A Switch in the Mechanism of Hypertension in the Syndrome of Apparent Mineralocorticoid Excess

Matthew A. Bailey, Janice M. Paterson, Patrick W.F. Hadoke, Nicola Wrobel, Christopher O.C. Bellamy, David G. Brownstein, Jonathan R. Seckl, and John J. Mullins

University of Edinburgh, Centre for Cardiovascular Science, Queen's Medical Research Institute, Edinburgh, Scotland, United Kingdom

ABSTRACT

The syndrome of apparent mineralocorticoid excess arises from nonfunctional mutations in 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2), an enzyme that inactivates cortisol and confers aldosterone specificity on the mineralocorticoid receptor. Loss of 11βHSD2 permits glucocorticoids to activate the mineralocorticoid receptor, and the hypertension in the syndrome is presumed to arise from volume expansion secondary to renal sodium retention. An 11βHSD2 null mouse was generated on an inbred C57BL/6J genetic background, allowing survival to adulthood. 11βHSD2$^{-/-}$ mice had BP approximately 20 mmHg higher on average compared with wild-type mice but were volume contracted, not volume expanded as expected. Initially, impaired sodium excretion associated with increased activity of the epithelial sodium channel was observed. By 80 days of age, however, channel activity was abolished and 11βHSD2$^{-/-}$ mice lost salt. Despite the natriuresis, hypertension remained but was not attributable to intrinsic vascular dysfunction. Instead, urinary catecholamine levels in 11βHSD2$^{-/-}$ mice were double those in wild-type mice, and α1-adrenergic receptor blockade rescued the hypertensive phenotype, suggesting that vasoconstriction contributes to the sustained hypertension in this model. In summary, it is proposed that renal sodium retention remains a key event in apparent mineralocorticoid excess but that the accompanying hypertension changes from a renal to a vascular etiology over time.

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In 90% of hypertensive patients, the causal pathophysiology cannot be defined and, in 25%, BP fails to fall to target values with existing therapies. Improved understanding of pathogenesis is key to addressing this problem. Fundamental principles of BP homeostasis have been established through investigation of the rare Mendelian hypertensive disorders. These disorders result almost exclusively from mutations in genes encoding proteins influencing directly or indirectly sodium balance,[1] suggesting that altered renal salt homeostasis is a key factor in the misregulation of BP[2]

The fine-tuning of sodium balance is achieved in the distal nephron, determined principally by the actions of aldosterone at the mineralocorticoid receptor (MR). *In vitro*, MR can be activated with equal potency by both mineralocorticoids and glucocorticoids.[3] *In vivo*, ligand access is determined by the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2), which catalyzes the rapid conversion of the active glucocorticoid cortisol (corticosterone in mice) to inert cortisone (11-dehydrocorticosterone), thereby

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M.A.B. and J.M.P. contributed equally to this work.

Correspondence: Prof. John J. Mullins, Centre for Cardiovascular Science, The University of Edinburgh, QMRI, 47, Little France Crescent, Edinburgh, EH16 4TJ, UK. Phone: +44-131-242-6724; Fax: +44-131-242-6782; E-mail: j.mullins@ed.ac.uk

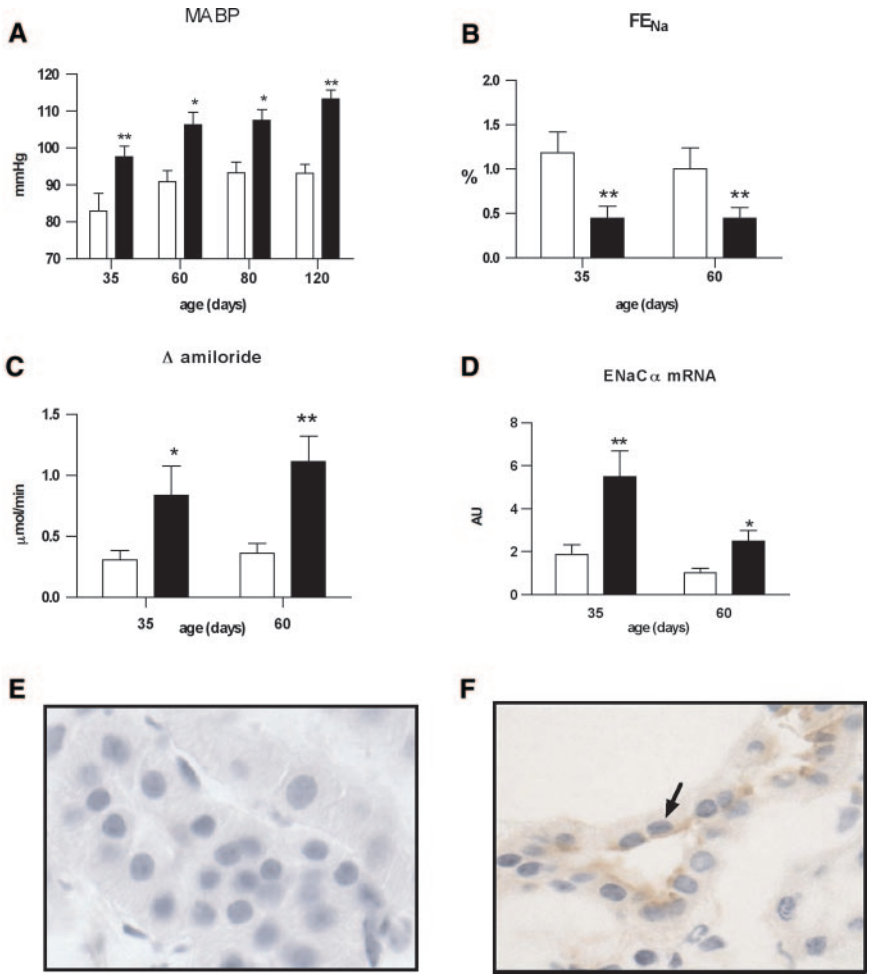

Figure 1. (A) MABP in 11βHSD2$^{-/-}$ mice (■) and age-matched C57BL/6J mice (□). Mice were studied at 35 d (n = 6 controls, 7 11βHSD2$^{-/-}$), 60 d (7/7), 80 d (7/5), and 120 d (7/7) of age. (B) Fractional sodium excretion. (C) The absolute effect of amiloride on sodium excretion (Δ amiloride). (D) The renal expression of ENaC-α mRNA normalized to the expression of 18S RNA in 35- and 60-d-old 11βHSD2$^{-/-}$ mice and aged-matched C57BL/6J mice. Data are means ± SEM. Comparisons were made using ANOVA with Bonferroni *post hoc* test. *$P < 0.05$; **$P < 0.01$. The expression of ENaC-α protein was below the detection threshold in kidneys from C57BL/6 mice (E), consistent with a previous report, but was localized to the apical membrane in the CCD of 60-d-old 11βHSD2$^{-/-}$ mice (F).

