Published in final edited form as:
Hypertension. 2012 September ; 60(3): 684–690. doi:10.1161/HYPERTENSIONAHA.112.196410.

Failure to down-regulate the epithelial sodium channel causes salt-sensitivity in *Hsd11b2* heterozygote mice

Eilidh Craigie, **Louise C Evans**, **John J Mullins**, and **Matthew A Bailey**
British Heart Foundation Centre for Cardiovascular Science, The University of Edinburgh, UK

Abstract

In vivo, the enzyme 11β-hydroxysteroid dehydrogenase type 2 influences ligand access to the mineralocorticoid receptor. Ablation of the encoding gene, HSD11B2, causes the hypertensive syndrome of Apparent Mineralocorticoid Excess. Studies in humans and experimental animals have linked reduced 11β-hydroxysteroid dehydrogenase type 2 activity and salt-sensitivity of blood pressure. In the present study, renal mechanisms underpinning salt-sensitivity were investigated in *Hsd11b2*$^{+/-}$ mice fed low, standard and high sodium diets.

In wild-type mice, there was a strong correlation between dietary sodium content and fractional sodium excretion, but not blood pressure. High sodium feeding abolished amiloride-sensitive sodium reabsorption, consistent with down-regulation of the epithelial sodium channel. In *Hsd11b2*$^{+/-}$ mice, the natriuretic response to increased dietary sodium content was blunted and epithelial sodium channel activity persisted. High sodium diet also reduced renal blood flow and increased blood pressure in *Hsd11b2*$^{+/-}$ mice. Aldosterone was modulated by dietary sodium in both genotypes and salt-sensitivity in *Hsd11b2*$^{+/-}$ mice was associated with increased plasma corticosterone levels. Chronic administration of an epithelial sodium channel blocker or a glucocorticoid receptor antagonist prevented salt-sensitivity in *Hsd11b2*$^{+/-}$ mice, whereas mineralocorticoid receptor blockade with spironolactone did not.

This study shows that reduced 11β-hydroxysteroid dehydrogenase type 2 causes salt-sensitivity of blood pressure due to impaired renal natriuretic capacity. This reflects deregulation of epithelial sodium channels and increased renal vascular resistance. The phenotype is not caused by illicit activation of mineralocorticoid receptors by glucocorticoids, but by direct activation of glucocorticoid receptors.

Keywords

glucocorticoid receptor; RU486; spironolactone; renin-angiotensin system

Introduction

Hypertension remains a significant public health burden worldwide, being a major risk factor for cardiovascular mortality and chronic kidney disease[1]. Although specific causes of

Correspondence: Matthew Bailey, PhD, Centre for Cardiovascular Science, The University of Edinburgh, The Queen's Medical Research Centre, 47, Little France Crescent, Edinburgh, EH16 4TJ, Telephone/Fax: +441312426720/+441312426782, matthew.bailey@ed.ac.uk.

Conflict of interest/Disclosures: NONE

Europe PMC Funders Author Manuscripts

hypertension are often difficult to resolve, salt-sensitivity of blood pressure (BP) is a contributory mechanism in a number of patient subgroups[2]. Salt-sensitivity is also an independent risk factor for adverse cardiovascular events in normotensive individuals[3] and is a negative prognostic indicator for clinical progression towards hypertension, microalbuminuria, and endothelial dysfunction[4]. The underlying mechanisms of salt-sensitivity are not well defined but subclinical renal impairment reducing the natriuretic efficiency of the kidney may be contributory. Abnormal modulation of the renin-angiotensin-aldosterone system (RAAS) by dietary salt has been linked to salt-sensitivity and cardio-renal damage in both patients[5] and in experimental models[6]. Mineralocorticoid receptor (MR) blockade is cardio-protective, even when aldosterone levels are low or normal[7], and pathophysiological activation of MR by alternative ligands has been found in rodent models of salt-sensitive hypertension[8,9].

Cross-talk at the receptor level between the RAAS and hypothalamic-pituitary-adrenal (HPA) axis is normally prevented by 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2). This enzyme protects MR directly by restricting the local availability of active glucocorticoids[10], and may also confer indirect protection by locking glucocorticoid-occupied MR in a transcriptionally inactive state[11]. Null mutations in the encoding gene (*HSD11B2)* cause the syndrome of Apparent Mineralocorticoid Excess (AME; OMIM +218030); an autosomal recessive disorder presenting with hypertension, severe hypokalaemia and low aldosterone. *Hsd11b2* null (*Hsd11b2*$^{-/-}$) mice have a similar phenotype to AME patients: unregulated activation of MR by glucocorticoids appears to be causative[12] and hypertension is associated with transient activation of the epithelial sodium channel (ENaC) in the aldosterone sensitive distal nephron (ASDN)[13].

A type 2 variant of AME (OMIM 207765) presents in adults as essential hypertension with mild abnormalities in steroid metabolism[14,15]. With a strong correlation between the severity of the AME phenotype and the underlying *HSD11B2* mutation[14], *HSD11B2* is an attractive candidate gene for salt-sensitivity. Indeed, *HSD11B2* polymorphisms associated with either raised BP *per se* or salt-sensitivity of BP have been found in several populations[16-19], although not all studies report a positive correlation[20,21].

