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Hsd11b2 HAPLOINSUFFICIENCY IN MICE CAUSES SALT-SENSITIVITY OF BLOOD PRESSURE

Matthew A. Bailey, PhD[#1], **Eilidh Craigie, PhD**[#1], **Dawn E.W. Livingstone, PhD**[1], **Yuri V. Kotelevtsev, PhD**[1,3], **Emad A.S. Al-Dujaili, PhD**[2], **Christopher J. Kenyon, PhD**[1], and **John J. Mullins, PhD**[1]

[1]British Heart Foundation/University Centre for Cardiovascular Science, The University of Edinburgh, Edinburgh, Scotland, UK

[2]Dietetics, Nutrition and Biological Sciences Research, Queen Margaret University, Edinburgh, Scotland, UK

[3]Stem Cell Genome Modification Laboratory, Puschino State University, Russia

[#] These authors contributed equally to this work.

Abstract

Salt-sensitivity of blood pressure is an independent risk factor for cardiovascular morbidity. Mechanistically, abnormal mineralocorticoid action and sub-clinical renal impairment may blunt the natriuretic response to high sodium intake, causing blood pressure to rise. 11β-hydroxysteroid dehydrogenase type 2 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 in aldosterone and a moderate but sustained increase in corticosterone.

This study demonstrates an inverse relationship between 11β-hydroxysteroid dehydrogenase type 2 activity, heart weight and blood pressure in a clinically important context. Reduced activity

Correspondence: Matthew Bailey, PhD 47, Little France Crescent The University of Edinburgh EH16 4TJ Telephone/Fax: +441312426720/+441312426782 matthew.bailey@ed.ac.uk.

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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 between 11β-hydroxysteroid dehydrogenase type 2, dietary salt and circulating glucocorticoids.

Keywords

genetics; hypertension; renal; kidney; sodium

INTRODUCTION

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 between renal, central and vascular systems. The mechanisms causing salt-sensitivity are not well defined but subclinical renal impairment and abnormal modulation of the reninangiotensin-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 hypothalamic-pituitary-adrenal (HPA) 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 (AME; OMIM +218030), which presents in children with salt-sensitive hypertension, hypokalaemia and low plasma aldosterone[9]. A type 2 variant of the disease (OMIM 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 physiologic 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 non-modulation of the RAAS and the increased blood pressure reflected activation of the glucocorticoid receptor (GR).

METHODS

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

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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 a further 18 days. Water and food intake, urine and fecal output and mouse body weight was monitored daily. Mice were then decapitated and the kidneys taken for histological examination, measurement of 11βHSD2 activity and gene expression.

Measurements in anaesthetized mice

mice, fed either a control or high sodium diet for 4, 21 or 70 days, were anaesthetized (Inactin, 100mg/kg; IP) for measurement of mean arterial blood pressure (MBP) by direct cannulation. Evans Blue dye was injected IV 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 to potassium concentration ratio (U_{Na}:$_K$) and transtubular potassium gradient (TTKG).

Renal 11βHSD2 enzyme activity was assessed using thin layer chromatography (TLC) 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 zero.

Inhibitor studies

mice received spironolactone, dexamethasone or RU38486 before and during high sodium feeding (please see 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 either 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% that 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 & C). The $U_{Na:K}$ ratio tended to be lower in *Hsd11b2*$^{+/-}$ mice than in wild types (*Hsd11b2*$^{+/-}$=0.39±0.07 *vs 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 *vs Hsd11b2*$^{+/+}$=8.46±0.69; P<0.05), indicating residual mineralocorticoid activity. MBP began to increase on the second day 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 *vs Hsd11b2*$^{+/+}$=8.6±1.2; P<0.01). After four 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±0.07ml vs *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 weight (Table 2) but significant albuminuria was not detected over the 21-day experiment. Consistent with this, the kidneys of salt-fed heterozygote mice appeared normal under histological examination. After 70 days of salt loading, the MBP differential between genotypes had increased to ~20mmHg (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 B). Plasma corticosterone was comparable between genotypes on a control sodium diet but was elevated in *Hsd11b2*$^{+/-}$ mice following 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, since 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 genotype 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 HPA axis[20], reducing 7am plasma corticosterone to ~20nmol/l in both groups. Dexamethasone abolished the sodium-induced differential between genotypes for both blood pressure (*Hsd11b2*$^{+/-}$=96.8±1.8mmHg vs