conferring on MR the specificity to aldosterone that it inherently lacks.[4,5] In the kidney, 11βHSD2 is expressed in the connecting tubule and in the principal cells of the cortical (CCD) and outer medullary collecting duct.[6] Acute pharmacologic inhibition of the enzyme promotes collecting duct sodium reabsorption[7] *via* increased activity of the epithelial sodium channel (ENaC).[8]

Inactivating mutations in the gene *HSD11B2*, which encodes 11βHSD2, cause the syndrome of apparent mineralocorticoid excess (SAME; OMIM #218030). In this setting, cortisol activates MR,[9–12] resulting in severe hypertension thought to arise from volume expansion secondary to renal sodium retention.[9,11,13,14] Suppression of cortisol by administration of synthetic glucocorticoids that do not activate MR is therapeutically effective,[12] and in one case, full correction of the disorder was achieved by renal transplantation,[15] highlighting the central role of the kidney in SAME.

These human genetic studies suggest that 11βHSD2 is a physiologic regulator of BP, but investigation of key mechanisms is not possible in patients because the disease is rare and most patients are children. We originally modeled SAME by targeted disruption of the 11βHSD2 locus in an outbred (MF1) mouse strain,[16] but the high neonatal mortality permitted only limited physiologic examination. We have now transferred the targeted mutation onto the C57BL/6J genetic background, which attenuates the neonatal mortality while retaining the cardinal features of the disease. Using a time course stretching from weaning to early adulthood, we have identified key mechanistic events in the development and maintenance of hypertension in SAME.

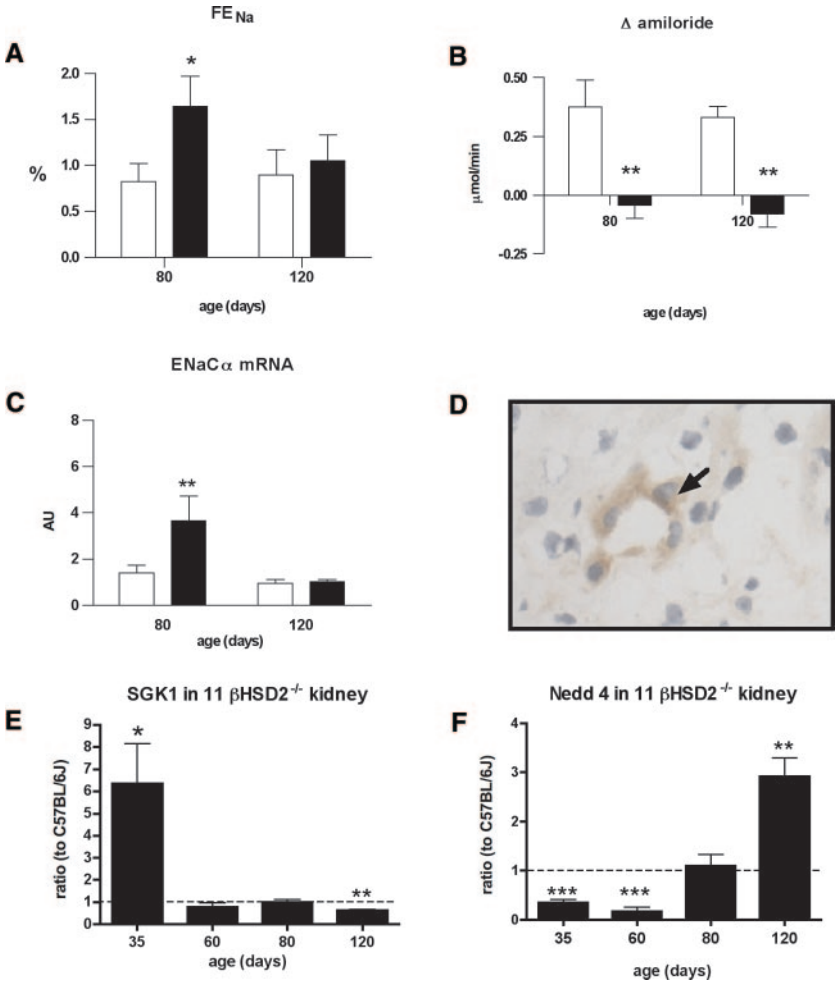

Figure 2. (A) Fractional sodium excretion in 80-d-old (7/5) and 120-d-old (7/7) 11βHSD2$^{-/-}$ mice (■) and age-matched C57BL/6J mice (□) (B) The absolute effect of amiloride on sodium excretion (Δ amiloride). (C) The renal expression of ENaC-α mRNA normalized to the expression of 18S RNA. (D) Expression of ENaC-α protein in the CCD of 120-d-old 11βHSD2$^{-/-}$ mice, showing that immunoreactivity is predominately cytoplasmic. Western analysis shows the relative expression of SGK1 (E) and Nedd4 (F) in the kidney ($n = 6$ for each group) of 11βHSD2$^{-/-}$ mice, as a ratio of that in kidneys of age-matched C57BL/6J mice. The dotted line shows the level of equal expression. Protein quantification was by densitometry. All data are means ± SEM. Comparisons were made using ANOVA with Bonferroni *post hoc* test or, for E and F, a one-sample *t* test assuming a sample mean of 1.0. *$P < 0.05$; **$P < 0.01$.

RESULTS

Sustained Hypertension but Transient Sodium Retention in 11βHSD2$^{-/-}$ Mice

11βHSD2$^{-/-}$ mice had elevated mean arterial BP (MABP) at each of the four time points studied (Figure 1A). Fractional sodium excretion was reduced by >50% in 11βHSD2$^{-/-}$ mice at 35 and 60 d of age (Figure 1B), during which time amiloride-sensitive sodium reabsorption was doubled (Figure 1C). Quantitative PCR showed increased renal expression of ENaC-α mRNA in 35- and 60-d-old 11βHSD2$^{-/-}$ mice (Figure 1D). Expression of ENaC-β and ENaC-γ were higher in 11βHSD2$^{-/-}$ mice than in controls, although this reached statistical significance only for ENaC-γ ($P < 0.01$; data not shown). The expression of MR was similar in both groups. ENaC-α immunoreactivity in the CCD and outer medullary collecting duct, not detectable in the C57BL/6J mice at any age (Figure 1E),[17] was evident in 60-d-old 11βHSD2$^{-/-}$ mice, demonstrating increased protein abundance. Strong apical membrane localization was observed (Figure 1F). At 80 d of age, 11βHSD2$^{-/-}$ mice exhibited natriuresis, with fractional sodium excretion being normalized by 120 d (Figure 2A). The mechanism of escape was identified: the natriuretic impact of amiloride was lost in 80- and 120-d-old 11βHSD2$^{-/-}$ mice (Figure 2B). The downregulation of functional channel activity does not rely principally on attenuation of transcriptional or translational processes because both ENaC-α mRNA levels (Figure 2C) and renal immunoreactivity remained higher in 11βHSD2$^{-/-}$ mice than in controls (Figure 2D).

