A urine-concentrating defect in 11β-hydroxysteroid dehydrogenase type 2 null mice

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Evans LC, Livingstone DE, Kenyon CJ, Jansen MA, Dear JW, Mullins JJ, Bailey MA. A urine-concentrating defect in 11β-hydroxysteroid dehydrogenase type 2 null mice. Am J Physiol Renal Physiol 303:F494–F502, 2012.—In aldosterone target tissues, 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2) is coexpressed with mineralocorticoid receptors (MR) and protects the receptor from activation by glucocorticoids. Null mutations in the encoding gene, Hsd11b2, cause apparent mineralocorticoid excess, in which hypertension is thought to reflect volume expansion secondary to sodium retention. Hsd11b2−/− mice are indeed hypertensive, but impaired natriuretic capacity is associated with significant volume contraction, suggestive of a urine concentrating defect. Water turnover and the urine concentrating response to a 24-h water deprivation challenge were therefore assessed in Hsd11b2−/− mice and controls. Hsd11b2−/− mice have a severe and progressive polyuric/polydipsic phenotype. In younger mice (~2 mo of age), polyuria was associated with decreased abundance of aqp2 and aqp3 mRNA. The expression of other genes involved in water transport (aqp4, slc14a2, and slc12a2) was not changed. The kidney was structurally normal, and the concentrating response to water deprivation was intact. In older Hsd11b2−/− mice (>6 mo), polyuria was associated with a severe atrophy of the renal medulla and downregulation of aqp2, aqp3, aqp4, slc14a2, and slc12a2. The concentrating response to water deprivation was impaired, and the natriuretic effect of the loop diuretic bumetanide was lost. In older Hsd11b2−/− mice, the V2 receptor agonist desmopressin did not restore full urine concentrating capacity. We find that Hsd11b2−/− mice develop nephrogenic diabetes insipidus. Gross changes to renal structure are observed, but these were probably secondary to sustained polyuria, rather than of developmental origin.

polyuric; polydipsia; aldosterone; corticosterone; aquaporin-2; prenatal programming

IN VITRO, ALDOSTERONE AND cortisol have equal affinities for mineralocorticoid receptors (MR) (3). In vivo, however, MR is selectively activated by aldosterone in target tissues such as the distal nephron (aldosterone-sensitive distal nephron; ASDN). This selectivity results from prereceptor metabolism of competing cortisol (corticosterone in rodents), which is an MR ligand, to cortisone (11-dehydrocorticosterone), which is not (16, 21).

In humans, congenital loss of 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2) causes apparent mineralocorticoid excess (AME; OMIM 218030), presenting with hypertension, hypokalemic alkalosis, and a suppressed renin-angiotensin-aldosterone system (38, 47). AME reflects activation of MR by glucocorticoids (47); with mineralocorticoid target proteins no longer subject to the homeostatic influence of the renin-angiotensin-aldosterone system, hypertension and electrolyte abnormalities develop secondary to renal salt and water retention. A similar profile is observed when AME is acquired following pharmacological inhibition of 11βHSD2 (18). In rats, inhibition of 11βHSD2 stimulates sodium reabsorption in the collecting duct following activation of the epithelial sodium channel (ENaC) (7).

To better define the role of 11βHSD2 in sodium and blood pressure homeostasis, we generated mice with a null mutation in the encoding gene (hsd11b2) (33). These mice faithfully model AME (33); fractional sodium excretion is reduced at weaning due to activation of ENaC (6), consistent with a renal origin of hypertension. However, ENaC activation is transient, and between 2 and 3 mo of age, amiloride-sensitive sodium transport is lost, fractional sodium excretion is normalized (6), and polyuria is established (33).

The phenotypic arc for AME resembles mineralocorticoid escape (32). In contrast to classic aldosterone excess, however, Hsd11b2−/− mice are consistently volume contracted, even in the early antinatriuretic phase (6). This raises the possibility that polyuria is not an adaptive response to prolonged MR activation but part of the early etiology of AME. Corticosteroids can influence the prenatal development of the urine concentrating mechanisms (42, 46); the current study was therefore designed to establish the causes of increased water turnover in Hsd11b2−/− mice.