Hsd11b2$^{+/+}$=94.5±0.9mmHg; NS) and plasma potassium (*Hsd11b2*$^{+/-}$=5.70±0.12mM vs *Hsd11b2*$^{+/+}$=5.83±0.38mM; NS). One interpretation of these data would be to attribute salt-sensitivity in heterozygotes to spill-over 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 ~10mmHg higher than in *Hsd11b2*$^{+/+}$ mice (Figure 3A). Similarly, spironolactone did not prevent heterozygote mice becoming hypokalaemic during high sodium feeding (Figure 3B). During MR blockade, the $U_{Na:K}$ ratio remained lower (*Hsd11b2*$^{+/-}$ = 0.94±0.60 vs *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 in 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]. AME arises in children who are homozygous[9] or compound heterozygous[24] for mutations that ablate 11βHSD2 activity. AME 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 AME associated with reduced enzyme velocity causes hypertension in older individuals[10,11] and an age-dependent decline in 11βHSD2 activity has 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 24-48 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 appeared 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]. Further diminution of the enzymatic barrier does not contribute to salt-sensitivity in heterozygote mice but spillover activation of MR

following 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 since spironolactone partially rescued the salt-induced increase in heart-to-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 since 11βHSD2 was not regulated by salt intake. It is possible that the HPA 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 ENaC activation in the ASDN underpins 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 ENaC[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 eNOS 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*$^{-/-}$ is maintained by catecholamine action[19] and a contribution of the sympathetic nervous system to the salt-sensitivity in heterozygotes cannot be excluded.

Perspective

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 HPA axis.

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Supplementary Material

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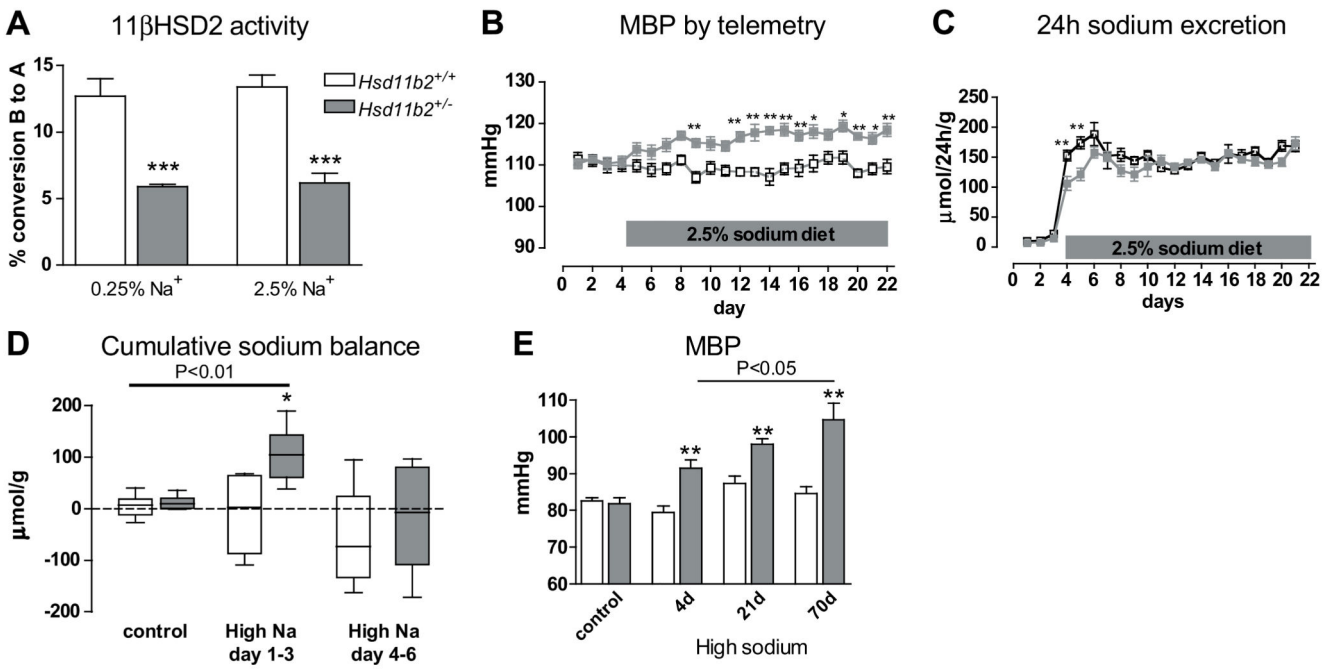