Altered ENaC Trafficking in 11βHSD2$^{-/-}$ Mice

In 120-d-old 11βHSD2$^{-/-}$ mice, ENaC-α immunoreactivity

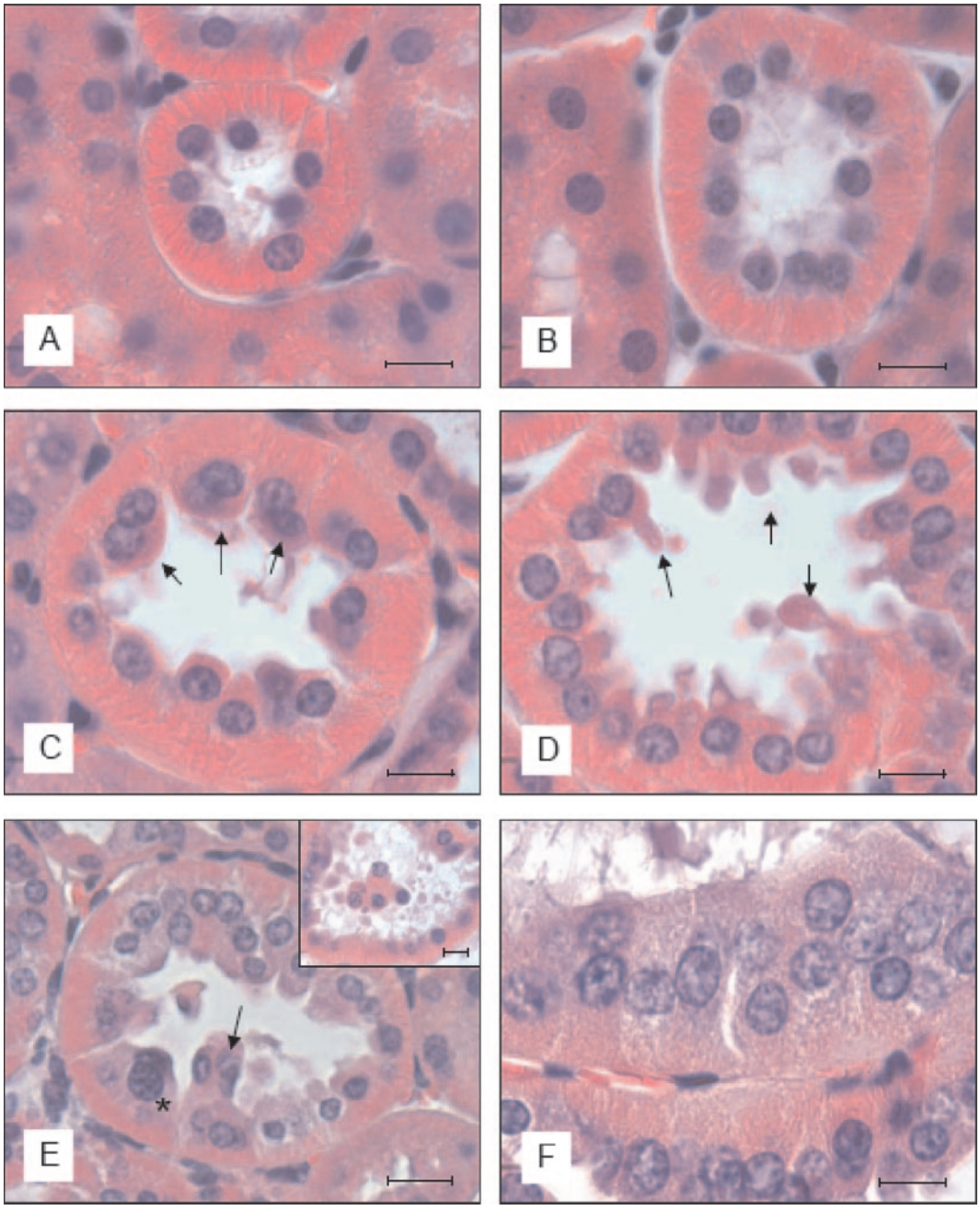

Figure 3. Temporal progression of epithelial cytopathology in distal convoluted tubules (DCT) of 11βHSD2$^{-/-}$ mice. (A) Control C57BL/6J mouse (60 d old). The cuboidal epithelium has apical nuclei and basal striations. (B) A 35-d-old 11βHSD2$^{-/-}$ mouse. The epithelium is columnar, and the number of cross-sectional nuclei is increased, indicative of hypertrophy and hyperplasia, respectively. (C) A 60-d-old 11βHSD2$^{-/-}$ mouse. In addition to epithelial hypertrophy and hyperplasia, there are multiple binucleate cells (arrows). (D) A 60-d-old 11βHSD2$^{-/-}$ mouse. Apical cytoplasmic blebs project into the lumen (arrows). (E) A 120-d-old 11βHSD2$^{-/-}$ mouse. Epithelial nuclei are irregular as a result of crowding and piling. A large hyperchromatic nucleus (*) suggests polyploidy. A cluster of epithelial cells projects into the lumen apparently with minimal contact with the basement membrane (arrow). (E, inset) A detached raft of epithelial cells lies in the tubule lumen. (F) A 120-d-old 11βHSD2$^{-/-}$ mouse. In addition to striking hypertrophy and irregular nuclear placement within the cells, there is loss of basal striations and granular cytoplasmic aggregates indicative of subcellular disorganization. Hematoxylin and eosin stain; Bars = 10 μm.

was displaced from the apical membrane of the collecting duct principal cells (Figure 2D), suggesting that altered channel trafficking underpins the transition from a sodium-retaining to sodium-shedding state. Western blotting analysis was therefore used to measure the renal abundance of two key ENaC trafficking proteins[18]: Serum glucocorticoid regulated kinase 1