MATERIALS AND METHODS

A congenic mouse strain, generated by a >10-generation backcross of the MF1 Hsd11b2−/− mouse with C57BL/6J (Harlan), was used in this study. All hsd11b2−/− mice were obtained from homozygous null breeding, and age-matched C57BL/6J were used as controls. Following a pilot study to define the temporal development of a gross histological phenotype, male mice were studied at two age groups: at 2 mo (range 52–64 days), the kidney was structurally normal, whereas at >6 mo of age (range 180–340 days), marked structural abnormalities were consistently observed. All experiments were performed under a UK Home Office Project Licence.

Water turnover and deprivation challenge. Mice were individually housed in metabolism cages (Tecniplast) with free access to a powdered rodent maintenance (RM1) diet (Special Diet Services) and water, unless otherwise specified. Following acclimatization, baseline measurements of body weight, urine output, and food and water intake were made over a 4-day period. Drinking water was then removed for 24 h. One week later, a second 24-h water deprivation was performed, immediately before which the synthetic vasopressin 2 receptor (V2R) agonist desmopressin (dDAVP) was administered (1 μg/kg sc). In separate cohorts of mice, the effect of dDAVP was assessed during ad libitum access to water.

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Response to bumetanide. 

Hsd11b2−/− and C57BL/6J mice at >180 days of age were anesthetized (Inactin, 120 mg/kg ip) and prepared surgically for renal clearance as described (6). After surgery, mice were infused intravenously with a solution containing (in mmol/l) 110 NaCl, 15 NaHCO3, 20 sodium amino hippurate, and 5 KCl. FITC-insulin (0.25%) was also added to the infuse for the evaluation of the glomerular filtration rate (GFR). After a 40-min equilibration, a timed urine collection was made. Bumetanide was then injected (1 mg/kg iv), and a second timed urine collection was made.

Urinary analysis. Sodium and potassium concentrations were measured using flame photometry (Spectronic Analytical Instruments) and osmolality by freezing point depression (Vogel). Urea was measured by a commercial, colormetric kit (BioAssay Systems).

Quantitative PCR analysis of mRNA abundance. RNA was isolated from homogenized kidneys using an RNeasy minikit (Qiagen) and reverse transcribed using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Quantitative PCR was performed using a Universal Probe Library kit (Roche) and primers designed using ProbeFinder version 2.45 for Mouse (Roche Diagnostics). The following target genes were assessed: apg2, apg3, apg4, avpr1a, avpr2, casp3, hif1a, nr3c2, slc12a1, and slc12a3. Quantification was performed using the second derivative maximum method, and target gene expression was normalized to mean housekeeper gene values. In the younger mice, values were normalized to an average of the abundance of Tbp, Ppia, and Rn18s. In contrast, at >180 days the abundance of the housekeeper genes differed significantly between genotypes, and target expression was therefore normalized to Rn18s alone. Primer sequences are detailed in Table 1. Data are presented as a percentage of the mean normalized value obtained in C57BL/6J mice.

Western blot analysis. Whole kidneys were homogenized, protein extracted, and separated by SDS-PAGE (8 µg of protein loaded/lane). Protein was electrophoresed to polyvinylidene difluoride membranes using a semidyem method. Immunoblotting was performed using a primary antibody against aquaporin-2 (AQP2; Cell Signalling Technology) following the manufacturer’s protocol. Membranes were then stripped and reprobed with anti-GAPDH (Sigma-Aldrich, St. Louis, MO). Since GAPDH presented as a 36-kDa band, membrane stripping was essential for intrablot quantification and the distinction of the AQP2/GAPDH was then used for statistical analysis. The individual water-deprived kidney values were expressed as a percentage of the average value measured in the hydrated kidneys from the corresponding group (n = 4–6).

