Mineralocorticoid and Glucocorticoid Receptors Stimulate Epithelial Sodium Channel Activity in a Mouse Model of Cushing Syndrome

Matthew A. Bailey, John J. Mullins, Christopher J. Kenyon

Abstract—Experiments in Cushing patients and healthy control subjects receiving adrenocorticotropic hormone (ACTH) indicate that transient renal sodium retention may contribute to the generation of hypertension. Here we have investigated the effect of chronic ACTH infusion on renal sodium handling in adult male C57BL/6J mice using selective antagonists to dissect mineralocorticoid and glucocorticoid receptor–mediated pathways. Mice were infused via osmotic minipump with ACTH (2.5 μg/d) or saline for 2 weeks before being anesthetized for renal function experiments. ACTH caused an increase in blood pressure and a reduction in fractional sodium excretion associated with enhanced activity of the epithelial sodium channel. Given separately, spironolactone and RU38486 blunted the pressor response to ACTH and the increased epithelial sodium channel activity; combined mineralocorticoid and glucocorticoid receptor blockade was required to resolve the response to ACTH excess. Dietary sodium depletion also prevented ACTH-induced hypertension. The effect of increased sodium reabsorption in the distal nephron is offset by downregulation of Na-K-Cl cotransport in the loop of Henle. Sodium excretion is normalized chronically, but blood pressure remains high; acute blockade of V1 receptors and α1 adrenoceptors in combination restored blood pressure to control values. In summary, ACTH excess promotes renal sodium reabsorption, contributing to the increased blood pressure; both glucocorticoid and mineralocorticoid receptor pathways are involved. These data are relevant to conditions associated with overactivity of the hypothalamic-pituitary-adrenal axis, such as obesity and chronic stress. (Hypertension. 2009;54:890-896.)

Key Words: renal clearance ■ amiloride ■ 11β-hydroxysteroid dehydrogenase ■ tubular sodium reabsorption ■ furosemide

The severity of hypertension in Cushing syndrome is an important predictor of morbidity and mortality,1 but the underlying cause remains uncertain.2 Renal sodium retention may contribute,3–5 with sodium balance being restored6 at the expense of elevated blood pressure. Impaired natriuretic capacity and hypokalemic alkalosis in Cushing syndrome may reflect mineralocorticoid excess, and aldosterone levels can indeed be high7 because of a direct action of adrenocorticotropic hormone (ACTH) on CYP11B2.8 However, the increase in aldosterone is transient.6,9,10 It is also possible that the mineralocorticoid receptor (MR) is activated by high levels of glucocorticoid.2 Nevertheless, glucocorticoid hypertension is not fully prevented by the MR antagonist spironolactone,11 and glucocorticoid receptor (GR)–mediated pathways may also contribute to sodium retention.

Glucocorticoids stimulate renal sodium transport by increasing the activity of the basolateral membrane Na,K-ATPase.12 Direct activation of apical membrane transport proteins has also been demonstrated. Thus, glucocorticoids increase the following: (1) sodium-hydrogen exchange by Na+/H+ exchanger 3 in the proximal tubule13; (2) furosemide-sensitive cotransport by Na-K-Cl cotransporter (NKCC 2) in the thick limb of Henle14; (3) thiazide-sensitive cotransport by Na-CI cotransporter (NCC) in the distal tubule14,15; and (4) the epithelial sodium channel (ENaC) in the connecting tubule and collecting ducts.16 The net effect on sodium reabsorption is, however, difficult to predict, because glucocorticoids can also inhibit mineralocorticoid action and promote natriuresis.17

The aim of the present study was to assess the impact of ACTH excess on renal sodium homeostasis. We found evidence for activation of ENaC through both the GR and MR; the net effect of increased sodium reabsorption was offset by increased glomerular filtration and downregulation of sodium transport in the thick limb of Henle.

Methods

All of the experiments were performed in accordance with United Kingdom Home Office regulations. Cohorts of male C57BL/6J mice were maintained on either a standard rodent diet (RM1 diet; 0.3% sodium, SDS Diets) or a low-sodium diet (0.03% sodium, SDS Diets) with free access to water. On day 0, osmotic minipumps
(model 2002, ALZET) containing either ACTH (Synacthen, Ciba-Geigy; 2.5 µg/d) or vehicle (0.9% NaCl) were implanted SC.