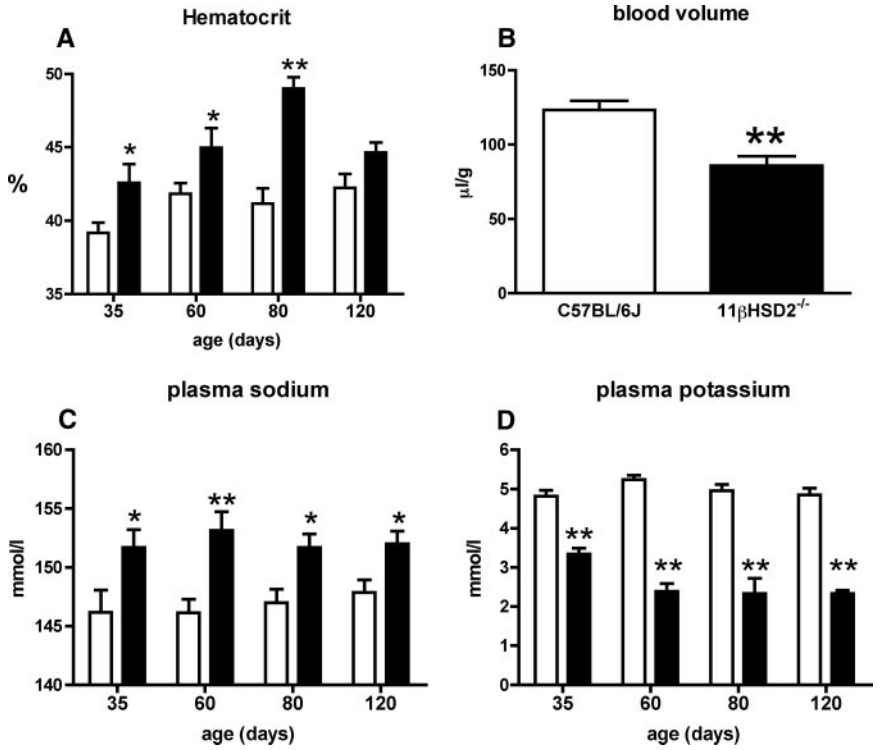

Figure 4. (A) Hematocrit in 11βHSD2$^{-/-}$ mice (■) and age-matched C57BL/6J mice (□). Mice were studied at 35 d (n = 6 controls, 7 11βHSD2$^{-/-}$), 60 d (7/7), 80 d (7/5), and 120 d (7/7) of age. (B) Blood volume in 80-d-old 11βHSD2$^{-/-}$ mice (n = 7) and C57BL/6J mice (n = 6), normalized per gram of body weight. (C) Plasma sodium. (D) Plasma potassium. Data are means ± SEM, and comparisons were made using either t test or ANOVA with a Bonferroni *post hoc* test. *$P < 0.05$; **$P < 0.01$.

(SGK1), which promotes insertion of the ENaC into the apical membrane of the principal cell; and neural precursor cell–expressed, developmentally downregulated protein type 4 (Nedd4), which causes channel removal by ubiquitination.[18] SGK1 expression was approximately seven-fold higher in 11βHSD2$^{-/-}$ mouse kidney ($P < 0.01$) at 35 d of age (Figure 2E). Levels of Nedd4 were significantly reduced in 11βHSD2$^{-/-}$ mice at both 35 and 60 d of age ($P < 0.01$; Figure 2F). At 80 d, expression of both proteins was comparable between groups; however, by 120 d of age, renal expression of SGK1 was reduced whereas that of Nedd4 was enhanced in 11βHSD2$^{-/-}$ mice.

11βHSD2$^{-/-}$ Mice Exhibit Progressive Cytopathology of the Distal Convoluted Tubule

In 35-d-old 11βHSD2$^{-/-}$ mice, the normally cuboidal (Figure 3A) epithelial cells of the distal nephron were columnar and the number of cross-sectional nuclei was increased, indicative of hypertrophy and hyperplasia, respectively (Figure 3B). From this time point, through 60 d (Figure 3, C and D), 80 d and 120 d (Figure 3E), there was progressive epithelial hypertrophy and hyperplasia with increasing prevalence of binucleation and hyperchromatic nuclei (indicative of polyploidy; Figure 3E). Many severely hypertrophied cells had loss of basal striations and had extensive accumulations of basophilic granules throughout the cytoplasm, changes suggestive of subcellular disorganization (Figure 3F). These marked changes of the renal epithelium were limited to the distal convoluted tubule: The segments of the collecting duct appeared anatomically normal during the course of study.

Volume-Contracted Hypertension in 11βHSD2$^{-/-}$ Mice

Renal sodium retention was not associated with volume expansion. Indeed, hematocrit was significantly increased in all cohorts of 11βHSD2$^{-/-}$ mice, indicating plasma volume contraction (Figure 4A). Blood volume was measured directly in a separate group of mice, and the approximately 30% reduction in 11βHSD2$^{-/-}$ mice confirmed (Figure 4B). The renin-angiotensin-aldosterone system, normally activated with plasma volume contraction, was suppressed: Plasma aldosterone was significantly lower in 11βHSD2$^{-/-}$ mice (Table 1) as was kidney renin mRNA, levels being <30% of controls in all age groups ($P < 0.05$). Plasma corticosterone concentrations were comparable between genotypes at all time points (Table 1). There was a significant hypernatremia (Figure 4C) and elevated plasma osmolality (Table 1) at all time points studied. Despite this, urine flow rate was approximately doubled and urine osmolality markedly reduced in 11βHSD2$^{-/-}$ mice (Table 1). The increase in free water clearance in 11βHSD2$^{-/-}$ mice (Table 1), inappropriate given the hypertonicity, is suggestive of a urine-concentrating defect. This may result from the progressive hypokalemia observed in

Table 1. Plasma and urine osmolality (mOsm/kg H_2O); free water clearance (μ l/min); and plasma concentrations of potassium (mmol/L), aldosterone (nmol/L), and corticosterone (nmol/l) in four cohorts of 11βHSD2$^{-/-}$ and C57BL/6J mice[a]

Parameter	Age (d)			
	35	60	80	120
P_{Osm}				
C57BL/6J	305 ± 3	304 ± 3	312 ± 2	306 ± 7
11βHSD2$^{-/-}$	315 ± 3[b]	311 ± 2[b]	313 ± 4	295 ± 2
U_{Osm}				
C57BL/6J	1801 ± 116	1630 ± 240	2007 ± 170	1572 ± 75
11βHSD2$^{-/-}$	779 ± 67[c]	647 ± 45[c]	604 ± 99[c]	609 ± 40[c]
FWC				
C57BL/6J	−5.9 ± 0.9	−5.8 ± 0.7	−3.6 ± 0.6	−4.1 ± 0.5
11βHSD2$^{-/-}$	−3.1 ± 0.7[b]	−3.2 ± 0.6[b]	−2.2 ± 0.6[b]	−1.6 ± 0.3[c]
P_{Aldo}				
C57BL/6J	1.57 ± 0.24	1.60 ± 0.02	1.31 ± 0.18	1.07 ± 0.09
11βHSD2$^{-/-}$	0.63 ± 0.12[c]	0.44 ± 0.07[c]	0.69 ± 0.07[c]	0.45 ± 0.05[c]
P_{Cort}				
C57BL/6J	374 ± 90	323 ± 20	338 ± 80	NA
11βHSD2$^{-/-}$	286 ± 58	289 ± 35	242 ± 22	NA

[a]Data are means ± SE. Statistical comparisons were made using ANOVA with least significant difference *post hoc* test. ANOVA indicated significant effect of genotype in all four variables. There was no effect of age per se for any variable, but there was a significant interaction between age and genotype for U_{Osm} ($P < 0.01$), FWC ($P < 0.01$), and P_K ($P < 0.01$). FWC, free water clearance; P_{Aldo}, plasma concentration of aldosterone; P_{Cort}, plasma concentration of corticosterone; P_K, plasma concentration of potassium; P_{Osm}, plasma osmolality; U_{Osm}, urine osmolality

[b]$P < 0.05$.