Magnetic resonance imaging. Mice were anesthetized and placed in a magnetic resonance imaging (MRI)-compatible holder (Rapid Biomedical, Rimpar, Germany). Rectal temperature and respiration were monitored and controlled throughout to ensure normal physiologic parameters. Respiration-gated MRI data were collected using a Agilent 7T preclinical scanner (Agilent Technologies, Oxford, UK), with a 33-mm volume coil (Rapid Biomedical, Würzburg, Germany).

For the anatomic scan, images were obtained from a single Hsd11b2 null mouse and wild-type littermate, aged 220 days. Twenty contiguous coronal T2-weighted fast-spin echo images (echo train length 4) of 0.5-mm slice thickness were collected with the following parameters: repetition time (TR) ∼2,500 ms depending on respiration rate; effective echo time = 36 ms; field of view = 20 mm × 20 mm; matrix = 256 × 256; 6 signal averages; total scan time was ∼16 min. Gadolinium-based G4 dendermer intravenous contrast was used as described (15). Briefly, a single coronal slice (1-mm thick) through the entire kidneys was acquired using a Fast Low Angle Shot (FLASH) pulse sequence with the following parameters: repetition time 40 ms, echo time 3.16 ms, Flip angle 30°, field of view 40 × 40 mm, matrix 256 × 256, 4 signal averages.

Histology. Kidneys were immersion fixed in buffered formaldehyde and embedded in paraffin. Midtransverse sections (4 µm) were cut, mounted, and stained with hematoxylin and eosin (H+E; Histology Core Service, The Queen’s Medical Research Institute, University of Edinburgh). Images were captured using a microscope (Zeiss Axioscope, Zeiss) at ×40 magnification. The number of glomeruli in 10 H+E-stained sections/mouse (n = 5/group) was counted to evaluate nephron loss.

Statistical analysis. Data are means ± SE, and n is specified for each experiment. Statistical comparisons were made using either ANOVA with a Bonferroni post hoc test or Student’s t-test, as appropriate.

RESULTS

Altered water homeostasis and renal hemodynamics in Hsd11b2−/− mice. At 2 mo of age, water turnover was higher in Hsd11b2−/− mice, which produced approximately three times more urine than in controls (Fig. 1A), and they were polydipsic (Fig. 1B). Urine osmolality was significantly lower in the Hsd11b2−/− mice (Fig. 1C), but 24-h osmolar excretion was not significantly different at this age (Table 2). A significant

Table 1. Quantitative PCR primers and probes

<table>
<thead>
<tr>
<th>Left Primer</th>
<th>Right Primer</th>
<th>Probe Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rn18s (18S RNA)</td>
<td>cttcacacaggggaacacccctac</td>
<td>gctccaccaacactagaagc</td>
</tr>
<tr>
<td>Tbp (TATA box binding protein)</td>
<td>ggtgggttgtcaacttctgcaa</td>
<td>gctccgggaaatcaggtagt</td>
</tr>
<tr>
<td>Ppia (Peptidylprolyl isomerase A)</td>
<td>ACGCaaccttgcttttc</td>
<td>gcaaacagctcgaaggagac</td>
</tr>
<tr>
<td>Apo2 (Aquaporin-2)</td>
<td>tgcagcctgtccttcattg</td>
<td>ggcagccggtggaataagt</td>
</tr>
<tr>
<td>Apo3 (Aquaporin-3)</td>
<td>tgggagctcactccttt</td>
<td>tgggtgaggagccaccat</td>
</tr>
<tr>
<td>Apo4 (Aquaporin-4)</td>
<td>tgggagttggtggtcacc</td>
<td>tgcaccaacacttggaactga</td>
</tr>
<tr>
<td>Avpr1a (Arginine vasopressin receptor 1a)</td>
<td>gcacaggtgtgcctctcat</td>
<td>aacccgtaacccgctgt</td>
</tr>
<tr>
<td>Avpr2 (Arginine vasopressin receptor 2)</td>
<td>cagcctggttcgcctcat</td>
<td>aacccgtaacccgctgt</td>
</tr>
<tr>
<td>Hif1a (Hypoxia-inducible factor 1, α-subunit)</td>
<td>tggagcattggttggtggtg</td>
<td>ggttcaccttactatat</td>
</tr>
<tr>
<td>Nfat5 (Nuclear factor of activated T cells 5)</td>
<td>tggtaggttggtggtggtg</td>
<td>ggttcaccttactatat</td>
</tr>
<tr>
<td>Nr3c2 (Mineralocorticoid receptor)</td>
<td>cttggcttcacacctttcgc</td>
<td>gcccagcttactatat</td>
</tr>
<tr>
<td>Slc12a1 (Sodium-potassium-chloride transporter; NKCC2)</td>
<td>gaaatgccggaagaaaagg</td>
<td>tggcctgcgtgtggtggtg</td>
</tr>
<tr>
<td>Slc14a2 (Urea transporter)</td>
<td>gaaatgccggaagaaaagg</td>
<td>tggcctgcgtgtggtggtg</td>
</tr>
</tbody>
</table>