We recently demonstrated a causal link between 11βHSD2 activity and salt-sensitivity in *Hsd11b2* heterozygote null (*Hsd11b2*$^{+/-}$) mice[22]. These mice were found to have salt-sensitive BP and electrolyte abnormalities consistent with mineralocorticoid excess. AME is classically considered a renal disease and we have therefore analyzed renal sodium handling in *Hsd11b2*$^{+/-}$ mice. We find that sodium excretion is abnormally modulated by dietary salt in these mice, dependent upon a dysregulation of ENaC. Our studies also point towards a major role for the glucocorticoid receptor (GR), and not the MR, in the salt-sensitive phenotype.

Methods

Experiments on age-matched cohorts of *Hsd11b2*$^{+/+}$ and *Hsd11b2*$^{+/-}$ mice were performed under a UK Home Office Licence, following ethical review by the University.

Renal Clearance

Renal function and BP was measured in anesthetized *Hsd11b2*$^{+/-}$ and *Hsd11b2*$^{+/+}$ mice, maintained on either a low (LS; 0.03%; n=7/8), standard (SS; 0.25%; n=8/8) or high sodium (HS; 2.5%; n=11/9) diet. After baseline measurements, amiloride (2 mg/kg; IV) was injected to measure ENaC activity. (online Data Supplement, http://hyper.ahajournals.org).

Chronic Inhibitor Administration

Renal function was measured mice maintained on a HS diet, receiving one of three co-treatments: spironolactone (n=6/8), RU486 (n= 9/10) or benzamil (n=7/6).

Sodium Balance in Conscious Mice

Mice were housed continuously in metabolic cages. Cumulative sodium balance was measured over cycles of 3 days. Mice were first fed SS diet, after which benzamil or vehicle was administered. After another balance period, HS diet was given for 3 days, this being the period of sodium retention[22].

Quantitative PCR

mRNA abundance was measured using a Universal Probe Library kit (Roche, UK) and primers designed for the following targets: *nr3c1, nr3c2, scnn1a, scnn1b* and *sgk1*. (See Table S1)

Statistics

All data are presented as means ± standard error. Statistical comparisons were made using Prism 5 (GraphPad Software, USA).

Results

Salt-sensitive BP and renal hemodynamics in *Hsd11b2*$^{+/-}$ mice

BP was measured in groups of *Hsd11b2*$^{+/+}$ and *Hsd11b2*$^{+/-}$ mice maintained on LS, SS or HS diet. On LS and HS diet, BP was comparable between genotypes. HS diet caused a significant increase in BP in *Hsd11b2*$^{+/-}$ mice (Figure 1A). Overall, there was a significant correlation between MABP and dietary sodium content in *Hsd11b2*$^{+/-}$ mice (Pearson r= 0.67; P<0.001); no such relationship was observed in *Hsd11b2*$^{+/+}$ mice.

Glomerular filtration rate (GFR) was higher in *Hsd11b2*$^{+/-}$ mice than controls but there was no relationship between this and dietary salt (Figure 1B). Renal blood flow (RBF) was higher in *Hsd11b2*$^{+/-}$ mice than *Hsd11b2*$^{+/+}$on LS and SS diets (Figure 1C). Dietary salt loading decreased RBF in *Hsd11b2*$^{+/-}$ mice: filtration fraction (FF) and renal vascular resistance (RVR) were both significantly elevated (Table 1).

Hsd11b2$^{+/-}$ mice have impaired fractional sodium excretion

Sodium excretion was lower in *Hsd11b2*$^{+/-}$ mice than wild-types on both SS and HS diets (Table 1) and fractional sodium excretion (FE_{Na}) was calculated to assess tubular function. In *Hsd11b2*$^{+/+}$ mice, there was an appropriate increase in FE_{Na} with increasing dietary sodium (Figure 2A. Pearson r= 0.67; P<0.01). This relationship was blunted in *Hsd11b2*$^{+/-}$ mice and on SS or HS diet, FE_{Na} was significantly lower than in *Hsd11b2*$^{+/+}$ mice (P<0.01). Although *Hsd11b2*$^{+/-}$ mice were not able to adapt their renal sodium excretion to their dietary sodium load as effectively as *Hsd11b2*$^{+/+}$ mice, plasma sodium concentration was not affected (Table 1).

Potassium excretion was elevated in *Hsd11b2*$^{+/-}$ mice, but only on a SS diet (Table 1) and fractional potassium excretion (FE_K) was comparable across all dietary regimens (Figure 2B).

Amiloride-sensitive sodium reabsorption in *Hsd11b2*$^{+/-}$ mice

The natriuretic effect of amiloride ($\Delta amiloride_{Na}$) was used to quantify ENaC-mediated sodium reabsorption. In *Hsd11b2*$^{+/+}$ mice (Figure 2C), there was an inverse relationship

between dietary sodium content and $\Delta amiloride_{Na}$ (Pearson r=−0.74, P<0.001), consistent with down-regulation of functional ENaC following increased sodium intake.

