Hsd11b2 Haploinsufficiency in Mice Causes Salt Sensitivity of Blood Pressure

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Abstract—Salt sensitivity of blood pressure is an independent risk factor for cardiovascular morbidity. Mechanistically, abnormal mineralocorticoid action and subclinical renal impairment may blunt the natriuretic response to high sodium intake, causing blood pressure to rise. 11β-Hydroxysteroid dehydrogenase type 2 (11βHSD2) controls ligand access to the mineralocorticoid receptor, and ablation of the enzyme causes severe hypertension. Polymorphisms in *HSD11B2* are associated with salt sensitivity of blood pressure in normotensives. In this study, we used mice heterozygote for a null mutation in *Hsd11b2* (*Hsd11b2*$^{+/-}$) to define the mechanisms linking reduced enzyme activity to salt sensitivity of blood pressure. A high-sodium diet caused a rapid and sustained increase in blood pressure in *Hsd11b2*$^{+/-}$ mice but not in wild-type littermates. During the adaptation to high-sodium diet, heterozygotes displayed impaired sodium excretion, a transient positive sodium balance, and hypokalemia. After 21 days of high-sodium feeding, *Hsd11b2*$^{+/-}$ mice had an increased heart weight. Mineralocorticoid receptor antagonism partially prevented the increase in heart weight but not the increase in blood pressure. Glucocorticoid receptor antagonism prevented the rise in blood pressure. In *Hsd11b2*$^{+/-}$ mice, high-sodium feeding caused suppression of aldosterone and a moderate but sustained increase in corticosterone. This study demonstrates an inverse relationship among 11βHSD2 activity, heart weight, and blood pressure in a clinically important context. Reduced activity causes salt sensitivity of blood pressure, but this does not reflect illicit activation of mineralocorticoid receptors by glucocorticoids. Instead, we have identified a novel interaction among 11βHSD2, dietary salt, and circulating glucocorticoids. **(*Hypertension*. 2011;57:515-520.) • Online Data Supplement**

Key Words: genetics ■ hypertension ■ renal ■ kidney ■ sodium

Salt sensitivity of blood pressure is an independent risk factor for cardiovascular mortality in normotensive individuals[1] and an independent prognostic factor for essential hypertension.[2] The salt-induced increase in blood pressure reflects a complex interplay among renal, central, and vascular systems. The mechanisms causing salt sensitivity are not well defined, but subclinical renal impairment and abnormal modulation of the renin-angiotensin-aldosterone system (RAAS) by dietary salt may be contributory.[3] Even when aldosterone is low or normal, mineralocorticoid receptor (MR) blockade can be cardioprotective,[4] and pathophysiological activation of MR by alternative ligands has been found in rodent models of salt-sensitive hypertension.[5,6]

Cross-talk at the receptor level between the RAAS and the hypothalamic-pituitary-adrenal axis is prevented by 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2). This enzyme protects MR directly, by restricting the local availability of glucocorticoids,[7] and indirectly, by locking glucocorticoid-occupied MR in an inactive state.[8] Null mutations in the encoding gene, *HSD11B2,* cause apparent mineralocorticoid excess (Online Mendelian Inheritance in Man +218030), which presents in children with salt-sensitive hypertension, hypokalemia, and low plasma aldosterone.[9] A type 2 variant of the disease (Online Mendelian Inheritance in Man 207765) presents in adults[10,11] as essential hypertension with mild abnormalities in steroid metabolism.

HSD11B2 is an attractive candidate gene for salt sensitivity, and polymorphisms associated with either blood pressure, per se, or salt sensitivity of blood pressure have been found in several populations.[12–18] To define the role of the enzyme in the physiological regulation of blood pressure, we previously generated mice with a targeted deletion of *Hsd11b2*.[19,20] In the present study, heterozygote null mice (*Hsd11b2*$^{+/-}$), which have only 50% of normal enzyme levels, were found to have salt-sensitive blood pressure and electrolyte abnormalities consistent with mineralocorticoid excess. However, we found no evidence for nonmodulation of the RAAS and the increased blood pressure reflected activation of the glucocorticoid receptor (GR).