Probes were designed using ProbeFinder version 2.45 for Mouse (Roche Diagnostics).
natriuresis was observed (Fig. 1D), which at this age was associated with phosphaturia (Fig. 1E), suggesting impaired sodium reabsorption in the proximal tubule.

At >6 mo of age, the polyuric/polydipsic phenotype was more pronounced in Hsd11b2−/− mice (Fig. 1, A and B). Daily urine output was ~40% of body weight, but cumulative water balance was not different from that of the age-matched C57BL/6 mice (Table 2). Sodium excretion was further elevated in Hsd11b2−/− mice (Fig. 1D), but phosphate excretion had normalized (Fig. 1E).

Urine concentrating capacity was assessed using a 24-h water deprivation challenge. At 2 mo, Hsd11b2−/− mice had an intact concentrating response, with urine flow rate falling (Fig. 2A) and osmolality rising (Fig. 2B), similar to control mice. In the older Hsd11b2−/− mice, maximal urine concentrating ability was impaired: urine flow fell and osmolality rose but not to the same extent as in the controls (Fig. 2, A and B). Water balance was significantly more negative in the null mice at >6 mo (Fig. 2C), and therefore body weight loss was greater during the challenge (Fig. 2D). Administration of the V2 receptor agonist dDAVP during the water deprivation challenge did not increase urine concentration, suggesting that the response was already maximal (Table 3).

In separate experiments, the acute effect of dDAVP on urine flow was assessed over a 6-h period during which mice (>6 mo) had unrestricted access to water. Baseline urine flow (0.4 ± 0.2 vs. 3.0 ± 0.6 μl/min, P < 0.001) and water intake (5.2 ± 0.5 vs. 10.8 ± 1.0 μl/min, P < 0.01) were significantly higher in null mice than in controls. Administration of dDAVP caused anuria in the Hsd11b2−/− mice (Fig. 1D). Water intake was not affected by dDAVP in either C57BL/6J mice (5.2 ± 0.5 vs. 4.8 ± 0.4 μl/min, P > 0.05) or Hsd11b2−/− mice (10.8 ± 1.0 vs. 8.4 ± 1.2 μl/min, P > 0.05).

Aquaporin expression in Hsd11b2−/− mice. In Hsd11b2−/− mice, mRNA abundance of aqp2 and aqp3 was significantly lower than in the controls, an effect observed in both age groups (Table 4). Aqp4 mRNA was comparable at 2 mo but significantly lower in the null mice at 6 mo (Table 4). There was no difference in the mRNA abundance of avpr2 in the null and control mice at either age. Avpr1a mRNA abundance was

Table 2. Kidney weight/body weight ratio and basal urinary excretion in Hsd11b2−/− mice