[c]$P < 0.01$.

11βHSD2$^{-/-}$ mice (Figure 4D): Fractional potassium excretion was elevated in 11βHSD2$^{-/-}$ mice at 35 d (39.8 ± 4.9 *versus* 22.1 ± 3%; $P < 0.05$) and comparable to controls from 60 d onward (approximately 20% in both groups).

No Evidence for Intrinsic Vascular Dysfunction in 11βHSD2$^{-/-}$ Mice

We assessed endothelium-dependent vasodilation and sensitivity to catecholamines of sections of thoracic aorta from 11βHSD2$^{-/-}$ mice or age-matched controls. The effects of acetylcholine and noradrenaline in each of the four age groups are shown if Figure 5. The maximum response to acetylcholine was reduced in 11βHSD2$^{-/-}$ mice only in the 60-d-old group (Figure 5B, Table 2). Because vasodilator responses to sodium nitroprusside in 11βHSD2$^{-/-}$ mice were comparable to those in controls during this study (Table 2), the reduced response to acetylcholine at 60 d of age relates to endothelial cell dysfunction, rather than altered sensitivity to nitric oxide. The maximum response and sensitivity to noradrenaline was not increased in 11βHSD2$^{-/-}$ null mice at any age (Figure 5, Table 2). Indeed, there is trend toward a *reduction* in noradrenaline-mediated contraction in the 35- and 60-d-old groups (Figure 5, A and B).

11βHSD2$^{-/-}$ Mice Have Increased Catecholamine Levels

In the absence of increased sensitivity to catecholamines of the vasculature *ex vivo*, urinary excretion of adrenaline and noradrenaline was measured and found to be substantially elevated in 11βHSD2$^{-/-}$ mice (Figure 6A). To assess directly the contribution of catecholamines to the maintenance of hypertension, we measured the impact on MABP of acute α1-adrenoreceptor blockade *in vivo*. A bolus of prazosin significantly reduced MABP in both groups of mice (>120 d of age; Figure 6, B and C, for sample traces), but the effect of the drug was substantially greater in the 11βHSD2$^{-/-}$ mice (−22.3 ± 1.4 *versus* −8.7 ± 1.1 mmHg; $n = 5/6$; $P < 0.001$). Importantly, α1-adrenoreceptor blockade rescued the hypertensive phenotype in 11βHSD2$^{-/-}$ mice (Figure 6D).

DISCUSSION

SAME is characterized by unregulated glucocorticoid activation of MR after ablation of 11βHSD2.[9–12] Hypertension in this disorder is thought to be renal in origin, relating to enhanced ENaC-mediated sodium reabsorption and volume expansion.[7,8,14] In this study, we used 11βHSD2$^{-/-}$ mice to model SAME and demonstrated that transient abnormal renal function can promote a chronic hypertensive cascade that is not sustained through volume expansion.

Initially, 11βHSD2$^{-/-}$ mice had an impaired ability to excrete sodium, relating to increased expression of ENaC subunits and enhanced activity of the functional channel shown by the increased natriuretic potency of amiloride. We also found increased expression of SGK1 and reduced expression of Nedd4 during this period, which combined would increase the membrane retention time of ENaC.[18] SGK1 is established as an early aldosterone response gene in the kidney.[19] In contrast, Nedd4 protein levels are not effected by acute administration of aldosterone[20,21]; however, our findings and those in a recent