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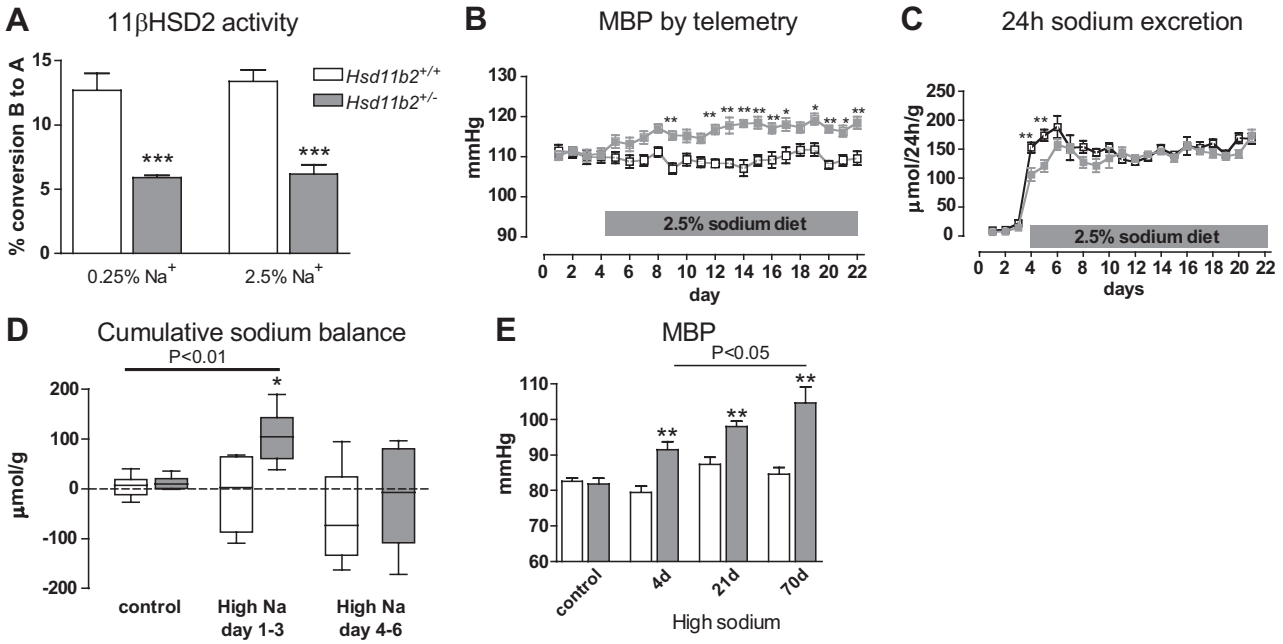

Figure 1. The response to high-sodium feeding in *Hsd11b2*$^{+/+}$ (open bars/symbols) and *Hsd11b2*$^{+/-}$ (gray bars/symbols) mice, numbers (*WT:HETS*) in parentheses. A, Renal 11βHSD2 activity in mice fed a control or high-sodium diet (n=6 in all); B, 24-hour MBP in conscious, unrestrained mice (n=4:6); C, 24-hour sodium excretion (n=7:10); D, 3-day cumulative sodium balances on control diet (basal) and during the adaptation to high-sodium diet (n=7:10); E, MBP in anesthetized mice after 4, 21, or 70 days on high-sodium diet (n=5:5 at each time point). Data are mean±SE or medians and ranges (D). *P<0.05, **P<0.01, ***P<0.001 vs wild type.

Methods

Experiments were performed on heterozygote (*Hsd11b2*$^{+/-}$) and wild-type (*Hsd11b2*$^{+/+}$) male mice (aged 100 to 200 days) under a license from the United Kingdom Home Office.

Studies in Conscious Mice

Blood pressure, measured by radiotelemetry, was recorded in mice initially maintained on standard chow (0.25% Na by weight) before high-sodium feeding (2.5% Na by weight) over a 19-day period. Sodium balance was measured using metabolism cages. After acclimatization, baseline measurements were made over a 3-day period, after which mice were fed high-sodium chow for an additional 18 days. Water and food intake, urine and fecal output, and mouse body weight were monitored daily. Mice were then decapitated and the kidneys taken for histological examination, measurement of 11βHSD2 activity, and gene expression.

Measurements in Anesthetized Mice

Mice, fed either a control or high-sodium diet for 4, 21, or 70 days, were anesthetized (Inactin, 100 mg/kg, IP) for measurement of mean arterial blood pressure (MBP) by direct cannulation. Evans Blue dye was injected intravenously for measurement of plasma volume and blood sampled for measurement of plasma potassium and osmolality. Urine was collected from the bladder for calculation of the urine sodium:potassium concentration ratio (U_{Na}:$_K$) and transtubular potassium gradient (TTKG).

Renal 11βHSD2 enzyme activity was assessed using thin layer chromatography to measure the conversion of [^{3}H]corticosterone to [^{3}H]dehydrocorticosterone.[21] Kidney homogenates from *Hsd11b2* null mice were used as negative controls and showed a conversion not significantly different from 0.

Inhibitor Studies

Mice received spironolactone, dexamethasone, or RU38486 before and during high-sodium feeding (please see the online Data Supplement at http://hyper.ahajournals.org).

Quantitative PCR

Hsd11b2 mRNA was quantified by a validated Taqman assay. Data were normalized to *wscr1* on a sample-to-sample basis. The expression of *wscr1* was not different between genotypes and was not affected by high-sodium diet.

Statistics

Data are mean±SE, except for cumulative sodium balance data, which are medians plus ranges. Comparisons were made using unpaired t test, ANOVA with Holm-Sidak post hoc test, or the Kruskal-Wallis test, as appropriate.

Results

Renal 11βHSD2 activity (Figure 1A) and *Hsd11b2* mRNA levels in *Hsd11b2*$^{+/-}$ mice were ≈50% those of *Hsd11b2*$^{+/+}$ mice and not influenced by dietary sodium. In conscious *Hsd11b2*$^{+/-}$ and wild-type mice fed a control sodium diet, MBP and urinary sodium excretion were similar (Figure 1B and 1C). The $U_{Na:K}$ ratio tended to be lower in *Hsd11b2*$^{+/-}$ mice than in wild types (*Hsd11b2*$^{+/-}$=0.39±0.07 versus Hsd11b2$^{+/+}$=0.80±0.21; P=0.08), but sodium balance was neutral (Figure 1D).