<table>
<thead>
<tr>
<th></th>
<th>C57BL/6J</th>
<th>Hsd11b2−/−</th>
<th>C57BL/6J</th>
<th>Hsd11b2−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney weight/body weight ratio</td>
<td>1.17 ± 0.05</td>
<td>1.47 ± 0.05*</td>
<td>1.07 ± 0.03</td>
<td>1.38 ± 0.04*</td>
</tr>
<tr>
<td>Osmolar excretion, mosmol-kgH2O–1·24 h−1</td>
<td>2.951 ± 94</td>
<td>3.569 ± 206</td>
<td>3.727 ± 286</td>
<td>5.229 ± 152*‡</td>
</tr>
<tr>
<td>Cumulative water balance, ml</td>
<td>11 ± 0.6</td>
<td>14 ± 0.9†</td>
<td>11 ± 0.8</td>
<td>14 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.001 compared with age-matched controls. †P < 0.05 compared with age-matched controls. ‡P < 0.001 compared with younger mice of the same genotype.
significantly higher in the null mice than the controls at 2 mo, but comparable levels of avpr1a mRNA were observed in the older groups (Table 4).

Western blot analysis was used to assess the AQP2 protein response to a water deprivation challenge. Renal AQP2 protein levels were increased in Hsd11b2-/- and C57BL/6J mice at 2 mo (Fig. 4), consistent with an intact concentrating mechanism at this time point. In older mice, water deprivation doubled AQP2 expression in control mice but this was not significantly affected by 6 mo. This expression was stable in the null mice, suggestive of renal insufficiency. The kidney weight/body weight ratio was higher in null mice than in controls at both age points (Table 2), suggesting that medullary atrophy is offset by further expansion of the renal cortex.

Expression of sodium-potassium-chloride cotransporter and UT-A. The loss of an intact medulla in older Hsd11b2-/- mice would be expected to impair the efficient generation of the corticomedullary osmotic gradient required for urine concentration. Indeed, in Hsd11b2-/- mice, there was an additional reduction in mRNA abundance for slc12a1, the gene encoding the sodium-potassium-chloride cotransporter (NKCC2; Table 4), which contributes to generation of a hyperosmotic interstitium. Notably, this ~50% reduction in gene expression was accompanied by a complete loss of the diuretic and natriuretic response to the NKCC2 inhibitor, bumetanide (Fig. 7, A and B). GFR was not different between the two groups (Fig. 7C). However, it was less stable in the null mice, suggestive of renal insufficiency. The expression of slc14a2, the gene encoding the urea transporter UT-A, was reduced by ~50% in Hsd11b2-/- mice in both age groups (Table 4), and the urinary excretion of urea was approximately twofold greater (Fig. 7D). There were no differences in the expression of other genes thought to be important for urine concentration and the maintenance of medullary integrity (Table 4).

Table 3. Affect of dDAVP on the response to water deprivation

<table>
<thead>
<tr>
<th></th>
<th>2 mo</th>
<th>6 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C57BL/6J</td>
<td>Hsd11b2-/-</td>
</tr>
<tr>
<td>UV, ml/24 h</td>
<td>0.4 ± 0.1</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>UOsm, mosmol/kgH2O</td>
<td>6,928 ± 1,327</td>
<td>2,923 ± 389*</td>
</tr>
<tr>
<td>Water balance, ml/24 h</td>
<td>-0.4 ± 0.1</td>
<td>-0.6 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. UV, urine flow; UOsm, urine osmolality; dDAVP, desmopressin. Cumulative water balance was calculated as the difference between water intake and urine flow over 24 h. *P < 0.01 compared with age-matched controls, †P < 0.001 compared with younger mice of the same genotype.
URINE CONCENTRATION IN Hsd11b2 NULL MICE

Fig. 3. Effect of desmopressin (dDAVP) on urine flow in C57Bl/6J (white bars) and Hsd11b2−/− (black bars) mice aged >6 mo. Measurements were made over 6 h during a baseline period and following dDAVP. Values are means ± SE. ***P < 0.001 compared with C57Bl/6J controls. ###P < 0.05 compared with pre-dDAVP values in the same mice.

Fig. 4. Aquaporin-2 (AQP2) protein response to a water deprivation challenge in C57Bl/6J (open bars) and Hsd11b2−/− (filled bars) mice aged 2 and >6 mo. Values are expressed as a percentage of the mean value from hydrated kidney blots and are means ± SE. Inset: typical gel from >6 mo C57Bl/6J kidneys. Hydrated kidneys are on the left of the ladder, dehydrated on the right.