On days 12 to 14, mice were anesthetized with Inactin (thiobutabarbital; 100 mg/kg IP), and renal function studies were performed as described; topical administration of a lidocaine solution was used during surgery. Mean arterial blood pressure (MABP) was recorded throughout (PowerLab, AD Instruments). Mice were infused through (0.2 mL/kg per 10 g IV) with a solution containing (in mM) 130 NaCl, 5 KCl, and 10 NaHCO₃. Fluorescein isothiocyanate-inulin (0.5%) was included for the measurement of glomerular filtration rate (GFR), and in some experiments, p-aminohippurate was included for the measurement of effective renal plasma flow. After a 60-minute equilibration period, 2 consecutive urine collections of 45 minutes each were made, bracketed by collections (20 µL) of arterial blood. After the first (control) urine sample, a bolus of either amiloride (2 mg/kg IV) or furosemide (2 mg/kg IV) was administered, and the second collection was then made. A 500-µL blood sample was then taken for electrolyte analysis.

In separate cohorts, the antihypertensive actions of either acute α₁ adrenoreceptor or V₁-vasopressinergic receptor blockade were measured. Blood pressure was monitored for 5 minutes at 2 Hz before a bolus of either prazosin (0.1 mg/kg IV) or Manning compound was infused through (0.2 mL/kg per 10 g IV) with a solution containing (in mM) 130 NaCl, 5 KCl, and 10 NaHCO₃. Fluorescein isothiocyanate-inulin (0.5%) was included for the measurement of glomerular filtration rate (GFR), and in some experiments, p-aminohippurate was included for the measurement of effective renal plasma flow. After a 60-minute equilibration period, 2 consecutive urine collections of 45 minutes each were made, bracketed by collections (20 µL) of arterial blood. After the first (control) urine sample, a bolus of either amiloride (2 mg/kg IV) or furosemide (2 mg/kg IV) was administered, and the second collection was then made. A 500-µL blood sample was then taken for electrolyte analysis.

To identify the contribution of MR and/or GR to the ACTH excess phenotype, spironolactone and RU38468 were used either separately or in combination. The compounds were encapsulated in an elastomer pellet (Silastic, a gift from Dow Corning, Inc) or SPSS 16 software (SPSS Inc).

Analysis
Fluorescein isothiocyanate-inulin concentration in 5 µL of plasma and urine was measured by fluorometry after dilution in 195 µL of HEPES buffer (pH 7.4). Urinary and plasma electrolyte concentrations were measured by ISE (model 9180, Roche), with osmolalities by freezing point depression (Vogel). Urine and plasma electrolyte concentrations were measured colorimetrically.

Statistical Analyses
All of the data are mean ± SE. For tests after Gaussian distribution, comparisons were made using either paired t test or 1-way ANOVA with Holm-Sidak post hoc test, as appropriate. For correlation, the Pearson product-moment correlation coefficient was used. Analysis was performed using either Prism 4 (GraphPad Software, Inc) or SPSS 16 software (SPSS Inc).

Results
ACTH treatment increased plasma corticosterone and aldosterone levels. ACTH-treated mice had a significantly higher hemocrit level than saline controls, which was attributed to hemocoencentration; depletion of plasma volume (≈20%) was confirmed. ACTH-treated mice were hypokalemic and hypernatremic (Table 1).

MABP was significantly elevated in mice exposed to ACTH (Figure 1A), as was GFR (Figure 1B). Renal blood flow was reduced by ACTH excess (Figure 1C), suggesting that hyperfiltration is not secondary to hypertension. Increased GFR in the face of enhanced renal vascular resistance indicates a direct effect of ACTH excess on filtration fraction (Table 1).

In absolute terms, sodium excretion was not different between the 2 groups of mice (Figure 1D), consistent with sodium balance. There was, however, a significant increase in filtered sodium load in ACTH-treated mice (40.5 ± 2.8 versus 25.5 ± 1.7 µmol/min; P < 0.001), and, thus, fractional excretion was lower than in controls (Figure 1E). Despite the mild hypokalemia, potassium excretion remained robust in the ACTH-treated animals and was not different from controls (Figure 1F). There was no difference in the urinary sodium-potassium concentration ratio in the 2 groups of mice, and urine flow rate and fractional potassium excretion were similarly unaffected by ACTH excess (Table 1).