In *Hsd11b2*$^{+/-}$ mice, the inverse relationship was blunted (Pearson r=−0.42; P<0.05). Critically, amiloride evoked a significant natriuresis on HS diet, suggesting that *Hsd11b2*$^{+/-}$ mice fail to regulate their ENaC activity appropriately in relation to sodium intake. This does not reflect altered gene transcription: mRNA abundance for ENaC subunits was not different between genotypes. Sgk1 expression was increased, which would promote ENaC retention in the apical membrane (see Figure S1) and maintain an electrophysiological driving force for potassium secretion via ROMK. Indeed, despite hypokalemia, the potassium sparing effect of amiloride was sustained (Figure 2D) in *Hsd11b2*$^{+/-}$ mice on HS diet, whereas wild-type mice maintain potassium homeostasis through processes independent of ENaC activity.

The effect of the specific ENaC antagonist, benzamil, on sodium balance was assessed in conscious mice. In untreated *Hsd11b2*$^{+/-}$ mice, the transition to HS diet caused a positive sodium balance (*Hsd11b2*$^{+/-}$ =104±15 versus *Hsd11b2*$^{+/+}$= −6.9±30.5 μmoles, P<0.05). In benzamil-treated mice, sodium balance remained neutral during this transition (*Hsd11b2*$^{+/-}$=28.87±68 versus *Hsd11b2*$^{+/+}$= 17.46±2.9 μmoles, NS).

Role of GR and MR

In the current study, increased sodium intake reduced aldosterone in both genotypes (Table 1). Nevertheless, *Hsd11b2*$^{+/-}$ mice had significantly lower levels than *Hsd11b2*$^{+/+}$ mice suggesting tonic suppression of the RAAS. It is therefore unlikely that aldosterone excess is responsible for the increased ENaC activity observed in *Hsd11b2*$^{+/-}$ mice.

Corticosterone was similar between genotypes on LS and SS diets (Table 1) but was elevated in *Hsd11b2*$^{+/-}$ mice on HS diet. To militate against confounding effects of anesthesia, corticosterone was measured in unrestrained conscious mice before and after HS feeding. The sodium-induced increase in corticosterone was confirmed in the PM measurement, prior to the active phase: AM corticosterone was not different between genotypes. (Figure S2).

Since activation of MR and/or GR by glucocorticoids was likely to be causative to the salt-induced phenotype, the renal expression of both was measured: MR expression was similar in both genotypes but GR expression was higher in *Hsd11b2*$^{+/-}$ mice (See Figure S3). To resolve the mechanisms for salt-sensitivity, *Hsd11b2*$^{+/+}$ and *Hsd11b2*$^{+/-}$ mice on a HS diet were treated chronically with i) the ENaC blocker benzamil, ii) the MR antagonist spironolactone, or iii) the GR antagonist RU486. The salt-sensitive phenotype was ameliorated by either benzamil or RU486 treatment, but not by spironolactone treatment (Figure 3A). The sodium-induced reduction in RBF was also ameliorated by benzamil treatment, and RBF was actually increased by RU486 treatment (Figure 3B). Spironolactone did not improve FE_{Na} (Figure 3C) and $\Delta amiloride_{Na}$ remained high. In contrast, both benzamil and RU486 treatment restored FE_{Na} in *Hsd11b2*$^{+/-}$ mice to *Hsd11b2*$^{+/+}$ levels and abolished genotype differences in $\Delta amiloride_{Na}$ (Figure 3D).

Discussion

In *Hsd11b2*$^{+/-}$ mice BP is directly influenced by dietary sodium intake. This strong salt-sensitivity of BP was not observed on the parental C57BL/6J background, and thus the HSD11B2 locus is a plausible candidate gene for salt-sensitivity in humans. As gene defects associated with monogenic BP disorders affect the renal handling of sodium[23], we hypothesized that salt-sensitivity in *Hsd11b2*$^{+/-}$ mice may also reflect abnormal renal

function. Data obtained across a regimen encompassing dietary sodium restriction and sodium loading supported this hypothesis, indicating that abnormal renal sodium homeostasis is driven by activation of GR, and not MR, in this model.

Renal sodium handling is the major determinate of long-term BP control and *Hsd11b2*$^{+/-}$ mice had lower FE_{Na} than *Hsd11b2*$^{+/+}$. Elevated tubular reabsorption is likely to be the major factor for impaired natriuresis and salt-sensitive BP in *Hsd11b2*$^{+/-}$ mice. Since 11βHSD2 governs ligand access to MR *in vivo*, we focused on the classical MR target, ENaC[24]. *Hsd11b2*$^{+/-}$ mice failed to down-regulate amiloride-sensitive sodium reabsorption with increasing dietary sodium, and impaired natriuresis therefore reflects an inability to regulate ENaC activity appropriately for sodium intake. We recognize that amiloride can also inhibit other sodium transport proteins, notably the sodium-hydrogen exchanger 3 (NHE3) in the proximal tubule[25]. Two lines of evidence argue against a major role of NHE3 here. Firstly, the potassium-sparing effect of amiloride localizes the natriuretic effect to the ASDN[26]. Secondly, chronic administration of the selective amiloride analogue benzamil attenuated the salt-sensitive phenotype. Our data plausibly suggest that dysregulated ENaC activity is a key mechanism for salt-sensitivity in this model. This effect is not underpinned by increased transcription of the rate-limiting αENaC subunit. The higher abundance of *sgk1* in salt-loaded heterozygote mice indicates that the dominant effect is to sustain the channel assembly in the apical membrane of the principal cell[24].