DISCUSSION

11βHSD2 governs ligand access to MR (21), and acute pharmacological inhibition of the enzyme promotes the reabsorption of sodium and water (7, 11). In contrast, chronic administration of 11βHSD2 inhibitors increases urine flow rate, but only when given orally (24). Intracerebroventricular delivery does not affect urine flow rate (24), indicating that polyuria observed in patients with AME or Hsd11b2−/− mice is of renal origin. This increased water turnover must relate directly, or indirectly, to a functional loss of 11βHSD2 activity. Here, we discuss three interrelated possibilities: first, that sustained activation of MR underpins the molecular changes associated with polyuria, either directly, or as a consequence of potassium depletion; second, that polyuria is programmed by perinatal exposure to glucocorticoids due to repression (23). A volume-dependent pathway is unlikely here, since 11βHSD2 is expressed in these segments, illicit activation of MR appears a plausible mechanism for downregulation of aquaporin and slc14a2, which encode distal nephron water channels, and reduced slc14a2, which encodes for two proteins, UT-A1 and UT-A3, that permit equilibration of urea between the lumen and the surrounding interstitium in the inner medullary collecting duct (IMCD) (20). The downregulation of urea transporters and increase in urea excretion observed in Hsd11b2−/− mice would cause an obligate diuresis (34). Indeed, urea-induced diuresis has been demonstrated experimentally in UT-A1/−/− mice, which, like Hsd11b2−/− mice, present with basal polyuria and polydipsia and have an impaired response to water deprivation (19). In the Hsd11b2−/− mice, this might be an allostatic response to hypertension, since increased sodium excretion would contribute to the normalization of natriuretic capacity that occurs at approximately this age (6).

Chronic exposure to aldosterone decreases both AQP2 expression in the cortical collecting duct (14) and UT-A expression in the IMCD (23). Since 11βHSD2 is expressed in these segments, illicit activation of MR appears a plausible mechanism for downregulation of aquaporin and slc14a2 in the present study. However, the effects of chronic MR activation on water transport are not consistent (29), and the effect on UT-A is dependent on volume expansion (49) rather than transcriptional repression (23). A volume-dependent pathway is unlikely here, since Hsd11b2−/− mice are chronically volume contracted (6).

Intact adrenal function is important for the development of urine concentrating capacity at weaning (44), but sustained prenatal exposure to dexamethasone, which is not metabolized by 11βHSD2, reduces nephron number (41), impairs postnatal development of the outer medulla (48), and increases the expression of key transporters, including NKCC2 (13). Loss of placental 11βHSD2 would expose the embryo to maternal glucocorticoids in utero, but this does not appear to be a major factor in the current study: glomeruli number is not reduced, the kidneys appear structurally normal, and, at 2 mo of age, expression of slc12a1 is comparable to controls. The glucocorticoid receptor (GR) may, however, play a more direct role in the polyuric phenotype. Recent data indicate that 11βHSD2