In *Hsd11b2*$^{+/+}$ mice, high-sodium feeding rapidly increased urinary sodium excretion without affecting either sodium balance or MBP. *Hsd11b2*$^{+/-}$ mice responded differently: the immediate natriuretic response was significantly blunted (Figure 1C), and the mice developed a positive sodium balance (Figure 1D). The $U_{Na:K}$ ratio increased immediately in both groups of mice in response to high-sodium feeding but remained relatively suppressed in heterozygotes (*Hsd11b2*$^{+/-}$=6.51±0.44 versus Hsd11b2$^{+/+}$=8.46±0.69; P<0.05), indicating residual mineralocorticoid activity. MBP began to increase on the second day

Table 1. Plasma Potassium (P_K) and Hematocrit in *Hsd11b2*$^{+/-}$ and *Hsd11b2*$^{+/+}$ Mice Maintained on Either a Control (0.25% Na) or High-Sodium (2.5% Na) Diet for 4, 21, or 70 Days

	P_K, mmol/L		Hematocrit	
Diet	*Hsd11b2*$^{+/-}$	*Hsd11b2*$^{+/+}$	*Hsd11b2*$^{+/-}$	*Hsd11b2*$^{+/+}$
Control	4.55±0.14	4.25±0.08	0.43±0.07	0.42±0.1
Control	(8)	(8)	(8)	(8)
High Na	3.74±0.10*	4.14±0.12	0.46±0.05†	0.41±0.08
4 d	(5)	(6)	(5)	(6)
High Na	3.72±0.11†	4.83±0.34	0.42±0.07	0.42±0.07
21 d	(7)	(9)	(7)	(9)
High Na	3.40±0.21†	4.45±0.29	0.43±0.07	0.42±0.03
70 d	(6)	(5)	(6)	(5)

Data are mean±SE with number of mice in parentheses. Statistical comparisons were made using *t* test.

*$P<0.05$.

†$P<0.01$.

of high-sodium feeding, reaching statistical significance at day 5, at which time neutral sodium balance had been restored.

Plasma volume, plasma potassium, and MBP were measured in separate cohorts of mice after 4, 21, or 70 days of high sodium intake. On the control diet, *Hsd11b2*$^{+/-}$ and *Hsd11b2*$^{+/+}$ mice had a similar plasma volume, plasma potassium, and hematocrit values (Table 1). The TTKG was significantly higher in heterozygote mice (*Hsd11b2*$^{+/-}$= 13.7±0.9 versus Hsd11b2$^{+/+}$=8.6±1.2; $P<0.01$). After 4 days of high-sodium feeding, *Hsd11b2*$^{+/-}$ mice became hypokalemic, and MBP was increased (Figure 1E). The TTKG was reduced but remained >7, indicating persistent potassium secretion in the collecting duct. The increased blood pressure in *Hsd11b2*$^{+/-}$ mice was not associated with volume expansion, plasma volume being lower (*Hsd11b2*$^{+/-}$= 1.48±0.07 mL versus *Hsd11b2*$^{+/+}$=2.13±0.02 mL; n=5 per group; $P<0.01$) and hematocrit higher (Table 1) than in *Hsd11b2*$^{+/+}$ mice.

After 21 days on high-sodium diet, *Hsd11b2*$^{+/-}$ mice remained hypokalemic, but hematocrit had normalized (Table 1). In heterozygote mice, high-sodium feeding significantly increased heart and kidney weights (Table 2), but significant albuminuria was not detected over the 21-day experiment. Consistent with this, the kidneys of salt-fed heterozygote mice seemed normal under histological examination. After 70 days of salt loading, the MBP differential between genotypes had increased to ≈20 mm Hg (Figure 1E), but hematocrit remained normal (Table 1).

Plasma aldosterone (Figure 2A) and 24-hour urinary aldosterone excretion (Figure 2B) were lower in *Hsd11b2*$^{+/-}$ mice on a control sodium diet, indicating tonic suppression of the RAAS. Adaptation to high-sodium feeding caused an appropriate reduction in aldosterone in both genotypes: aldosterone remained significantly lower in heterozygotes (Figure 2A and 2B). Plasma corticosterone was comparable between genotypes on a control sodium diet but was elevated in *Hsd11b2*$^{+/-}$ mice after high-sodium feeding (Figure 2C). Plasma samples were collected under terminal anesthesia, but we do not attribute the increased levels observed in heterozygotes to this, because 24-hour urinary corticosterone excretion (a surrogate for plasma corticosterone[22]) obtained in conscious, unrestrained mice was also elevated in *Hsd11b2*$^{+/-}$ mice by dietary sodium loading (Figure 2D). Deoxycorticosterone excretion was not different between genotypes and was not affected by dietary sodium (data not shown).