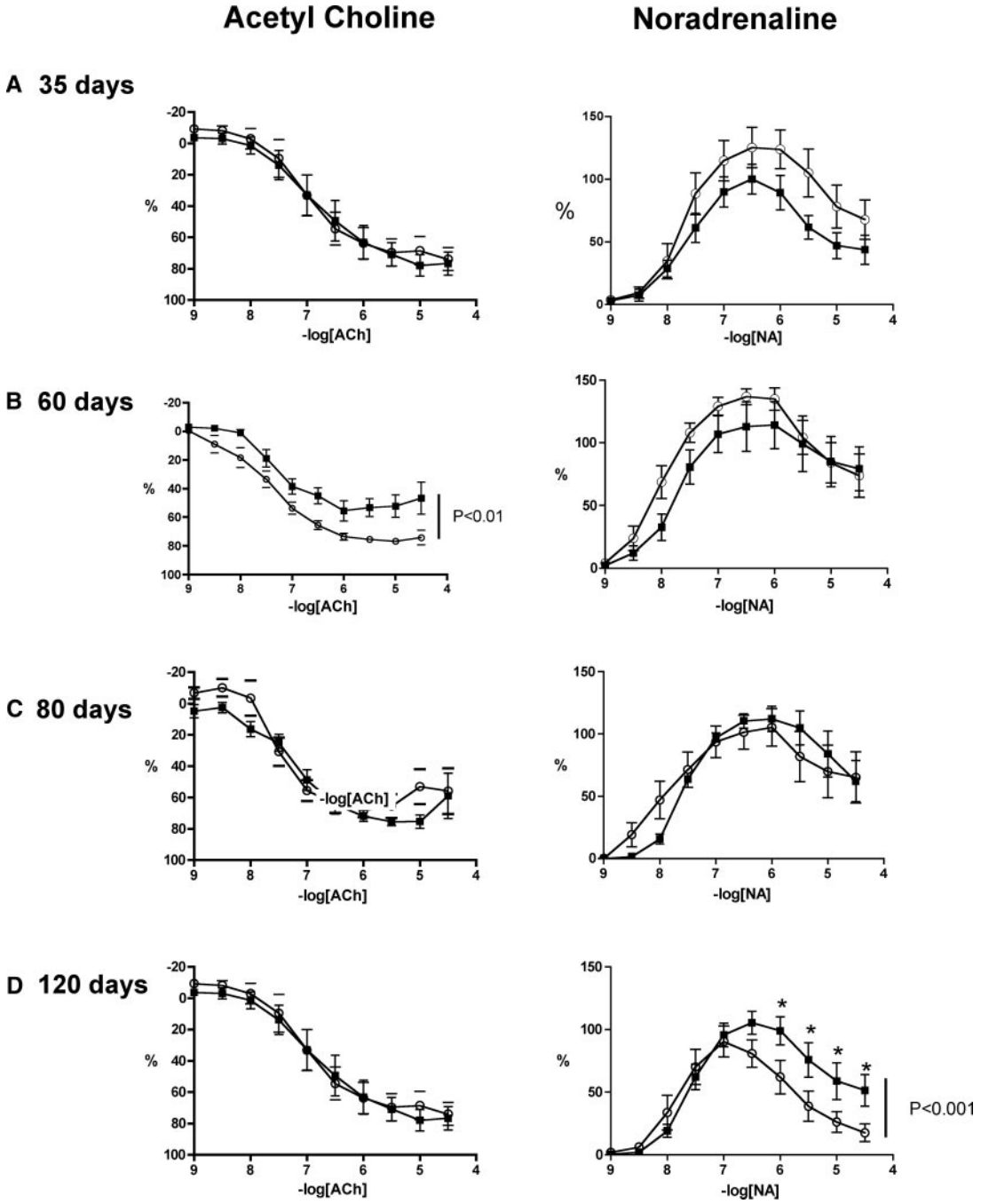

Figure 5. Vascular contractility in aortic rings from 11βHSD2$^{-/-}$ mice (■) and age-matched C57BL/6J mice (○). Mice were studied at 35 (A), 60 (B), 80 (C), and 120 d of age (D) with six mice per genotype used at each time point. Concentration-response curves to acetylcholine are shown on the left and to noradrenalin on the right. Data are means ± SEM of the percentage relaxation/contraction to the response induced by 5-hydroxytryptamine/potassium chloride, respectively. Comparisons were made using either *t* test or ANOVA with a Bonferroni *post hoc* test. $^{**}P < 0.01$.

study[22] indicate that chronic activation of MR leads to decreased collecting duct expression of Nedd4-2.

A key finding of our study is that the kidney is able to circumvent a genetic obligation for avid sodium reabsorption. By the 80-d time point, 11βHSD2$^{-/-}$ mice displayed enhanced sodium excretion. The escape from perceived mineralocorticoid excess does not rely on attenuation of transcriptional or translational processes because ENaC-α mRNA and CCD protein levels remained higher in 11βHSD2$^{-/-}$ mice than in controls during this period. Our data suggest that ENaC is rendered physiologically inactive because, in the CCD at least, it is not trafficked to the apical membrane. We have not confirmed

Table 2. Myography data from 11βHSD2$^{-/-}$ and aged-matched C57BL/6J mice[a]

Parameter	Age			
	35 (n = 6)	60 (n = 6)	80 (n = 6)	120 (n = 7)
Maximum response (EMax, mN/mm or % relaxation)				
NA				
C57BL/6J	1.84 ± 0.24	3.08 ± 0.35	2.34 ± 0.42	2.78 ± 0.41
11βHSD2$^{-/-}$	1.42 ± 0.20	2.53 ± 0.52	2.72 ± 0.28	2.23 ± 0.20
KCl				
C57BL/6J	1.65 ± 0.07	2.09 ± 0.10	2.22 ± 0.17	3.25 ± 0.34
11βHSD2$^{-/-}$	1.69 ± 0.23	2.56 ± 0.15	2.60 ± 0.07	2.43 ± 0.32
ACh				
C57BL/6J	74.0 ± 7.4	78.4 ± 2.2	74.0 ± 4.4	85.1 ± 3.5
11βHSD2$^{-/-}$	79.3 ± 6.7	63.5 ± 4.6[b]	78.8 ± 3.8	84.3 ± 6.1
SNP				
C57BL/6J	101.8 ± 4.4	102.6 ± 1.7	101.9 ± 1.6	103.0 ± 2.1
11βHSD2$^{-/-}$	102.7 ± 3.2	100.3 ± 3.2	99.1 ± 1.1	101.3 ± 1.3
Sensitivity (pD2; logIC_{50})				
NA				
C57BL/6J	7.75 ± 0.12	8.07 ± 0.13	7.91 ± 0.19	7.70 ± 0.18
11βHSD2$^{-/-}$	7.64 ± 0.18	7.82 ± 0.07	8.00 ± 0.03	7.54 ± 0.07
KCl				
C57BL/6J	1.79 ± 0.16	1.76 ± 0.19	1.79 ± 0.14	1.64 ± 0.08
11βHSD2$^{-/-}$	1.67 ± 0.08	1.60 ± 0.18	1.91 ± 0.27	1.45 ± 0.07
ACh				
C57BL/6J	7.03 ± 0.19	7.46 ± 0.14	7.53 ± 0.11	7.37 ± 0.14
11βHSD2$^{-/-}$	6.84 ± 0.24	7.25 ± 0.14	7.19 ± 0.15	7.30 ± 0.15
SNP				
C57BL/6J	7.78 ± 0.12	8.19 ± 0.17	8.05 ± 0.17	7.95 ± 0.12
11βHSD2$^{-/-}$	7.75 ± 0.09	7.86 ± 0.13	7.67 ± 0.11	8.04 ± 0.12

[a]Data are means ± SEM. Comparisons were made using t test. ACh, acetylcholine; KCl potassium chloride; NA, noradrenaline; SNP, sodium nitroprusside.
[b]P = 0.0134.

altered ENaC trafficking in either the late distal convoluted tubule or the connecting tubule. That the natriuretic impact of amiloride was lost, however, indicates that functional channel activity is abolished throughout the distal nephron. The mechanisms underpinning trapping in the cytosol may, in the longer term, involve an alteration in the balance between insertion and retrieval, but ENaC is functionally inactivated before any changes in expression of SGK1 or Nedd4 are apparent. The loss of functional ENaC explains why amiloride is only an effective means of long-term BP control[12,23] in SAME when used at the high dosages[24] required to inhibit proximal tubule sodium transport.[25] A generalized transport defect could be indicated, so extensive histopathologic examination of 11βHSD2$^{-/-}$ mouse kidneys was performed.

In some patients with SAME, the kidney is grossly normal,[10] but cysts, nephrocalcinosis, and hypertensive end-organ damage are seen in others, particularly as the disease progresses.[26] We showed progressive changes of the renal epithelium in 11βHSD2$^{-/-}$ mice, but, surprisingly, these were limited to the distal convoluted tubule: the segments of the collecting duct appeared anatomically normal. Because the mouse distal convoluted tubule does not express 11βHSD2,[6] these changes must be secondary to the deletion of the gene in other nephron segments. The structural changes are strongly reminiscent of those occurring in the rat after long-term exposure to loop diuretics such as furosemide.[27] We propose that impaired sodium reabsorption in the loop of Henle, secondary to potassium depletion, promotes a compensatory hypertrophy of the distal convoluted tubule. The use of thiazide diuretics to inhibit sodium chloride co-transport in this segment would therefore be specifically indicated for long-term BP regulation in SAME.[10,24] Our analysis suggests that downregulation of ENaC is not caused by gross changes in collecting duct structure.