Table 4. mRNA abundance of medullary genes involved in urine concentration

<table>
<thead>
<tr>
<th>Gene</th>
<th>C57Bl/6J 2 mo</th>
<th>Hsd11b2−/− 2 mo</th>
<th>C57Bl/6J 6 mo</th>
<th>Hsd11b2−/− 6 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>AQP2</td>
<td>100 ± 20.5</td>
<td>33.3 ± 4.8***</td>
<td>100 ± 10.1</td>
<td>49.1 ± 8.6‡</td>
</tr>
<tr>
<td>AQP3</td>
<td>100 ± 5.0</td>
<td>60 ± 4.0***</td>
<td>100 ± 6.3</td>
<td>48.5 ± 6.5‡</td>
</tr>
<tr>
<td>AQF4</td>
<td>100 ± 14.4</td>
<td>111.5 ± 12.6</td>
<td>100 ± 7.6</td>
<td>37.3 ± 6.3‡</td>
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<tr>
<td>Aqp2a</td>
<td>100 ± 4.3</td>
<td>106.9 ± 8.4</td>
<td>100 ± 12.3</td>
<td>86 ± 12.1</td>
</tr>
<tr>
<td>Aqp1a</td>
<td>100 ± 6.8</td>
<td>142.1 ± 15.1</td>
<td>100 ± 16.9</td>
<td>77 ± 13.6</td>
</tr>
<tr>
<td>NKCC2</td>
<td>100 ± 2.1</td>
<td>104.7 ± 9.6</td>
<td>100 ± 8.8</td>
<td>47.8 ± 8.6‡</td>
</tr>
<tr>
<td>UT-A</td>
<td>100 ± 31.2</td>
<td>50.1 ± 11.9</td>
<td>100 ± 25.5</td>
<td>29.6 ± 12.6*</td>
</tr>
<tr>
<td>MR</td>
<td>100 ± 10.9</td>
<td>100.9 ± 8.8</td>
<td>100 ± 6.2</td>
<td>109.2 ± 15.7</td>
</tr>
<tr>
<td>TonEBP</td>
<td>100 ± 4.7</td>
<td>98.1 ± 6.8</td>
<td>100 ± 7.5</td>
<td>101.4 ± 15.9</td>
</tr>
<tr>
<td>HIF-1α</td>
<td>100 ± 3.1</td>
<td>97.0 ± 6.8</td>
<td>100 ± 11.4</td>
<td>84.1 ± 12.6</td>
</tr>
<tr>
<td>Caspase-3</td>
<td>100 ± 4.2</td>
<td>115.8 ± 8.6</td>
<td>100 ± 7.0</td>
<td>76.8 ± 9.1</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed as a percentage of the mean value recorded in the age matched C57Bl/6J mice. UT, urea transporter; MR, mineralocorticoid receptor; TonEBP, tonicity-enhanced binding protein. *P < 0.05 †P < 0.01, ‡P < 0.001 compared with age-matched controls.
also governs ligand access to GR (4, 22), and glucocorticoids have long been known to increase the urinary excretion of urea (31) by inhibiting UT-A expression in the IMCD (37) following GR-mediated transcriptional repression of \( \text{slc14a2} \) (20, 42).

Dietary potassium depletion reduces medullary osmolarity (9) and the expression of AQP2 (36), NKCC2 (1), and UT-A (30). Potassium depletion also reduces the abundance of tonicity-enhanced binding protein (NFAT5) (28), a transcriptional activator critical for the development of the renal medulla (35). These changes occur in parallel with the development of polyuria, suggesting a causal link. Despite evidence of hypokalemia, severe potassium depletion can be discounted as an important mechanism for increased water turnover in the younger \( \text{Hsd11b2}^-/- \) mice: the expression of \( \text{aqp2}, \text{aqp3}, \text{and slc14a2} \) was normal, and medullary hypertonicity was sufficient to permit concentration of urine to \( \sim 5,000 \) mosmol/kgH\(_2\)O following water deprivation. Sustained potassium depletion does cause renal insensitivity to AVP (10, 45), and this may contribute to the development of diabetes insipidus, discussed below, as \( \text{Hsd11b2}^-/- \) mice age.

Although the reduced abundance of mRNA encoding \( \text{aqp2}, \text{aqp3}, \text{and slc14a2} \) is consistent with a nephrogenic diabetes insipidus in \( \text{Hsd11b2}^-/- \) mice at \( \sim 2 \) mo, these mice have a full concentration response to water deprivation indicative of a functional AVP axis. In addition, the demonstration that \( \text{avpr}2 \) abundance was comparable in the null and control mice suggests that the polyuria is not the result of reduced AVP binding at \( \text{avpr}2 \), but rather impaired signaling downstream of the receptor. The increased abundance of \( \text{avpr}1a \) suggests an intriguing alternative hypothesis. AVP stimulation of vasopressin 1a receptors (V\(_1\alpha\)R) located on the luminal membrane of the collecting duct has been shown to blunt the antidiuretic effects of AVP at V\(_2\)R, at a postreceptor level (8). Therefore, the increased mRNA abundance of \( \text{avpr}1a \) at this age may con-
tribute to the renal insensitivity to AVP’s antidiuretic effects, and subsequently the development of polyuria.