To identify mechanisms underlying salt sensitivity in *Hsd11b2*$^{+/-}$ mice, we first used dexamethasone to suppress the hypothalamic-pituitary-adrenal axis,[20] reducing 7:00 AM plasma corticosterone to ≈20 nmol/L in both groups. Dexamethasone abolished the sodium-induced differential between genotypes for both blood pressure (*Hsd11b2*$^{+/-}$=96.8±1.8 mm Hg versus *Hsd11b2*$^{+/+}$=94.5±0.9 mm Hg; *P* value not significant) and plasma potassium (*Hsd11b2*$^{+/-}$=5.70±0.12 mmol/L versus *Hsd11b2*$^{+/+}$=5.83±0.38 mmol/L; *P* value not significant). One interpretation of these data would be to attribute salt sensitivity in heterozygotes to spillover activation of MR by glucocorticoids. In fact, the normalization of blood pressure between genotypes was attributable to a significant ($P<0.05$) dexamethasone-induced pressor response in wild-type mice, which was not observed in heterozygotes. Spironolactone was, therefore, administered to assess the involvement of MR in the salt-sensitive phenotype. MR blockade did not prevent the salt-induced increase in blood pressure observed in *Hsd11b2*$^{+/-}$ mice, which remained ≈10-mm Hg higher than in *Hsd11b2*$^{+/+}$ mice (Figure 3A). Similarly, spironolactone did not prevent heterozygote mice becoming hypokalemic during high-sodium feeding (Figure 3B). During MR blockade, the $U_{Na:K}$ ratio also

Table 2. Body, Heart, and Kidney Wet Weight at Sacrifice in *Hsd11b2* Heterozygote Mice Maintained for 21 Days on Either a Control (0.25% Na) or High-Sodium (2.5% Na) Diet

Parameter	Control Diet	High Sodium	High Sodium+SPIRO	High Sodium+RU38486	ANOVA *P*
n	8	9	10	8	
Body weight, g	32.7±1.2	33.9±1.0	32.6±0.5	33.1±0.7	Not significant
Heart weight, mg	129.8±3.9	162.5±6.0†	141.5±5.9	144.3±2.7	<0.01
Kidney weight, g	325.8±12.7	393.6±21.8*	350.4±8.0	368.6±11.9	<0.05

Mice were sham operated or had slow-release pellets containing spironolactone (SPIRO) or RU38486 implanted subcutaneously. Data are mean±SE with number of mice in parentheses. Statistical comparisons were made using 1-way ANOVA, with *P* value shown in the final column.

*$P<0.01$ vs control in Bonferroni posttest.

†$P<0.001$ vs control in Bonferroni posttest.

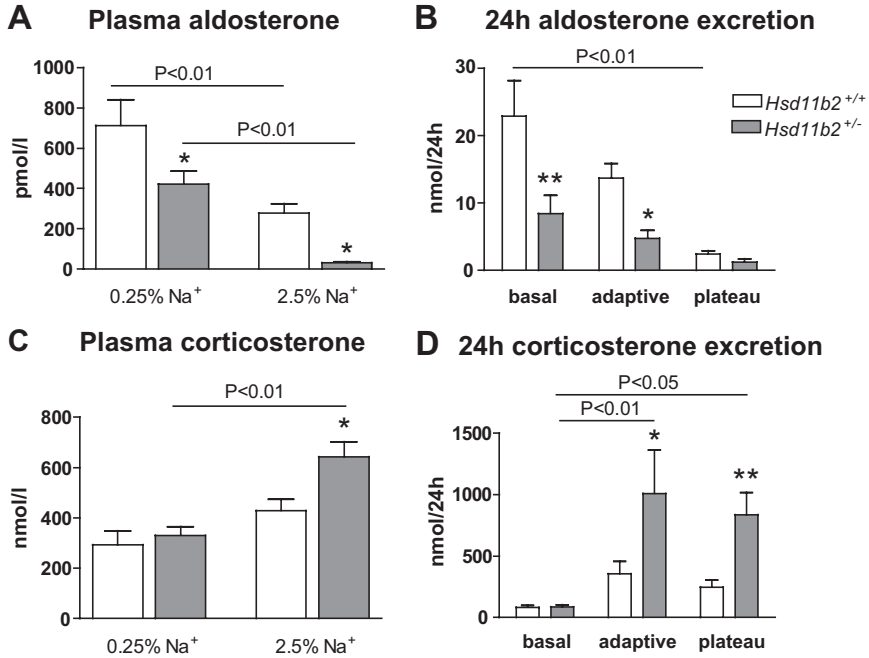

Figure 2. Steroid profiles in *Hsd11b2*$^{+/-}$ (▨) and *Hsd11b2*$^{+/+}$ mice (□), with numbers (*WT:HET*) given in parentheses. A, Plasma aldosterone measured in terminal arterial blood samples after 21 days on either control or high-sodium diet (n=8 for all); B, 24-h urinary aldosterone excretion in mice on control sodium diet and during the adaptive and plateau phases after high-sodium feeding. (n=10:7); C, Plasma corticosterone in terminal arterial blood samples, as before; D, Urinary corticosterone excretion, as before. Data are mean±SE; **P*<0.05, ***P*<0.01 vs wild type. Within-genotype comparisons are as stated.

remained lower (*Hsd11b2*$^{+/-}$=0.94±0.60 versus *Hsd11b2*$^{+/+}$=2.43±1.80; n=7/5; *P*=0.06). Despite the lack of effect on blood pressure, spironolactone partially prevented the salt-induced increase in heart weight observed in *Hsd11b2*$^{+/-}$ mice (*P*<0.05; Table 2).