Control by MR of ENaC and its regulatory proteins is well documented[24]. In the current study, the physiological ligand aldosterone was appropriately modulated by dietary sodium in both groups of mice. However, there was a tonic suppression of the RAAS in *Hsd11b2*$^{+/-}$ mice across all dietary regimens, consistent with MR activation by glucocorticoids following reduced 11βHSD2. To investigate the contribution of MR, mice fed HS diet were chronically treated with spironolactone. MR antagonism caused a small reduction in BP in both groups of mice, but the pressure differential between the genotypes persisted, as we have previously reported[22]. Critically, spironolactone treatment did not normalize ENaC-mediated sodium reabsorption in *Hsd11b2*$^{+/-}$ mice. The lack of sensitivity to spironolactone may reflect the increased abundance of GR, relative to MR and it is possible that a higher dose would uncover an MR-mediated effect. Nevertheless, the current dose was previously found to be effective against similar concentrations of glucocorticoid[8] and although spironolactone treatment can improve both the hypertension and hypokalemia observed in AME[27], it is of variable benefit in long term treatment[28].

We found that chronic GR blockade normalized ENaC activity and increased RBF. RU486 also prevented the salt-induced rise in BP, consistent with our previous findings[22]. Quantitatively, the effect of RU486 was similar to that of chronic benzamil administration. Together, these data indicate that the cluster of ENaC-related phenotypes in the *Hsd11b2*$^{+/-}$ mice are mediated via GR, not MR. Regulation of ENaC by GR-dependent pathways has been documented in renal cell lines[29,30], in dexamethasone-treated adrenalectomised rats[31] and in a mouse model of Cushing syndrome[8]. A recent study immunolocalized both MR and GR to 11βHSD2-expressing cells in the rat ASDN, demonstrating that physiological variations in circulating aldosterone regulated the translocation of GR and not MR between the nucleus and the cytoplasm[32]. This challenges the conventional view of ASDN regulation by corticosteroids, but is consistent with our data and suggests a mechanism for salt-sensitivity in this model.

The infusion of the dexamethasone can increase the abundance of renal αENaC mRNA and protein expression. This does not automatically equate to an increased physiological activity for ENaC[33] and in *Hsd11b2*$^{+/-}$ mice, ENaC transcription was comparable to controls. Additional ENaC regulatory pathways may be critical, and our data in *Hsd11b2*$^{+/-}$ mice, as

well as other models[4], suggests that dietary salt and/or activation of the HPA axis plays an important role. A realistic point of convergence under these circumstances may be WNK4, which exerts a negative regulatory effect upon ENaC activity[34]. WNK4 is physiologically regulated by dietary sodium status[35], and by glucocorticoids via a negative GRE in the promotor region of the gene[36]. In addition, β2-receptor activation has been demonstrated to induce salt-sensitive hypertension in mice due to GR-mediated inhibition of WNK4 expression[37]. Conversely, *Cre-lox* technology has suggested that GR expression in the ASDN is not critical to dexamethasone-induced hypertension when salt intake is normal[38]. However, mice lacking GR in the ASDN had elevated BP prior to dexamethasone treatment and the effect of a HS diet was not assessed in this study.

HS diet also increased RVR in *Hsd11b2*$^{+/-}$ mice, which would reduce natriuretic capacity, particularly if the medullary vasa recta were constricted. 11βHSD2 is expressed in both arteriole smooth muscle[39] and the vascular endothelium[40]. On a mixed MF1 background, *Hsd11b2*$^{-/-}$ mice had endothelial dysfunction and enhanced vasoconstriction to norepinephrine[41]. This may relate to the genetic background; vascular function was normal on a congenic C57Bl/6J strain[13]. Nevertheless, we cannot discount a vascular component of salt-sensitive BP in *Hsd11b2*$^{+/-}$ mice. ENaC in the vascular endothelium is stimulated by aldosterone excess and high sodium[42] and this is associated with reduced nitric oxide release[43]. Notably, in *Hsd11b2*$^{+/-}$ mice, chronic ENaC blockade prevented the salt-induced increase in RVR.

Perspectives

Failure to regulate ENaC activity with sodium status underpins salt-sensitivity in *Hsd11b2*$^{+/-}$ mice. This is a GR, rather than MR-mediated phenotype. HS feeding increased corticosterone in *Hsd11b2*$^{+/-}$ mice and it is notable that salt-sensitive individuals display both an enhanced stress-induced activation of the HPA axis[44] and attenuated glucocorticoid clearance[45]. Since renal enzyme activity is not influenced by dietary sodium in *Hsd11b2*$^{+/-}$ mice[22], impaired peripheral metabolism cannot fully explain this phenomenon and we suggest that dietary salt activates the HPA axis. Indeed, 11βHSD2 is also expressed in cardiovascular control centers of the brain influencing sympathetic outflow[46]. Central mechanisms are therefore likely to contribute to salt-sensitive BP, at least in the stable phase when sodium balance is restored.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Sources of Funding

EC received an MRC Capacity Building PhD studentship. This work was supported by Wellcome Trust Intermediate (MB) and Principal (JM) Fellowships and by a British Heart Foundation Centre of Research Excellence Award.