The reduction in fractional sodium excretion during ACTH treatment indicated increased tubular reabsorption, and we therefore measured the activity/expression of key sodium transport proteins. Enhanced ENaC activity was demonstrated; amiloride caused a significant natriuresis in both ACTH and saline-treated mice, but the effect was greater in the ACTH-treated mice (Figure 2A). An increase in ENaC-α mRNA was observed after ACTH treatment (Table 2), but this was to a lesser extent than the increase in functional channel activity. The possibility of enhanced ENaC trafficking to the apical membrane of the principal cell is suggested by the increased expression of serum glucocorticoid regulated kinase 1 (Table 2). The antikaliuretic effect of amiloride was
evident in both groups of mice but was lower in the ACTH group (Figure 2B). Amiloride increased the urine flow rate in both groups of mice, with the diuresis being greater in the ACTH group (Figure 2C). The \( \text{N}_{9251} \) and \( \text{H}_{9252} \) subunits of the Na,K-ATPase or the expression of NCC was not changed by ACTH treatment. For the former, it is possible that regional differences in mRNA expression are masked by the use of whole kidney homogenates. Expression of NKCC2 was signifi-
Table 2. Renal mRNA Expression in C57BL/6J Mice Treated With Vehicle (n=12) or ACTH (n=12) for 14 Days

<table>
<thead>
<tr>
<th>Name</th>
<th>Gene ID</th>
<th>Vehicle</th>
<th>ACTH</th>
<th>P</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-ENaC</td>
<td>Scnn1a</td>
<td>0.46±0.05</td>
<td>&lt;0.05</td>
<td>0.65±0.09</td>
<td></td>
</tr>
<tr>
<td>β-ENaC</td>
<td>Scnn1b</td>
<td>0.59±0.10</td>
<td>NS</td>
<td>0.56±0.07</td>
<td></td>
</tr>
<tr>
<td>γ-ENaC</td>
<td>Scnn1g</td>
<td>0.59±0.07</td>
<td>NS</td>
<td>0.66±0.07</td>
<td></td>
</tr>
<tr>
<td>NCC</td>
<td>Slc12a3</td>
<td>0.78±0.09</td>
<td>NS</td>
<td>0.66±0.07</td>
<td></td>
</tr>
<tr>
<td>NKCC2</td>
<td>Slc12a1</td>
<td>1.24±0.11</td>
<td>&lt;0.001</td>
<td>0.70±0.07</td>
<td></td>
</tr>
<tr>
<td>α1 Na,K-ATPase</td>
<td>Ap1a1</td>
<td>0.78±0.10</td>
<td>NS</td>
<td>0.80±0.09</td>
<td></td>
</tr>
<tr>
<td>β Na,K-ATPase</td>
<td>Ap1b1</td>
<td>0.98±0.13</td>
<td>NS</td>
<td>0.98±0.13</td>
<td></td>
</tr>
<tr>
<td>sgk1</td>
<td>Sgk1</td>
<td>0.53±0.07</td>
<td>&lt;0.01</td>
<td>1.36±0.25</td>
<td></td>
</tr>
<tr>
<td>MR</td>
<td>Nr3c2</td>
<td>0.64±0.07</td>
<td>NS</td>
<td>0.80±0.09</td>
<td></td>
</tr>
<tr>
<td>GR</td>
<td>Nr3c1</td>
<td>0.72±0.07</td>
<td>NS</td>
<td>0.80±0.09</td>
<td></td>
</tr>
<tr>
<td>11β-HSD2</td>
<td>Hsd11b2</td>
<td>0.74±0.10</td>
<td>NS</td>
<td>0.81±0.12</td>
<td></td>
</tr>
</tbody>
</table>

Data are normalized to the expression of 18S RNA, which was not different between groups. Data are mean±SE. Statistical comparisons were made using the t test and P values (2 tailed) as given. NS indicates not significant; sgk1, serum glucocorticoid regulated kinase 1.

Discussion

ACTH infusion induces a robust pressor response in humans and experimental animals, associated with reduced sodium excretion.4 In our study, rigorous dietary sodium restriction prevented this response to ACTH excess, suggesting that enhanced ENaC-mediated sodium reabsorption is an important hypertensive event. Indeed, there was a highly significant correlation between the amiloride-sensitive sodium reabsorption and blood pressure. The functional ENaC channel complex is a heterodimer of α, β, and γ subunits; only αENaC was stimulated by ACTH excess in the present study and not to the same extent as the increase in physiological channel activity. Stimulation by corticosteroids of αENaC has been shown previously,20 with expression in the inner medullary collecting duct being the rate-limiting step for channel formation.20 It is possible that an increase in αENaC expression in this segment could have a disproportionate effect on renal sodium transport. In addition, we have evidence to suggest that trafficking of the channel complex to the apical membrane of the principal cell is stimulated by ACTH; expression of serum glucocorticoid regulated kinase 1 was greater in kidneys from ACTH-treated mice than from control mice.