The GR antagonist RU38486 prevented the sodium-induced increase in blood pressure (Figure 4A) and partially prevented the increased heart weight (Table 2) observed in the heterozygotes. RU38486 also normalized plasma potassium (Figure 4B) and the $U_{Na:K}$ ratio.

Discussion

Deficiency in 11βHSD2 promotes salt retention, potassium wasting, and hypertension, thought to reflect unregulated activation of renal MR by glucocorticoids.[9,23] Apparent mineralocorticoid excess arises in children who are homozygous[9] or compound heterozygous[24] for mutations that ablate 11βHSD2 activity. Apparent mineralocorticoid excess is rare, and the majority of those carrying a single mutated allele appear normal.[9] Detailed long-term follow-up of heterozygotes is lacking but evidence suggests abnormal steroid excretion and a propensity toward low-renin hypertension in later life.[9,25] A variant of apparent mineralocorticoid excess associated with reduced enzyme velocity causes hypertension in older individuals,[10,11] and an age-dependent decline in 11βHSD2 activity has also been reported.[26] Defects in 11βHSD2 may, therefore, be a risk factor for hypertension in the general population.

In the present study we identified a strong sensitivity of blood pressure to dietary sodium intake in mice heterozygote for a null mutation in *Hsd11b2*. On a control diet, heterozygote mice displayed subtle signs of mineralocorticoid excess but had no derangements in blood pressure or plasma electrolytes and were in neutral sodium balance. The transition to high-salt feeding uncovered in heterozygote mice a blunted renal natriuretic response: transient sodium retention preceded a rise in blood pressure by 24 to 48 hours. *Hsd11b2*$^{+/-}$ mice also developed hypokalemia. The suppressed $U_{Na:K}$ ratio and TTKG >7 suggested enhanced mineralocorticoid bioactivity in the distal nephron. The RAAS seemed to be appropriately modulated by dietary salt: overt aldosterone excess does not cause the sodium retention in *Hsd11b2*$^{+/-}$ mice.

In mice[20] and humans[9] lacking 11βHSD2, glucocorticoids have been shown to act as unregulated mineralocorticoids. In the current study, sodium loading did not affect 11βHSD2 activity, consistent with previous reports.[27] Additional diminution of the enzymatic barrier does not contribute to salt sensitivity in heterozygote mice, but spillover activation of MR after an increase in circulating corticosteroid was indicated. However, spironolactone (administered at a dose shown to be effective against high concentrations of glucocorticoid[5]) did not alleviate the symptoms of mineralocorticoid excess in salt-loaded heterozygote mice, and we, therefore, suggest that inappropriate activation of MR is not causal. Our study does, however, suggest a cardioprotective role for MR,[4] independent of blood pressure, because spironolactone partially rescued the salt-induced increase in heart:body weight ratio in *Hsd11b2*$^{+/-}$ mice.

At present we cannot define the mechanisms leading to increased corticosterone. However, salt-sensitive individuals display an attenuated glucocorticoid clearance,[28] and glucocorticoid regeneration by renal 11βHSD1 has been linked to salt sensitivity in rats.[29] In the present study, impaired peripheral metabolism alone cannot account for the rise in plasma corticosterone, because 11βHSD2 was not regulated by salt intake. It is possible that the hypothalamic-pituitary-adrenal axis is activated during the transition to high-sodium diet, as has been reported in salt-sensitive humans.[30]

Mechanistically, the alterations in $U_{Na:K}$ and TTKG provide compelling evidence that epithelial sodium channel activation in the aldosterone-sensitive distal nephron under-