References

1. Couser WG, Riella MC. Cardiovascular disease: World Kidney Day 2011: protect your kidneys, save your heart. Nat Rev Nephrol. 2011; 7:130–132. [PubMed: 21358694]
2. Haddy FJ. Role of dietary salt in hypertension. Life Sci. 2006; 79:1585–1592. [PubMed: 16828490]
3. Weinberger MH, Fineberg NS, Fineberg SE, Weinberger M. Salt sensitivity, pulse pressure, and death in normal and hypertensive humans. Hypertension. 2001; 37:429–432. [PubMed: 11230313]

4. Sanders PW. Vascular consequences of dietary salt intake. Am J Physiol Renal Physiol. 2009; 297:F237–F243. [PubMed: 19339634]
5. Waanders F, de Vries LV, van Goor H, Hillebrands JL, Laverman GD, Bakker SJ, Navis G. Aldosterone, from (patho)physiology to treatment in cardiovascular and renal damage. Curr Vasc Pharmacol. 2011; 9:594–605. [PubMed: 21529330]
6. Nishiyama A, Yao L, Nagai Y, Miyata K, Yoshizumi M, Kagami S, Kondo S, Kiyomoto H, Shokoji T, Kimura S, Kohno M, Abe Y. Possible contributions of reactive oxygen species and mitogen-activated protein kinase to renal injury in aldosterone/salt-induced hypertensive rats. Hypertension. 2004; 43:841–848. [PubMed: 14769808]
7. Usukura M, Zhu A, Yoneda T, Karashima S, Yagi K, Yamagishi M, Takeda Y. Effects of a high-salt diet on adipocyte glucocorticoid receptor and 11β-hydroxysteroid dehydrogenase 1 in salt-sensitive hypertensive rats. Steroids. 2009; 74:978–982. [PubMed: 19646461]
8. 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. [PubMed: 19635986]
9. Shibata S, Mu S, Kawarazaki H, Muraoka K, Ishizawa K, Yoshida S, Kawarazaki W, Takeuchi M, Ayuzawa N, Miyoshi J, Takai Y, Ishikawa A, Shimosawa T, Ando K, Nagase M, Fujita T. Rac1 GTPase in rodent kidneys is essential for salt-sensitive hypertension via a mineralocorticoid receptor-dependent pathway. J Clin Invest. 2011; 12:3233–3243. [PubMed: 21765214]
10. Funder JW, Pearce PT, Smith R, Smith AI. Mineralocorticoid action: target tissue specificity is enzyme, not receptor, mediated. Science. 1988; 242:583–585. [PubMed: 2845584]
11. Funder JW. Aldosterone and mineralocorticoid receptors in the cardiovascular system. Prog Cardiovasc Dis. 2010; 52:393–400. [PubMed: 20226957]
12. 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. [PubMed: 10074485]
13. 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. [PubMed: 18032795]
14. Dave-Sharma S, Wilson RC, Harbison MD, Newfield R, Azar MR, Krozowski ZS, Funder JW, Shackleton CH, Bradlow HL, Wei JQ, Hertecant J, Moran A, Neiberger RE, Balfe JW, Fattah A, Daneman D, Akkurt HI, De Santis C, New MI. Examination of genotype and phenotype relationships in 14 patients with apparent mineralocorticoid excess. J Clin Endo Metab. 1998; 83:2244–2254.
15. Ulick S, Tedde R, Mantero F. Pathogenesis of the type 2 variant of the syndrome of apparent mineralocorticoid excess. J Clin Endo Metab. 1990; 70:200–206.
16. 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. [PubMed: 10948076]
17. 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. [PubMed: 17551100]
18. Bocchi B, Kenouch S, Lamarre-Cliche M, Muffat-Joly M, Capron MH, Fiet J, Morineau G, Azizi M, Bonvalet JP, Farman N. Impaired 11β-hydroxysteroid dehydrogenase type 2 activity in sweat gland ducts in human essential hypertension. Hypertension. 2004; 43:803–808. [PubMed: 14981055]
19. 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. [PubMed: 11711524]
20. Kerstens MN, Navis G, Dullaart RP. Salt sensitivity and 11β-hydroxysteroid dehydrogenase type 2 activity. Am J Hypertens. 2004; 17:283–284. [PubMed: 15001207]