By the end of the experiment, absolute sodium excretion was normal despite persistently high levels of ENaC. This is compatible with long-term sodium balance at the expense of chronically elevated blood pressure. Our data indicate that both hemodynamic and tubular mechanisms may compensate for a reduced natriuretic capacity. First, ACTH causes glomerular hyperfiltration and increases the filtered sodium load. Hyperfiltration occurs despite increased renal vascular resistance, and it is therefore unlikely to be a secondary response to hypertension. Notably, hyperfiltration is considered a GR-mediated response,22 and selective GR blockade tended to lower GFR. However, spironolactone was also required to fully prevent hyperfiltration. Aldosterone modulates angiotensin II action in the renal microvasculature23 and can directly increase glomerular pressure by inducing pathological glomerular remodeling.24
Second, expression and activity of NKCC2 were downregulated. This was unexpected, because corticosteroids stimulate sodium reabsorption in the thick limb of Henle. Reduced NKCC2 activity, possibly as a result of mild potassium depletion, has ramifications for the transport of other electrolytes: the reduced electrochemical driving force for paracellular reabsorption through the cation shunt may cause the hypercalciuria induced by ACTH excess. The expression of NCC was not affected by ACTH treatment despite regulation by glucocorticoids. We were also unable to demonstrate altered expression of either subunit of the Na,K-ATPase, although the α subunit has been linked to ACTH-induced hypertension. Nevertheless, activity of NCC and Na,K-ATPase can be increased without requiring gene transcription, and we cannot discount a role for these transporters in the response to ACTH excess.

The renal and hypertensive actions of ACTH excess can be attributed to combined activation of MR and GR, and there is no evidence for alternative pathways for stimulation of ENaC. In addition, MR and GR have reciprocal functionality, because single receptor blockade only blunted the enhanced ENaC activity: the hypertension was similarly reduced by single receptor blockade. With stimulation of aldosterone being transient, activation of MR by glucocorticoids, normally pre-
vented by 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2), must be considered. The ratio of urinary free cortisol:cortisone, an index of 11βHSD2 function, is increased in patients with Cushing syndrome, suggesting inhibition of 11βHSD2 activity by ACTH. However, renal hsd11b2 mRNA expression was not altered by ACTH excess, nor does ACTH inhibit enzymatic function. It is possible, therefore, that the protective barrier provided by 11βHSD2 is breached, allowing corticosterone to activate MR.

The notion that GR activation directly regulates ENaC is controversial. The channel can be activated by either receptors in a mouse cell line, and there is evidence for functional crossover in MR knockout mice and transgenic mice overexpressing GR in the collecting duct. In contrast, however, -ENaC expression was not stimulated by a GR-specific dosage of dexamethasone, and collecting duct–specific deletion of MR prevents expression of ENaC.

In the present study, GR-dependent contraction of plasma volume predominated. Volume depletion, hyperosmolality, and hypernatremia stimulate vasopressin release, causing sustained activation of the sympathetic nervous system. Our data suggest that both systems sustain elevated blood pressure during ACTH excess. In the rat, neither chemical sympathectomy nor chronic V1 receptor antagonism fully prevent the pressor response to ACTH. ACTH-induced hypertension is also observed in Brattleboro rats, indicating that vasopressin is not essential. Other studies, however, find V1 antagonism an effective measure to counter enhanced vascular reactivity in glucocorticoid-mediated hypertension. In the present investigation, the acute effect of receptor antagonism in the maintenance phase of hypertension was measured, and there is clearly a need for chronic studies.

Perspectives

ACTH-dependent hypertension is sodium dependent and is associated with MR- and GR-mediated activation of ENaC. Renal sodium retention may ultimately sustain hypertension via stimulation of vasoactive factors, and this may occur independent of any blood pressure change. This study has relevance to hypertensive disorders associated with overactivity of the hypothalamic-pituitary adrenal axis: enhanced ENaC expression is found in a rat model of chronic stress, and a mouse obesity model displays impaired sodium excretion.

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Disclosures

None.

References


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