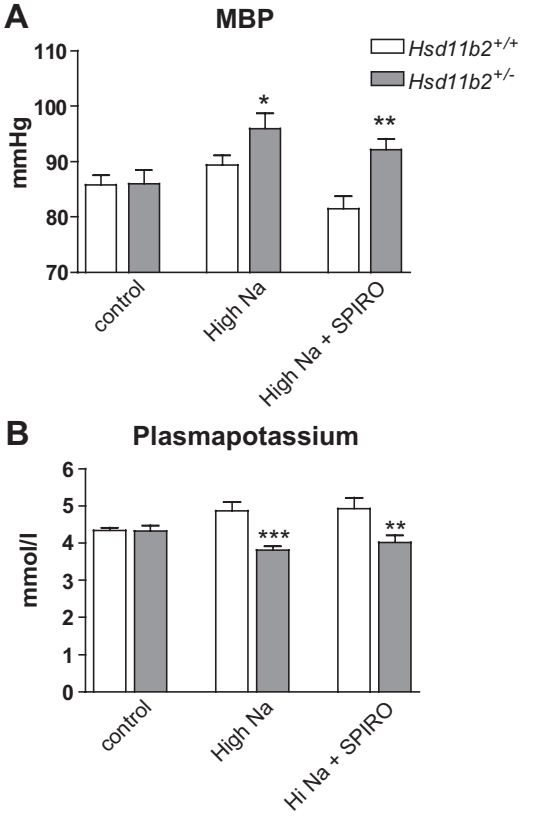

Figure 3. A, MBP and (B) plasma potassium in *Hsd11b2*$^{+/+}$ (□) and *Hsd11b2*$^{+/-}$ (▨) mice maintained for 21 days on a control (n=8:8) or high- (n=7:9) sodium diet. In separate groups, the MR antagonist spironolactone (SPIRO; n=8:7) was administered before and during high-sodium feeding. *P<0.05, **P<0.01, ***P<0.001 vs wild-type mice.

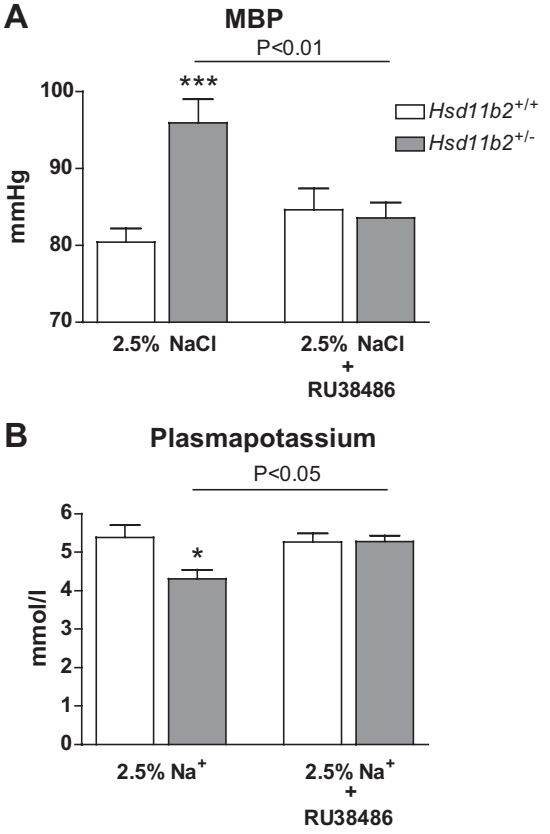

Figure 4. A, MBP and (B) plasma potassium in *Hsd11b2*$^{+/+}$ (□) and *Hsd11b2*$^{+/-}$ (▨) mice maintained for 21 days on high-sodium diet. Mice received the GR antagonist RU38486 (n=9:8) or vehicle (n=8:6) before and during high-sodium feeding. *P<0.05, ***P<0.001. Within-genotype comparisons as stated.

pins the sodium retention in *Hsd11b2*$^{+/-}$ mice. GR blockade prevented the development of the salt-induced phenotype, and this is consistent with regulation by GR of serum glucocorticoid regulated kinase 1 and the epithelial sodium channel.[5,31] Moreover, recent studies indicate that 11βHSD2 regulates the translocation of GR into the principal cell nucleus,[32] thereby governing transcriptional responses to glucocorticoids.

Surprisingly, sodium retention was associated with volume contraction rather than expansion. This may reflect a countervailing influence of GR on vascular permeability and compliance. Redistribution of fluid out of the vascular space is characteristic of glucocorticoid excess, and we have previously noted plasma volume contraction in other relevant models.[5,19] The absence of volume expansion in *Hsd11b2*$^{+/-}$ mice challenges the assumption that the salt-sensitive phenotype is an uncomplicated renal phenomenon. 11βHSD2 is expressed in other sites critical to blood pressure homeostasis and alternative explanations for the salt sensitivity should be considered. For example, moderate glucocorticoid excess inhibits endothelial NO synthase expression by the vascular endothelium,[33,34] an effect normally buffered by 11βHSD2.[34] Suppression of 11βHSD2 exacerbates the inhibition,[34] which could contribute to the GR-driven increase in blood pressure observed here. Similarly, central inhibition of 11βHSD2 exerts a strong pressor effect.[35] Hypertension in the *Hsd11b2*$^{-/-}$ mouse is maintained by catecholamine action,[19] and a contribution of the sympathetic nervous system to the salt sensitivity in heterozygotes cannot be excluded.

Perspectives

Genetic, acquired, or age-dependent reductions in 11βHSD2 may adversely affect blood pressure homeostasis. Our study demonstrates an inverse relationship between 11βHSD2 and blood pressure in a clinically important context: high-sodium intake[36] and salt-sensitivity of blood pressure[1,2] are important risk factors for cardiovascular death. Our data suggest that MR activation does not cause the salt sensitivity of blood pressure but contributes to the cardiac hypertrophy. We have identified a potential role for 11βHSD2 in governing GR access and speculate that this may involve activation of the hypothalamic-pituitary-adrenal axis.

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Disclosures

None.