Figure 1.

The response to high sodium feeding in *Hsd11b2*$^{+/+}$ (open bars/symbols) and *Hsd11b2*$^{+/-}$ (grey bars/symbols) mice, numbers (*WT:HETS)* in parentheses. A) renal 11β-hydroxysteroid dehydrogenase type 2 activity in mice fed a control or high sodium diet (n=6 in all); (B) 24h mean arterial blood pressure in conscious, unrestrained mice (n=4:6) with the transition from control to high sodium indicated by an arrow; C) 24h 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) and E) mean arterial blood pressure in anesthetized mice after 4d, 21d or 70d on high sodium diet (n=5:5 at each time point). Data are means ± SE or medians and ranges (panel D). *P<0.05, **P<0.01, ***P<0.001 compared to wild-type.

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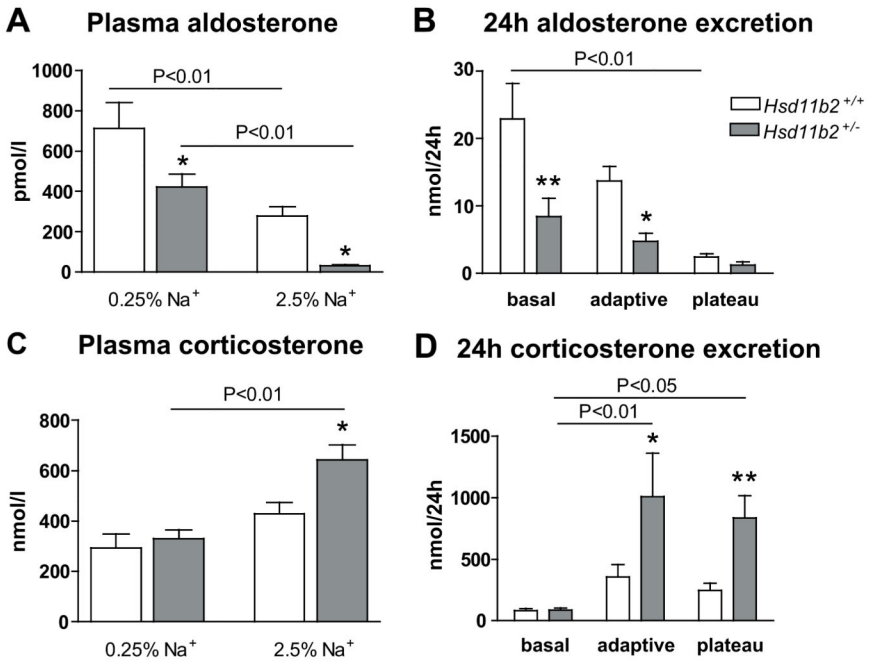

Figure 2.

Steroid profiles in *Hsd11b2*$^{+/-}$ (grey bars) and *Hsd11b2*$^{+/+}$ mice (open bars), 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) 24h urinary aldosterone excretion in mice on control sodium diet and during the adaptive and plateau phases following high sodium feeding. (n=10:7); C) Plasma corticosterone in terminal arterial blood samples, as before; D) urinary corticosterone excretion, as before. Data are means ± SE. *P<0.05, **P<0.01 compared to wild-type. Within genotype comparisons are as stated.