21. Melander O, Frandsen E, Groop L, Hulthen UL. No evidence of a relation between 11β-hydroxysteroid dehydrogenase type 2 activity and salt sensitivity. Am J Hypertens. 2003; 16:729–733. [PubMed: 12944030]
22. Bailey MA, Craigie E, Livingstone DE, Kotelevtsev YV, Al-Dujaili EA, Kenyon CJ, Mullins JJ. Hsd11b2 haploinsufficiency in mice causes salt sensitivity of blood pressure. Hypertension. 2011; 57:515–520. [PubMed: 21282561]
23. Mullins LJ, Bailey MA, Mullins JJ. Hypertension, kidney, and transgenics: a fresh perspective. Physiol Rev. 2006; 86:709–746. [PubMed: 16601272]
24. Soundararajan R, Pearce D, Ziera T. The role of the ENaC-regulatory complex in aldosterone-mediated sodium transport. Mol Cell Endocrinol. 2012; 350:242–247. [PubMed: 22101317]
25. Yun CH, Tse CM, Nath SK, Levine SA, Brant SR, Donowitz M. Mammalian Na^+/H^+ exchanger gene family: structure and function studies. Am J Physiol. 1995; 269:G1–G11. [PubMed: 7631785]
26. Kleyman TR, Cragoe EJ Jr. The mechanism of action of amiloride. Semin Nephrol. 1988; 8:242–248. [PubMed: 2849182]
27. Morineau G, Sulmont V, Salomon R, Fiquet-Kempf B, Jeunemaitre X, Nicod J, Ferrari P. Apparent Mineralocorticoid Excess: Report of Six New Cases and Extensive Personal Experience. J Am Soc Nephrol. 2006; 17:3176–3184. [PubMed: 17035606]
28. Quinkler M, Stewart PM. Hypertension and the cortisol-cortisone shuttle. J Clin Endo Metab. 2003; 88:2384–2392.
29. 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. [PubMed: 15743993]
30. Naray-Fejes-Toth A, Fejes-Toth G. Glucocorticoid receptors mediate mineralocorticoid-like effects in cultured collecting duct cells. Am J Physiol. 1990; 259:F672–F678. [PubMed: 2221105]
31. Muller OG, Parnova RG, Centeno G, Rossier BC, Firsov D, Horisberger JD. Mineralocorticoid effects in the kidney: correlation between alphaENaC, GILZ, and Sgk-1 mRNA expression and urinary excretion of Na^+ and K^+ J Am Soc Nephrol. 2003; 14:1107–1115. [PubMed: 12707381]
32. Ackermann D, Gresko N, Carrel M, Loffing-Cueni D, Habermehl D, Gomez-Sanchez C, 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:F1473–1485. [PubMed: 20861076]
33. Frindt G, Palmer LG. Regulation of epithelial Na^+ channels by adrenal steroids: Mineralocorticoid and glucocorticoid effects. Am J Physiol Renal Physiol. 2012; 302:F20–F26. [PubMed: 22012806]
34. Ring AM, Cheng SX, Leng Q, Kahle KT, Rinehart J, Lalioti MD, Volkman HM, Wilson FH, Hebert SC, Lifton RP. WNK4 regulates activity of the epithelial Na^+ channel in vitro and in vivo. Proc Natl Acad Sci. 2007; 104:4020–4024. [PubMed: 17360470]
35. O'Reilly M, Marshall E, Macgillivray T, Mittal M, Xue W, Kenyon CJ, Brown RW. Dietary electrolyte-driven responses in the renal WNK kinase pathway in vivo. J Am Soc Nephrol. 2006; 17:2402–2413. [PubMed: 16899520]
36. Li C, Li Y, Liu H, Sun Z, Lu J, Zhao Y. Glucocorticoid repression of human with-no-lysine (K) kinase-4 gene expression is mediated by the negative response elements in the promoter. J Mol Endocrinol. 2008; 40:3–12. [PubMed: 18096992]
37. Mu S, Shimosawa T, Ogura S, Wang H, Uetake Y, Kawakami-Mori F, Marumo T, Yatomi Y, Geller DS, Tanaka H, Fujita T. Epigenetic modulation of the renal beta-adrenergic-WNK4 pathway in salt-sensitive hypertension. Nat Med. 2011; 17:573–580. [PubMed: 21499270]
38. Goodwin JE, Zhang J, Velazquez H, Geller DS. The glucocorticoid receptor in the distal nephron is not necessary for the development or maintenance of dexamethasone-induced hypertension. Biochem Biophys Res Commun. 2010; 394:266–271. [PubMed: 20188070]
39. Hatakeyama H, Inaba S, Takeda R, Miyamori I. 11β-hydroxysteroid dehydrogenase in human vascular cells. Kidney Int. 2000; 57:1352–1357. [PubMed: 10760066]
40. Gong R, Morris DJ, Brem AS. Variable expression of 11β Hydroxysteroid dehydrogenase isoforms in vascular endothelial cells. Steroids. 2008; 73:1187–1196. [PubMed: 18573267]

41. Hadoke PW, Christy C, Kotelevtsev YV, Williams BC, Kenyon CJ, Seckl JR, Mullins JJ, Walker BR. Endothelial cell dysfunction in mice after transgenic knockout of type 2, but not type 1, 11β-hydroxysteroid dehydrogenase. Circulation. 2001; 104:2832–2837. [PubMed: 11733403]

42. Kusche-Vihrog K, Sobczak K, Bangel N, Wilhelmi M, Nechyporuk-Zloy V, Schwab A, Schillers H, Oberleithner H. Aldosterone and amiloride alter ENaC abundance in vascular endothelium. Pflugers Arch. 2008; 455:849–857. [PubMed: 17891415]

43. Oberleithner H, Riethmuller C, Schillers H, MacGregor GA, de Wardener HE, Hausberg M. Plasma sodium stiffens vascular endothelium and reduces nitric oxide release. Proc Natl Acad Sci. 2007; 104:16281–16286. [PubMed: 17911245]

44. 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. [PubMed: 18337758]

45. 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 Endo Metab. 2003; 88:4180–4185.