A striking outcome of this study is the marked volume contraction observed in 11βHSD2$^{-/-}$ mice, even during the period of enhanced sodium reabsorption. Human[28] and animal studies[29] have consistently shown volume expansion in the early, prehypertensive stage of experimental mineralocorticoidism. In this situation, volume expansion increases cardiac output, initiating hypertension, which is then maintained in the adaptive phase by peripheral vasoconstriction.[30] In most forms of hypertension, peripheral resistance is high and blood volume is either normal or slightly low (reviewed by Hamlyn and Blaustein[29] and Cowley[31]) during the chronic phase. The volume contraction observed in 11βHSD2$^{-/-}$ mice may therefore represent the postadaptive period of hypertension, and we cannot exclude a period of volume expansion before 35 d of age.

To explain volume contraction in the face of sodium reten-

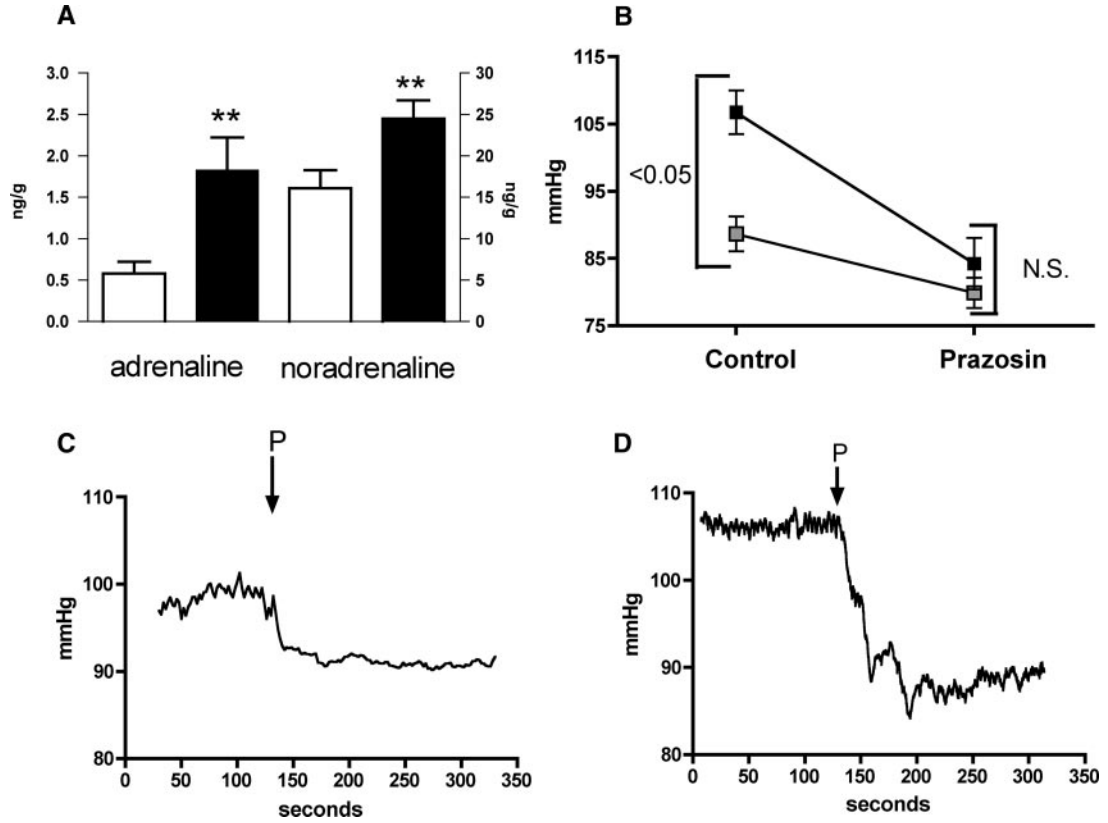

Figure 6. (A) Twenty-four-hour urinary excretion of adrenaline (left axis) and noradrenalin (right axis) in 120-d-old 11βHSD2$^{-/-}$ (■) mice and age-matched C57BL/6J mice (□), expressed as nanograms per gram of body weight (n = 10 in each group). (B) Effect of the α1-adrenoreceptor antagonist prazosin (100 μg/kg, intravenously) on MABP in 120-d-old 11βHSD2$^{-/-}$ mice (■; n = 5) and age-matched C57BL/6J mice (□; n = 6). Data are means ± SEM, and comparisons were made using either t test or ANOVA with a Bonferroni *post hoc* test. **$P < 0.01$. Sample BP recordings are shown for C57BL/6J mice (C) and 11βHSD2$^{-/-}$ mice (D).

tion, we have two connected hypotheses. The first is that sodium retention is "water-free," being held in osmotically inactive stores. This occurs in the DOCA-salt model of mineralocorticoid hypertension and minimizes the impact on extracellular fluid volume to buffer BP.[32] The second hypothesis is that water homeostasis is disturbed in 11βHSD2$^{-/-}$ mice, hydration being insufficient to expand or even maintain blood volume. The hypernatremia observed in 11βHSD2$^{-/-}$ mice throughout the experimental period supports the hypothesis but does not explain the phenomenon. Although 11βHSD2$^{-/-}$ mice are polyuric, most probably as a result of nephrogenic diabetes insipidus secondary to potassium depletion,[33] they are also polydipsic. It is unclear why a volume balance is not achieved: 11βHSD2-containing neurons in the nucleus of the solitary tract are stimulated by chronic hypovolemia,[34] and pharmacologic inhibition of the enzyme promotes thirst.[35] It may be that a fully operational renin-angiotensin system, suppressed in this model, is required to match precisely intake to output.

Although progressive potassium depletion models accurately the untreated human syndrome, it confounds elucidation of the hypertensive mechanism in 11βHSD2$^{-/-}$ mice. Potassium depletion can influence BP through various mechanisms[36,37]: Membrane depolarization promotes contraction of vascular smooth muscle, and vascular tone can be influenced indirectly by impaired endothelium-dependent vasodilation, increased sympathetic outflow, and depression of baroreceptor sensitivity. Similar effects are also found with hypernatremia,[38] a consistent feature in 11βHSD2$^{-/-}$ mice and one sustained beyond the downregulation of ENaC activity. Traditionally, sodium is considered the chief culprit in hypertension, with potassium being assigned a minor role; however, evidence from both human population studies, such as the DASH (Dietary Approaches to Stop Hypertension) or Intersalt trials, combined with experiments in animals models, suggest that an isolated derangement in one or the other is of lesser importance than their interaction.[36] In this study, the electrolyte disturbances seem inextricably linked: Enhanced sodium reabsorption in the distal nephron would stimulate potassium secretion, and the resulting potassium depletion would cause volume contraction and hypernatremia.

