mice (P<0.001; Figure 4C; Figure S2A and S2B).

We measured the level of ventricular atrial natriuretic peptide mRNA, a marker of hypertrophy, by RT-PCR and found that the Ang II infusion caused an increase in atrial natriuretic peptide expression in both the AS<sup>shsh</sup> and the WT mice, but there were no significant differences between the genotypes (Figure 4D). Ang II infusion also affected the echocardiographic parameters in both AS<sup>shsh</sup> and WT mice, but again there were no significant differences between genotypes (data not shown).

**Cardiac Fibrosis After Ang II Infusion in AS<sup>hihi</sup> and WT Mice Fed an MHS Diet**

To further characterize the cardiac remodeling induced by infusion of Ang II, we examined the degree of cardiac fibrosis in AS<sup>shsh</sup> and WT mice. A histological study revealed that, after Ang II infusion, the AS<sup>shsh</sup> mice have increased fibrosis compared with the WT mice and that the fibrosis was particularly notable in perivascular tissue (Figure S2C and S2D). Quantitative analysis of the extent of the fibrosis using Image J (NIH) showed that the differences in total cardiac fibrosis between the genotypes were not significant after Ang II treatment (Figure S3A). The levels of collagen I and collagen III mRNA (determined by RT-PCR) were increased in both genotypes by the Ang II treatment (Figure S3B and S3C), with the expression of collagen III being significantly higher (P<0.05) in the AS<sup>shsh</sup> mice compared with the WT mice (Figure S3C).

**Oxidative Stress**

Several studies have shown the involvement of oxidative stress in cardiac injury induced by Ang II. We, therefore, examined the urinary excretion of 8-isoprostane, as a marker of systemic oxidative stress, in the AS<sup>shsh</sup> and WT mice on the MHS diet after Ang II infusion. Compared with control mice, the urine 8-isoprostane level was significantly increased by the Ang II infusion, and this increase was significantly greater in the AS<sup>shsh</sup> mice (P<0.05) than in WT mice (Figure S3D). Thus, the AS<sup>shsh</sup> mice are more susceptible than WT mice to Ang II/salt-dependent hypertension: they had a greater increase in BP, increased cardiac hypertrophy, and oxidative stress relative to WT mice.

**Discussion**

To determine the effects of a modest increase in ANP expression on BP, electrolyte homeostasis, and cardiovascular...
lar phenotype, we used gene targeting to generate inbred mice in which the 3’-UTR of the relatively unstable mRNA of AS is replaced with the 3’-UTR of a more stable mRNA (bGH). Our study demonstrates that this modest increase in expression of AS in mice results in their BP becoming sensitive to increased dietary salt and in changes in their metabolism of water and electrolytes. The increase in expression of AS also makes the mice more susceptible to the development of Ang II–dependent hypertension when on a diet with increased salt.

On an NS diet, the increase in AS expression in the AS\textsuperscript{hi/hi} mice had no significant effects on BP, plasma aldosterone, or plasma electrolytes, and echocardiography did not reveal significant changes in heart function in AS\textsuperscript{hi/hi} mice. However, on an HS diet, the BP of the AS\textsuperscript{hi/hi} mice was increased, accompanied by increased plasma aldosterone, increased expression of \(\alpha\)ENaC in the kidney, and decreased plasma K\(^+\) compared with WT mice. The increased BP of the AS\textsuperscript{hi/hi} mice on the HS diet is probably because of an increase in sodium reabsorption in the distal nephron consequent to the increased expression of \(\alpha\)ENaC resulting from elevated plasma aldosterone, which can strongly upregulate \(\alpha\)ENaC.\textsuperscript{19} This presumption is in agreement with the view expressed by several investigators\textsuperscript{2,20} that increases in the function of ENaC(s) play a central role in the development of hypertension.

It is also possible that the elevation in BP in the AS\textsuperscript{hi/hi} mice fed HS is because of an increased expression of AS in the cardiovascular system (vessels and heart) or central nervous system. Thus, Wang et al\textsuperscript{21} showed that intracerebroventricular infusion of aldosterone and an Na\(^+\)-rich artificial cerebrospinal fluid caused sympathetic hyperactivity and an increase in BP in normotensive rats. Gomez-Sanchez et al\textsuperscript{22} have demonstrated that the intracerebroventricular infusion of triolestan, an inhibitor of 3\(\beta\)-steroid dehydrogenase (an enzyme in the biochemical pathway leading to aldosterone) prevented and also reversed the development of hypertension in Dahl salt-sensitive rats, suggesting that increased synthesis of a mineralocorticoid agonist in the central nervous system, possibly aldosterone, contributes to salt sensitivity of BP in Dahl salt-sensitive rats.