References

1. Weinberger MH, Fineberg NS, Fineberg SE, Weinberger M. Salt sensitivity, pulse pressure, and death in normal and hypertensive humans. *Hypertension*. 2001;37:429–432.
2. Morimoto A, Uzu T, Fujii T, Nishimura M, Kuroda S, Nakamura S, Inenaga T, Kimura G. Sodium sensitivity and cardiovascular events in patients with essential hypertension. *Lancet*. 1997;350:1734–1737.
3. Jaimes EA, Zhou MS, Pearse DD, Puzis L, Raij L. Upregulation of cortical COX-2 in salt-sensitive hypertension: role of angiotensin II and reactive oxygen species. *Am J Physiol Renal Physiol*. 2008;294: F385–F392.
4. Chai W, Danser AH. Why are mineralocorticoid receptor antagonists cardioprotective? *Naunyn Schmiedebergs Arch Pharmacol*. 2006;374: 153–162.
5. Bailey MA, Mullins JJ, Kenyon CJ. Mineralocorticoid and glucocorticoid receptors stimulate epithelial sodium channel activity in a mouse model of Cushing syndrome. *Hypertension*. 2009;54:890–896.
6. Shibata S, Nagase M, Yoshida S, Kawarazaki W, Kurihara H, Tanaka H, Miyoshi J, Takai Y, Fujita T. Modification of mineralocorticoid receptor function by Rac1 GTPase: implication in proteinuric kidney disease. *Nat Med*. 2008;14:1370–1376.
7. Funder JW, Pearce PT, Smith R, Smith AI. Mineralocorticoid action: target tissue specificity is enzyme, not receptor, mediated. *Science*. 1988; 242:583–585.
8. Funder JW. Aldosterone and mineralocorticoid receptors in the cardiovascular system. *Prog Cardiovasc Dis*. 2010;52:393–400.
9. Stewart PM, Corrie JE, Shackleton CH, Edwards CR. Syndrome of apparent mineralocorticoid excess: a defect in the cortisol-cortisone shuttle. *J Clin Invest*. 1988;82:340–349.
10. Li A, Tedde R, Krozowski ZS, Pala A, Li KX, Shackleton CH, Mantero F, Palermo M, Stewart PM. Molecular basis for hypertension in the "type II variant" of apparent mineralocorticoid excess. *Am J Hum Genet*. 1998;63:370–379.
11. Ulick S, Tedde R, Mantero F. Pathogenesis of the type 2 variant of the syndrome of apparent mineralocorticoid excess. *J Clin Endocrinol Metab*. 1990;70:200–206.
12. Agarwal AK, Giacchetti G, Lavery G, Nikkila H, Palermo M, Ricketts M, McTernan C, Bianchi G, Manunta P, Strazzullo P, Mantero F, White PC, Stewart PM. CA-Repeat polymorphism in intron 1 of HSD11B2: effects on gene expression and salt sensitivity. *Hypertension*. 2000;36:187–194.
13. Alikhani-Koupaei R, Fouladkou F, Fustier P, Cenni B, Sharma AM, Deter HC, Frey BM, Frey FJ. Identification of polymorphisms in the human 11β-hydroxysteroid dehydrogenase type 2 gene promoter: functional characterization and relevance for salt sensitivity. *FASEB J*. 2007;21: 3618–3628.
14. Carvajal CA, Romero DG, Mosso LM, Gonzalez AA, Campino C, Montero J, Fardella CE. Biochemical and genetic characterization of 11β-hydroxysteroid dehydrogenase type 2 in low-renin essential hypertensives. *J Hypertens*. 2005;23:71–77.
15. Lovati E, Ferrari P, Dick B, Jostarndt K, Frey BM, Frey FJ, Schorr U, Sharma AM. Molecular basis of human salt sensitivity: the role of the 11β-hydroxysteroid dehydrogenase type 2. *J Clin Endocrinol Metab*. 1999;84:3745–3749.
16. Mariniello B, Ronconi V, Sardu C, Pagliericcio A, Galletti F, Strazzullo P, Palermo M, Boscaro M, Stewart PM, Mantero F, Giacchetti G. Analysis of the 11β-hydroxysteroid dehydrogenase type 2 gene (HSD11B2) in human essential hypertension. *Am J Hypertens*. 2005;18:1091–1098.
17. Poch E, Gonzalez D, Giner V, Bragulat E, Coca A, de La Sierra A. Molecular basis of salt sensitivity in human hypertension: evaluation of renin-angiotensin-aldosterone system gene polymorphisms. *Hypertension*. 2001;38: 1204–1209.
18. Watson B Jr, Bergman SM, Myracle A, Callen DF, Acton RT, Warnock DG. Genetic association of 11β-hydroxysteroid dehydrogenase type 2 (HSD11B2) flanking microsatellites with essential hypertension in blacks. *Hypertension*. 1996;28:478–482.
19. Bailey MA, Paterson JM, Hadoke PW, Wrobel N, Bellamy CO, Brownstein DG, Seckl JR, Mullins JJ. A switch in the mechanism of hypertension in the syndrome of apparent mineralocorticoid excess. *J Am Soc Nephrol*. 2008;19:47–58.