In the older \textit{Hsd11b2^{−/−}} mice, which had an impaired concentration response, the water deprivation challenge did not substantially increase the expression of AQP2 at the protein level. These data indicate either a failure to increase central production of AVP or insensitivity to the hormone at the renal level. The abundance of mRNA encoding \textit{avpr2} and \textit{avpr1a} was comparable to controls. In combination with the reduced abundance of \textit{slc14a2, aqp2, aqp3}, and \textit{aqp4}, this suggests reduced plasma AVP in the older \textit{Hsd11b2^{−/−}} mice and a failure of the homeostatic response to volume contraction. This was not measured in the current study: physiological measurements in mice are difficult since stress induces a rapid release of AVP from the posterior pituitary. This challenge is compounded in \textit{Hsd11b2^{−/−}} mice by a life-long anxiety phenotype (27). However, since the administration of dDAVP during the water deprivation challenge did not further increase the urine osmolality in \textit{Hsd11b2^{−/−}} mice, our experimental data are indicative of nephrogenic diabetes insipidus.

Fig. 6. Dendrimer contrast MRI analysis of gross renal structure. \(A\): 6 mo C57BL/6J mouse. \(B\): 6 mo \textit{Hsd11b2^{−/−}} mouse.

Fig. 7. Renal function in C57BL/6J (open bars) and \textit{Hsd11b2^{−/−}} (filled bars) mice. \(A\): change in urine flow. \(B\): change in urinary sodium excretion. \(C\): basal glomerular filtration rate. \(D\): urinary urea excretion. \(***P < 0.001, *P < 0.05\) compared with C57BL/6J controls.
The development of renal insensitivity to AVP is perhaps unsurprising since we consistently observed extensive medullary atrophy in Hsd11b2−/− mice at ~6 mo of age. A similar phenotype is observed in mice with a null mutation in nfat5 (35), but mRNA abundance for this gene was not altered by the deletion of Hsd11b2. Similarly, the expression of caspase-3 was not increased, indicating that atrophy was not the result of hypertonicity-driven apoptosis. We also discounted the hypothesis that compression of peritubular capillaries, following

was not increased, indicating that atrophy was not the result of
deletion of

(35), but mRNA abundance for this gene was not altered by the

expression of

tubular distension (40), caused hypoxia within the medulla: the

hypothesis that compression of peritubular capillaries, following

speculate that albuminuria contributes to the morphological

MR-dependent, pressure-independent mechanisms (43). We

In summary, our studies suggest that loss of 11βHSD2 (12). Chronicaldo-
sterope also causes cellular senescence and impairs renal wound healing (17). Microalbuminuria was observed in the younger Hsd11b2 null mice, suggesting that incipient damage preceded the gross structural changes. Some of the injury and renal remodeling may therefore reflect sustained hypertensive barotrauma. Nevertheless, glucocorticoid administration induces glomerulosclerosis, podocyte injury, and albuminuria by MR-dependent, pressure-independent mechanisms (43). We speculate that albuminuria contributes to the morphological changes observed in the kidneys of Hsd11b2 null mice. First, albuminuria stimulates proliferation of the proximal tubule (25), which would contribute to the cortical expansion observed here. Second, shedding of large amounts of protein into the tubule lumen could obstruct the urinary tract, leading to

dhmonerophosis.

In summary, our studies suggest that loss of 11βHSD2 function results in a nephrogenic diabetes insipidus phenotype, with chronic overactivation of MR causing a renal insensitivity to AVP. AVP acting via the V1R is a determinant of blood pressure (2) and therefore may be a contributory factor in the development of hypertension in Hsd11b2−/− mice as it is in other states of glucocorticoid excess (5). This is supported by the increased mRNA abundance of avpr1a at ~2 mo.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


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URINE CONCENTRATION IN Hsd11b2 NULL MICE