46. Geerling JC, Loewy AD. Aldosterone-sensitive neurons in the nucleus of the solitary tract: efferent projections. J Comp Neurol. 2006; 497:223–250. [PubMed: 16705681]

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Novelty and Significance

1. What is new?
    - i. Reduced activity of a steroid-processing enzyme impairs the efficiency of salt excretion by the kidney
    - ii. The protein causing sodium retention was identified and a new role for regulation of sodium transport in the kidney by steroid hormones revealed.
    - iii. High intake of salt in the diet increases glucocorticoids in the blood, which increases salt retention by the kidney. This causes blood pressure to rise
2. What is relevant?
    - i. In some people, blood pressure rises with high salt intake and this increases the risk for developing hypertension, cardiovascular and renal disease.
    - ii. High levels of glucocorticoids are common in stress and metabolic disorders and could reduce the excretion of salt by the kidney
    - iii. The mechanisms for salt retention could therefore be potential targets for antihypertensive therapy

Summary: Reduced activity of a glucocorticoid-metabolizing enzyme in the kidney and brain causes increased levels of steroid in the blood, impairs the ability of the kidney to excrete sodium and causes blood pressure to rise.

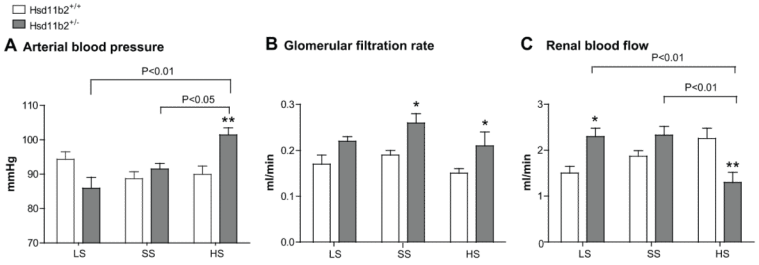

Figure 1.

A) Arterial blood pressure; B) glomerular filtration rate; and C) renal blood flow in *Hsd11b2*$^{+/+}$ (open bars) and *Hsd11b2*$^{+/-}$ (grey bars) mice after 3 weeks on either low sodium (LS; n=7/8), standard sodium (SS; n=8/8) or high sodium (HS; 11/9) diet. Data are means±SEM. Comparisons were made using ANOVA with Bonferroni *post hoc* test. **P*<0.05; ***P*<0.01.

Europe PMC Funders Author Manuscripts

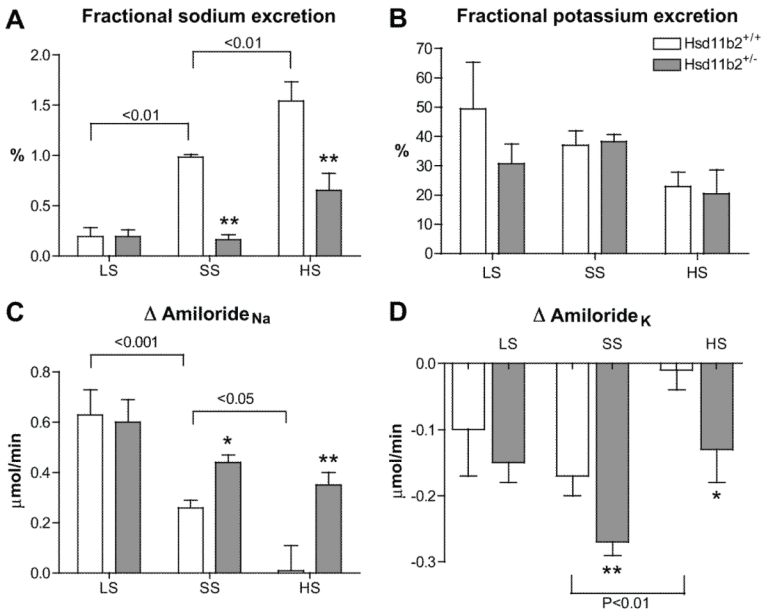

Figure 2.

A) Fractional sodium excretion; B) fractional potassium excretion; C) amiloride-sensitive sodium reabsorption ($\Delta amiloride_{Na}$) and D) amiloride sensitive potassium secretion ($\Delta amiloride_{k}$) in *Hsd11b2*$^{+/+}$ (open bars) and *Hsd11b2*$^{+/+}$ (grey bars) mice after 3 weeks on either low sodium (LS; n=7/8), standard sodium (SS; n=8/8) or high sodium (HS; n=11/9) diet. Data are means±SEM. Comparisons were made using ANOVA with Bonferroni *post hoc* test. **P*<0.05; ***P*<0.01.