20. Kotelevtsev Y, Brown RW, Fleming S, Kenyon C, Edwards CR, Seckl JR, Mullins JJ. Hypertension in mice lacking 11β-hydroxysteroid dehydrogenase type 2. *J Clin Invest*. 1999;103:683–689.
21. Stewart PM, Murry BA, Mason JI. Human kidney 11β-hydroxysteroid dehydrogenase is a high affinity nicotinamide adenine dinucleotide-dependent enzyme and differs from the cloned type I isoform. *J Clin Endocrinol Metab*. 1994;79:480–484.
22. Al-Dujaili EA, Mullins LJ, Bailey MA, Andrew R, Kenyon CJ. Physiological and pathophysiological applications of sensitive ELISA methods for urinary deoxycorticosterone and corticosterone in rodents. *Steroids*. 2009;74:938–944.
23. Farese RV Jr, Biglieri EG, Shackleton CH, Irony I, Gomez-Fontes R. Licorice-induced hypermineralocorticoidism. *N Engl J Med*. 1991;325: 1223–1227.
24. Lavery GG, Ronconi V, Draper N, Rabbitt EH, Lyons V, Chapman KE, Walker EA, McTernan CL, Giacchetti G, Mantero F, Seckl JR, Edwards CR, Connell JM, Hewison M, Stewart PM. Late-onset apparent mineralocorticoid excess caused by novel compound heterozygous mutations in the HSD11B2 gene. *Hypertension*. 2003;42:123–129.
25. Li A, Li KX, Marui S, Krozowski ZS, Batista MC, Whorwood CB, Arnhold IJ, Shackleton CH, Mendonca BB, Stewart PM. Apparent mineralocorticoid excess in a Brazilian kindred: hypertension in the heterozygote state. *J Hypertens*. 1997;15:1397–1402.
26. Henschkowski J, Stuck AE, Frey BM, Gillmann G, Dick B, Frey FJ, Mohaupt MG. Age-dependent decrease in 11β-hydroxysteroid dehydrogenase type 2 activity in hypertensive patients. *Am J Hypertens*. 2008; 21:644–649.
27. Brem AS, Bina RB, King T, Chobanian MC, Morris DJ. Influence of dietary sodium on the renal isoforms of 11 β-hydroxysteroid dehydrogenase. *Proc Soc Exp Biol Med*. 1997;214:340–345.
28. Kerstens MN, van der Kleij FG, Boonstra AH, Sluiter WJ, Koerts J, Navis G, Dullaart RP. Salt loading affects cortisol metabolism in normotensive subjects: relationships with salt sensitivity. *J Clin Endocrinol Metab*. 2003;88:4180–4185.
29. Liu Y, Singh RJ, Usa K, Netzel BC, Liang M. Renal medullary 11β-hydroxysteroid dehydrogenase type 1 in Dahl salt-sensitive hypertension. *Physiol Genomics*. 2008;36:52–58.
30. Weber CS, Thayer JF, Rudat M, Sharma AM, Perschel FH, Buchholz K, Deter HC. Salt-sensitive men show reduced heart rate variability, lower norepinephrine and enhanced cortisol during mental stress. *J Hum Hypertens*. 2008;22:423–431.
31. Gaeggeler HP, Gonzalez-Rodriguez E, Jaeger NF, Loffing-Cueni D, Norregaard R, Loffing J, Horisberger JD, Rossier BC. Mineralocorticoid versus glucocorticoid receptor occupancy mediating aldosterone-stimulated sodium transport in a novel renal cell line. *J Am Soc Nephrol*. 2005;16:878–891.
32. Ackermann D, Gresko N, Carrel M, Loffing-Cueni D, Habermehl D, Gomez-Sanchez CE, Rossier BC, Loffing J. In vivo nuclear translocation of mineralocorticoid and glucocorticoid receptors in rat kidney: differential effect of corticosteroids along the distal tubule. *Am J Physiol Renal Physiol.* 2010;299:F1473–F1485.
33. Wallerath T, Witte K, Schafer SC, Schwarz PM, Prellwitz W, Wohlfart P, Kleinert H, Lehr HA, Lemmer B, Forstermann U. Down-regulation of the expression of endothelial NO synthase is likely to contribute to glucocorticoid-mediated hypertension. *Proc Natl Acad Sci U S A*. 1999; 96:13357–13362.
34. Liu Y, Mladinov D, Pietrusz JL, Usa K, Liang M. Glucocorticoid response elements and 11β-hydroxysteroid dehydrogenases in the regulation of endothelial nitric oxide synthase expression. *Cardiovasc Res*. 2009;81:140–147.
35. Gomez-Sanchez EP, Gomez-Sanchez CE. Central hypertensinogenic effects of glycyrrhizic acid and carbenoxolone. *Am J Physiol*. 1992;263: E1125–E1130.
36. Strazzullo P, D'Elia L, Kandala NB, Cappuccio FP. Salt intake, stroke, and cardiovascular disease: meta-analysis of prospective studies. *BMJ*. 2009;339:b4567.