Because volume expansion does not seem to underpin hypertension in 11βHSD2$^{-/-}$ mice, we measured the intrinsic contractile properties of the conduit vasculature. Vascular tissue contains both glucocorticoid receptors and MR, activation of which can sensitize vascular smooth muscle to the effects of

vasopressors.[39] 11βHSD2 is localized to the vascular endothelium,[40] and inhibition of the enzyme increases vascular sensitivity to angiotensin II[41] and catecholamines.[42] Vascular reactivity to noradrenaline is enhanced in a patient with SAME,[43] and we have found significant endothelial dysfunction in the outbred MF1 mouse model of SAME at 120 d of age,[44] although not increased sensitivity to noradrenaline. In this study, however, we found only limited evidence for enhanced vascular contractility in 11βHSD2$^{-/-}$ mice, and this was transient. We conclude that changes in the intrinsic properties of the conduit vasculature do not make a major contribution to the development or maintenance of hypertension in this model of SAME. That the vascular phenotype is not observed on the C57BL/6J background implicates protective modifier loci that influence the physiologic function of the MR pathway.

Increased urinary excretion of catecholamines was observed in 11βHSD2$^{-/-}$ mice, which plausibly reflects increased sympathetic nerve activity.[45] Adrenoreceptor blockade was shown, at least acutely, to rescue the hypertensive phenotype. Extending this observation to the clinical situation, antiadrenergics, including the vasodilator dihydralazine, have been used to treat SAME in patients who do not respond to either MR blockade or cortisol suppression.[10] The mechanism underlying increased sympathetic activity is unknown but could result from either potassium depletion[36] or sodium retention.[38]

Our findings demonstrate that renal sodium retention is transient and amiloride-sensitive sodium reabsorption is downregulated in 11βHSD2$^{-/-}$ mice. In the chronic phase, hypertension is not sustained through either volume expansion or direct vascular dysfunction. We propose that the key hypertensive event is an electrolyte disturbance that activates the sympathetic nervous system, consistent with marked volume depletion.[29]

CONCISE METHODS

A congenic mouse strain was generated by backcross of the MF1 11βHSD2$^{-/-}$ mouse with C57BL/6J. All mice used in this study were obtained from homozygous null breeding after 12 generations of backcross onto C57BL/6J (Harlan, Bicester, UK). Age-matched C57BL/6J were used as controls. Following this extensive backcross, the congenic strain will be genetically >99.9% identical to the parent inbred strain.[46] Although we did not use littermate wild types as controls, it is highly likely that the phenotypes reported reflect the targeted disruption of *hsd11b2* contained in the non-C57BL/6J chromosomal fragment.

In a separate group of 12th-generation congenics, heterozygote intercrosses were used to demonstrate that the three genotypes (identified as described previously[16]) were present at birth in the normal Mendelian ratio. In contrast to the original outbred strain, the congenic 11βHSD2$^{-/-}$ mice exhibited no abnormal occurrence of neonatal death.

Four age groups of mice were used in this study: 35, 60, 80, and 120 (±3) d of age. The time points were selected to cover the maturation of renal function from weaning to adulthood, with the final time point being in the stable period of adult kidney function in the C57BL/6J mouse.[47] All experiments were performed in accordance with the UK Home Office (Scientific Procedures) Act 1986.

In Vivo Studies

Renal function experiments were performed as described previously.[48] Mice were infused intravenously with a saline solution containing 1% FITC-inulin at a rate of 0.2 ml/h per 10 g body wt. A 60-min equilibration period was followed by two consecutive periods (45 min each) of renal function measurements, sandwiched by collection of a 20-μl blood sample. After the first (control) collection, amiloride (2 mg/kg intravenously; Sigma, Poole, UK) was administered, and, after a 10-min equilibration period, a second urine collection was made. MABP was measured throughout (PowerLab; ADInstruments, Oxford, UK). At the end of the experiment, a 500-μl blood sample was taken for measurements of aldosterone and corticosterone, and the kidneys were removed.

In separate groups of mice, (*1*) plasma volume was determined by injection of Evans Blue (1 μl/g of a 0.5% wt/vol solution intravenously) and blood volume was calculated from plasma volume and hematocrit, (*2*) the effect of acute α1 adrenoreceptor blockade was measured by monitoring of the effect of a bolus of prazosin (0.1 mg/kg; Sigma) on MABP, and (*3*) 24-h urinary excretion of adrenalin and noradrenalin was measured by ELISA (CatCombi; IBL, Hamburg, Germany).

Histopathologic Analyses

Kidneys were immersion-fixed in 10% neutral buffered formalin for 48 h followed by paraffin embedding. Four-micron sections were stained with hematoxylin and eosin and examined by a specialist in rodent pathology (D.G.B.).

Protein Studies

Total protein was extracted from one half kidney by homogenization, separated by SDS-PAGE (10 μg of total protein per lane), and blotted to polyvinylidene difluoride membrane. Immunoblotting using rabbit polyclonal antibodies (Upstate, Lake Placid, NY) against either Nedd4 or SGK1 was performed according to the manufacturer's protocols, detecting a protein of 115 and 50 kD, respectively. Coomassie Blue staining of parallel samples was performed to determine equal protein loading. The localization of ENaC-α was determined by immunohistochemical staining on 5-μm sections of perfusion-fixed kidney from a separate group of age-matched mice, as described previously[17] using a polyclonal antibody provided by Dr. J. Loffing (University of Fribourg, Fribourg, Switzerland).

Quantitative PCR

Total RNA was prepared from whole kidneys, and mRNA of interest were quantified by commercial assays according to the manufacturer's instructions using an ABI 7700 (Applied Biosystems, Warrington, UK). Data were normalized to 18S rRNA on a sample-to-sample basis.

Aortic Ring Myography

Male 11βHSD2$^{-/-}$ mice and age-matched controls were killed by cervical dislocation, and thoracic aortas were removed into a physiologic saline solution. Two rings per mouse were taken for measurement of isometric force as described previously.[44]

Statistical Analyses

All data are presented as means ± SE. After tests for Gaussian distribution, comparisons were made using either unpaired *t* test or ANOVA with Bonferroni *post hoc* test, as appropriate. Statistical comparisons were made using Prism 4.0 (GraphPad Software, San Diego, CA).

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DISCLOSURES

None.

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