Salt sensitivity of blood pressure is an independent risk factor for cardiovascular mortality in normotensive individuals and an independent prognostic factor for essential hypertension. 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. Even when aldosterone is low or normal, mineralocorticoid receptor (MR) blockade can be cardioprotective, and pathophysiological activation of MR by alternative ligands has been found in rodent models of salt-sensitive hypertension.

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, and indirectly, by locking glucocorticoid-occupied MR in an inactive state. 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. A type 2 variant of the disease (Online Mendelian Inheritance in Man 207765) presents in adults 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. To define the role of the enzyme in the physiological regulation of blood pressure, we previously generated mice with a targeted deletion of Hsd11b2. 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).
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). MBP and urinary sodium excretion were similar (Figure 1B and C). The UNa: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).

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 C). The UNa: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).

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Table 1. Plasma Potassium (P<sub>k</sub>) and Hematocrit in Hsd11b2<sup>+/−</sup> and Hsd11b2<sup>+/+</sup> Mice Maintained on Either a Control (0.25% Na) or High-Sodium (2.5% Na) Diet for 4, 21, or 70 Days

<table>
<thead>
<tr>
<th>Diet</th>
<th>P&lt;sub&gt;k&lt;/sub&gt;, mmol/L</th>
<th>Hematocrit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hsd11b2&lt;sup&gt;+/−&lt;/sup&gt;</td>
<td>Hsd11b2&lt;sup&gt;+/+&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>4.55±0.14</td>
<td>4.25±0.08</td>
</tr>
<tr>
<td></td>
<td>0.43±0.07</td>
<td>0.42±0.1</td>
</tr>
<tr>
<td>Control</td>
<td>(8)</td>
<td>(8)</td>
</tr>
<tr>
<td>High Na</td>
<td>3.74±0.10†</td>
<td>4.14±0.12</td>
</tr>
<tr>
<td>4 d</td>
<td>0.46±0.05†</td>
<td>0.41±0.08</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td>(6)</td>
</tr>
<tr>
<td>High Na</td>
<td>3.72±0.11†</td>
<td>4.83±0.34</td>
</tr>
<tr>
<td>21 d</td>
<td>0.42±0.07</td>
<td>0.42±0.07</td>
</tr>
<tr>
<td></td>
<td>(7)</td>
<td>(9)</td>
</tr>
<tr>
<td>High Na</td>
<td>3.40±0.21†</td>
<td>4.45±0.29</td>
</tr>
<tr>
<td>70 d</td>
<td>0.43±0.07</td>
<td>0.42±0.03</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>(5)</td>
</tr>
</tbody>
</table>

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<sup>+/−</sup> and Hsd11b2<sup>+/+</sup> mice had a similar plasma volume, plasma potassium, and hematocrit values (Table 1). The TTKG was significantly higher in heterozygote mice (Hsd11b2<sup>+/−</sup>=13.7±0.9 versus Hsd11b2<sup>+/+</sup>=8.6±1.2; P<0.01). After 4 days of high-sodium feeding, Hsd11b2<sup>+/−</sup> 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<sup>+/−</sup> mice was not associated with volume expansion, plasma volume being lower (Hsd11b2<sup>+/−</sup>=1.48±0.07 mL versus Hsd11b2<sup>+/+</sup>=2.13±0.02 mL; n=5 per group; P<0.01) and hematocrit higher (Table 1) than in Hsd11b2<sup>+/+</sup> mice.

After 21 days on high-sodium diet, Hsd11b2<sup>+/−</sup> 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<sup>+/−</sup> 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<sup>+/−</sup> 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<sup>22</sup>) obtained in conscious, unrestrained mice was also elevated in Hsd11b2<sup>+/−</sup> 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<sup>+/−</sup> mice, we first used dexamethasone to suppress the hypothalamic-pituitary-adrenal axis, 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<sup>+/−</sup>=96.8±1.8 mm Hg versus Hsd11b2<sup>+/+</sup>=94.5±0.9 mm Hg; P value not significant) and plasma potassium (Hsd11b2<sup>+/−</sup>=5.70±0.12 mmol/L versus Hsd11b2<sup>+/+</sup>=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<sup>+/−</sup> mice, which remained ~10-mm Hg higher than in Hsd11b2<sup>+/+</sup> mice (Figure 3A). Similarly, spironolactone did not prevent heterozygote mice becoming hypokalemic during high-sodium feeding (Figure 3B). During MR blockade, the U<sub>NK</sub> ratio also
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 UNa:K ratio.