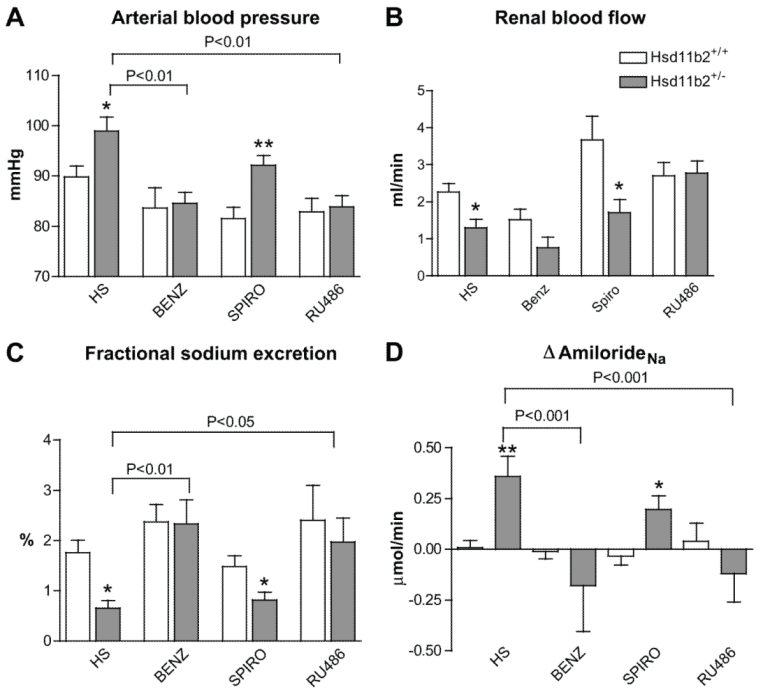

Figure 3.

A) Arterial blood pressure; B) renal blood flow; C) fractional sodium excretion; and D) amiloride-sensitive sodium reabsorption ($\Delta amiloride_{Na}$) in *Hsd11b2*$^{+/+}$ (open bars) and *Hsd11b2*$^{+/-}$ (grey bars) mice after 3 weeks on a high sodium (HS) diet, or with HS diet accompany by either benzamil (Benz; n=7/6), spironolactone (Spiro; n=6/8) or RU486 treatment (n=9/10). Data are means±SEM. Comparisons were made using ANOVA with Bonferroni *post hoc* test. **P*<0.05; ***P*<0.01.

Europe PMC Funders Author Manuscripts

Table 1

Renal and plasma data. *Hsd11b2*$^{+/+}$ and *Hsd11b2*$^{+/-}$ mice were maintained on a low sodium (LS; n=7/8), standard sodium (SS; n=8/8) or high sodium (HS; n=11/9) diet. Data are means ± SEM. Statistical comparisons were made using ANOVA with Bonferroni *post hoc* test. ***P*<0.01; [a]*P*<0.001 versus wild-type.

	Parameter	*Hsd11b2*$^{+/+}$ LS	*Hsd11b2*$^{+/-}$ LS	*Hsd11b2*$^{+/+}$ SS	*Hsd11b2*$^{+/-}$ SS	*Hsd11b2*$^{+/+}$ HS	*Hsd11b2*$^{+/-}$ HS
FF *	(%)	30.0 ± 6.1	22.2 ±3.8	23.8 ± 1.7	25.7 ± 1.8	16.8 ± 0.8	42.1 ± 4.1 **
RVR †	(mmHg/ml.min^{-1})	67.2 ± 7.0	37.8 ± 2.9	48.8 ± 3.3	41.1 ± 3.5	42.4 ± 4.2	93.8 ± 18.7 **
E_{Na} ‡	(μmol/min)	0.05±0.02	0.06±0.01	0.26±0.07	0.07±0.03 *	0.35±0.03	0.19±0.04 **
E_K §	(μmol/min)	0.3 ± 0.06	0.24 ± 0.04	0.28 ± 0.03	0.44 ± 0.03 *	0.17 ± 0.03	0.20 ± 0.07
P_{Na} //	(mmol/l)	152.6 ± 3.5	149.0 ± 0.4	146.0 ± 0.6	147.0 ± 0.9	149.1 ± 1.2	149.8 ± 0.9
P_k ¶	(mmol/l)	4.41 ± 0.34	4.02 ± 0.19	4.25 ± 0.08	4.55 ± 0.14	4.83 ± 0.34	3.72 ± 0.11 **
P_{Cort} #	(nmol/l)	515 ± 78	425 ± 21	294 ± 24	330 ± 34	429 ± 44	698 ± 48 **
P_{aldo} **	(pmol/l)	1615 ±81	809 ± 103 **	537 ± 164	421 ± 94	332±59	39±0.6a

* Filtration fraction,

† renal vascular resistance,

‡ urinary excretion of sodium and

§ potassium and plasma concentrations of

// sodium,

¶ potassium,

corticosterone and

** aldosterone.