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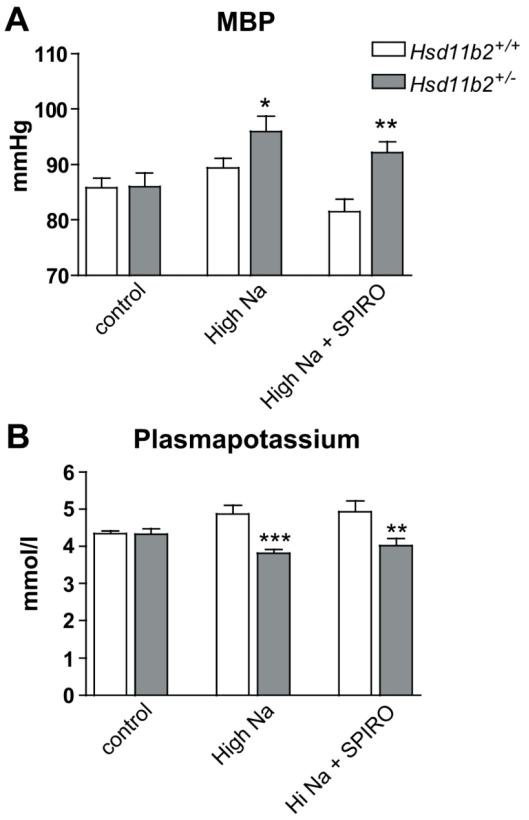

Figure 3.

A) mean arterial blood pressure and B) plasma potassium in *Hsd11b2*$^{+/+}$ (open bars) and *Hsd11b2*$^{+/-}$ (grey bars) mice maintained for 21 days on a control (n=8:8) or high sodium (n=7:9) sodium diet. In separate groups, the mineralocorticoid receptor antagonist spironolactone (SPIRO, n=8:7) was administered before and during high sodium feeding. *P<0.05, **P<0.01. ***P<0.001, compared to wild-type mice.

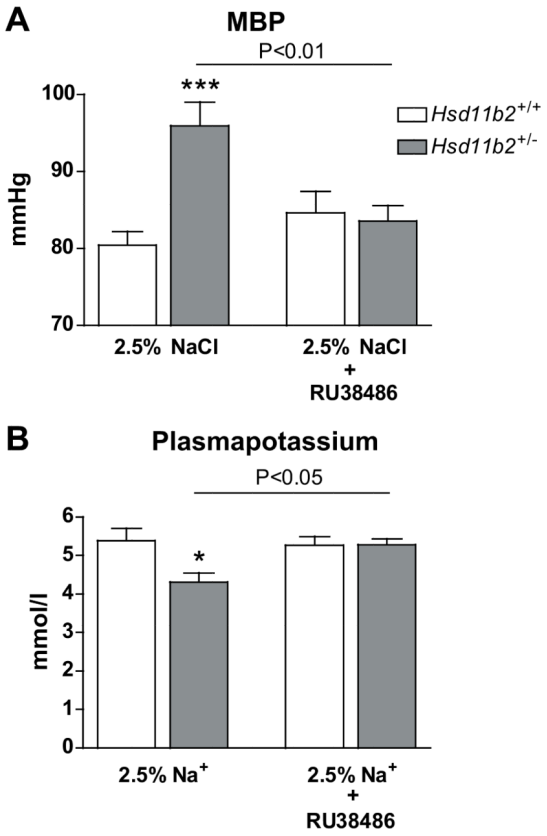

Figure 4.

A) mean arterial blood pressure and B) plasma potassium in *Hsd11b2*$^{+/+}$ (open bars) and *Hsd11b2*$^{+/-}$ (grey bars) mice maintained for 21 days on high sodium diet. Mice received the glucocorticoid receptor 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.

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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) for either 4, 21 or 70 days. Data are mean ± SE with number of mice in parentheses.

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

Statistical comparisons were made using t-test

* P<0.05,

† P<0.01.

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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. 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 one-way ANOVA, with P value shown in the final column.

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	NS
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

Bonferroni post-tests

*P<0.01,

†P<0.001 versus control.