**Discussion**

Deficiency in 11βHSD2 promotes salt retention, potassium wasting, and hypertension, thought to reflect unregulated activation of renal MR by glucocorticoids. Apparent mineralocorticoid excess arises in children who are homozygous or compound heterozygous for mutations that ablate 11βHSD2 activity. Apparent mineralocorticoid excess is rare, and the majority of those carrying a single mutated allele appear normal. Detailed long-term follow-up of heterozygotes is lacking but evidence suggests abnormal steroid excretion and a propensity toward low-renin hypertension in older individuals, and an age-dependent decline in renal natriuretic response: transient sodium retention preceded a rise in blood pressure by 24 to 48 hours. Hsd11b2+/− mice also developed hypokalemia. The suppressed UNa: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 and humans 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. 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 corticosterone was indicated. However, spironolactone (administered at a dose shown to be effective against high concentrations of glucocorticoid) 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, 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, and glucocorticoid regeneration by renal 11βHSD1 has been linked to salt sensitivity in rats. 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.

Mechanistically, the alterations in UNa:K and TTKG provide compelling evidence that epithelial sodium channel activation in the aldosterone-sensitive distal nephron under-
pens 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 intake36 and salt-sensitivity of blood pressure1,2 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
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_Hsd11b2_ HAPLOINSUFFICIENCY IN MICE CAUSES SALT-SENSITIVITY OF BLOOD PRESSURE

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Genotyping

Mice were obtained from heterozygote crosses of a congenic *Hsd11b2-C57BL/6J* line\(^1\). For genotyping, genomic DNA, isolated from ear biopsies, was digested by *BamHI* and separated on 0.8% agarose gel for Southern analysis using a *EcoRI* hybridization probe subcloned from the first intron of the *Hsd11b2* gene. The wild-type allele was represented by a 10kb (9899bp) restriction fragment. The targeted allele was represented by 2 kb (1850 bp) restriction fragment, resulting from the insertion of a *BamHI* site at the 5’ end of the Neo cassette, upstream of the genomic *XbaI* site. A sample blot, below, shows the three genotypes, *Hsd11b2\(^{+/+}\)* (het), *Hsd11b2\(^{+/−}\)* (WT) and *Hsd11b2\(^{−/−}\)* (null).

Telemetry studies: Blood pressure was measured by radiotelemetry using a device (Model TA 11PAC10, Data Sciences, UK) implanted under isofluorane anesthesia. After recovery from surgery, blood pressure was monitored until circadian variation was restored and mean pressure stabilized (all within 7 days). Mice were single
housed throughout and allowed free access to water and a control chow (0.25% Na by weight; RM1 diet, Special Diet Services, UK). After 7 days, baseline blood pressure was recorded over a three-day period and mice then fed a high sodium diet (2.5% Na by weight; Diet 829504, SDS, UK) over a 19-day recording period. For each mouse, a daily average mean arterial blood pressure was taken and used to calculate the mean daily value per genotype.

**Inhibitor studies:** In addition to control (untreated) experiments, mice received one of 3 co-treatments:

- **i)** Spironolactone pellets (Silastic, a gift from Dow-Corning, USA) were implanted subcutaneously under isofluorane anesthesia, five days before feeding 2.5% sodium diet. Two pellets were implanted, each containing 30mg of spironolactone. *In vitro* studies confirmed that spironolactone release from the matrix is at a constant rate over the experimental period. The concentration of canrenone in terminal plasma was measured by mass-spectrometry (Clinical Research Facility, University of Edinburgh) and was ~75 nmol/l, similar to a previous study in which the drug exerted a hypotensive effect.

- **ii)** Dexamethasone was administered in the drinking water (1µg/ml in 0.1% ethanol) throughout the period of high sodium feeding. Plasma corticosterone was measured in samples taken from conscious, unrestrained animals at 6:30pm.

- **iii)** RU38486 pellets were implanted five days before giving the high sodium diet. Two pellets were implanted, each containing 30mg and the concentration of RU38486 in terminal plasma, measured by mass spectrometry was ~100nmol/l.

**Analysis:** Sodium and potassium concentrations were measured by ISE or flame photometry. Osmolality was measured by freezing-point depression. Corticosterone,
deoxycorticosterone and aldosterone concentrations were measured by ELISA or RIA, as described\textsuperscript{4,5}. For urinary excretion, an average rate was measured during the baseline period and during the adaptive (days 1, 2 and 3) and plateau (days 11, 13, & 15) phases of the response to high sodium diet. Urinary albumin concentration was measured using a commercial kit (Olympus Diagnostics) as described\textsuperscript{6}.

REFERENCES