Our study also shows that, although both the WT and AS\textsuperscript{hi/hi} mice increased their water intake and urine volume several fold when fed an HS diet, the increase in urine volume in the AS\textsuperscript{hi/hi} mice was significantly greater than in WT mice and was accompanied by a corresponding decrease in urine osmolality. These findings suggest that the AS\textsuperscript{hi/hi} mice require a greater-than-usual intake and excretion of water to handle the HS intake. This may be related to their hypokalemia, which is known to impair the ability of the kidney to concentrate urine.\textsuperscript{23} However, an impaired ability to concentrate urine has also been shown in normokalemic patients with primary aldosteronism and may be related to an expansion of extracellular fluid volume.\textsuperscript{24}

In contrast to the impaired ability of the AS\textsuperscript{hi/hi} to adapt to HS, they adapt to an LS diet with significantly less activation of the RAAS than WT mice. Thus, after 3 weeks on an LS diet, the WT mice had increased their renal renin mRNA level to \(\approx 5\) times normal and their adrenal AS mRNA level to \(\approx 25\) times normal, whereas the increase in the AS\textsuperscript{hi/hi} was significantly less (renin at \(\approx 2\) times normal and AS at \(\approx 7\) times normal). The AS\textsuperscript{hi/hi} mice also had significantly less increase in plasma aldosterone (\(\approx 4\) times in AS\textsuperscript{hi/hi} versus \(\approx 6\) times in WT). They also adapt to LS with higher urine osmolality and less water intake than WT mice. Interestingly, similar changes in water handling in Sabra hypertension prone rats, a salt-sensitive hypertension prone strain, occur even on an NS diet.\textsuperscript{25}

Recent data indicate that NO may play a role in salt-sensitive hypertension, because NO promotes vasodilatation, diuresis, and natriuresis.\textsuperscript{26} Our experiments show that the excretion of NO in mice of both genotypes is higher on the HS diet than on the LS diet, in agreement with results obtained in rats.\textsuperscript{27} We find that, in WT mice, renal eNOS expression was not significantly changed by various salt intakes, in agreement with the results of other investigators.\textsuperscript{28} However, in the AS\textsuperscript{hi/hi} mice, renal expression of eNOS was decreased by LS diet, probably contributing to their ability to retain sodium.

Many studies of patients with salt-sensitive hypertension\textsuperscript{29–32} report that BP is decreased and that the RAAS system responds less to a decrease in salt intake. The pathways leading to less activation of RAAS are not fully understood, although some evidence suggests a complex feedback interaction between RAAS and the sympathetic nervous system in hypertensive subjects when they are sodium restricted.\textsuperscript{32} Because our AS\textsuperscript{hi/hi} mice are hypertensive on HS and activate their RAAS less when on LS diet, they provide a potential animal model for unraveling the pathways involved in this puzzling phenomena. In summary, the AS\textsuperscript{hi/hi} mice exhibit salt-sensitive hypertension but adjust to an LS diet with less activation of RAAS than WT mice.

Salt-sensitive hypertension in patients has been associated with an increased frequency of cardiovascular events. We, therefore, tested whether the AS\textsuperscript{hi/hi} mice would be more susceptible to the development of Ang II–dependent cardiovascular complications when fed an MHS diet. We found that chronic infusion of Ang II in AS\textsuperscript{hi/hi} mice led to an exaggerated increase in BP, increased cardiac hypertrophy, and augmented systemic oxidative stress relative to WT mice treated in the same way. Our data are consistent with previous data reported by other investigators\textsuperscript{5} indicating a role of aldosterone in remodeling the heart in both a BP-dependent and BP-independent manner. The increase in perivascular fibrosis in AS\textsuperscript{hi/hi} mice is consistent with the demonstration by Rocha et al\textsuperscript{33} that aldosterone is involved in Ang II/salt-induced vascular remodeling. Although differences in total cardiac fibrosis between AS\textsuperscript{hi/hi} and WT mice did not reach significance, our study revealed a significant increase in collagen III expression in AS\textsuperscript{hi/hi} mice. Again, this is consistent with previous works by Rombouts et al\textsuperscript{34} showing that low doses of aldosterone tend to increase procollagen III synthesis. Urinary excretion of 8-isoprostane, a marker of systemic oxidative stress, was significantly increased in the Ang II salt-treated AS\textsuperscript{hi/hi} mice compared with WT mice, confirming the data of other investigators\textsuperscript{35} that oxidative stress is involved in cardiac injury. In summary, AS\textsuperscript{hi/hi} mice are more susceptible than WT to the development of Ang II salt-dependent hypertension and to cardiovascular complications.

**Perspectives**

Our study demonstrates that, in mice, generated by replacing the 3’-UTR of the relatively unstable AS mRNA with the 3’-UTR of a stable mRNA (bGH), a modest increase in AS expression (to
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Disclosures

None.

References

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