11β-Hydroxyysteroid Dehydrogenase and Corticosteroid Action in Lyon Hypertensive Rats

Susan A. Lloyd-MacGilp, Susan M. Nelson, Marine Florin, Ming Lo, Joanna McKinnell, Jean Sassard, Christopher J. Kenyon

Abstract—Adrenocorticoicoid activity in Lyon hypertensive (LH) and low blood pressure (LL) rat strains differ in several respects. Abnormal activity of 11β-hydroxyysteroid dehydrogenase enzymes (11β-HSD1 and 11β-HSD2), which interconvert corticosterone and inactive 11-dehydrocorticosterone, might contribute to the LH phenotype by regulating corticosteroid hormone access to receptors. 11β-HSD2 (expressed in kidney but not liver) prevents endogenous glucocorticoids from binding to mineralocorticoid receptors. 11β-HSD1 (expressed in liver and kidney) favors active glucocorticoid formation from 11-dehydrocorticosterone. 11β-HSD properties in LH and LL have been compared by several approaches: (1) 11βHSD activities have been measured in vitro as corticosterone dehydrogenation and in vivo as interconversion of injected cortisol and cortisone; (2) the effects of cortisol and cortisone on urine electrolytes and volume have been measured; and (3) 11β-HSD mRNA expression has been measured by in situ hybridization. 11β-HSD2 enzyme activities in LH and LL rats were similar and urinary cortisol:cortisone ratios were not different after cortisol injection. Cortisol caused a natriuresis and kaliuresis in both strains, with a slightly reduced response in LH rats. Renal 11β-HSD2 mRNA expression was slightly lower in LH rats. 11β-HSD1 was less active in LH than LL rats: enzyme activities were lower in tissue extracts; urinary cortisone:cortisol was lower in LH rats after cortisone injections; cortisone increased urine volume in LL but not LH rats; and mRNA levels tended to be lower in LH tissues. We conclude that 11β-HSD1 is impaired in LH rats. The LH phenotype of heavier adrenals, raised corticosterone, and reduced thymus weight is similar to that described for 11β-HSD1 knockout mice. (Hypertension. 1999;34:1123-1128.)

Key Words: glucocorticoids ■ mineralocorticoids ■ corticosterone ■ cortisol ■ cortisone ■ renal function

The Lyon strains of hypertensive (LH), normotensive (LN), and low blood pressure (LL) rats exhibit different patterns of mineralocorticoid and glucocorticoid hormone secretion depending on age.1 In young LH rats, concentrations of mineralocorticoids (aldosterone and deoxycorticosterone) are elevated, whereas glucocorticoid levels are low in relation to LL or LN rats. In adulthood, the pattern is reversed. Because both mineralocorticoid and glucocorticoid excess can cause hypertension,2 it may be that these changing patterns of steroid metabolism could account for some of the blood pressure differences between Lyon strains of rat.

An important factor in the control of corticosteroid metabolism, particularly in relation to the balance between mineralocorticoid and glucocorticoid hormones, is the enzymatic interconversion of biologically active corticosterone (rodent) and cortisol (humans) to the inactive 11-ketone metabolites, 11-dehydrocorticosterone and cortisol, respectively.3,4 Two distinct isozymes of 11β-hydroxyysteroid dehydrogenase (11β-HSD1 and 11β-HSD2) catalyze this reaction. 11β-HSD2 favors dehydrogenation and inactivates corticosterone and cortisol. The mineralocorticoid receptor, which binds aldosterone and corticosterone with similar affinities, shows specificity for aldosterone only when 11β-HSD2 is present. Patients or transgenic mice with 11β-HSD2 deficiency are characterized by hypertension, hypokalemia, and suppressed plasma renin activity and aldosterone concentration.5,5

11β-HSD1 is expressed in a wide range of glucocorticoid-sensitive tissues, including the liver, kidney, and vasculature. In many intact cells it appears to act predominantly as a reductase, thereby promoting glucocorticoid hormone action by activating 11-dehydro metabolites.6–8 However, in cell-free extracts and in at least 1 cell line,9 11β-HSD1 functions as a dehydrogenase. Clearly, the cellular context plays a major part in determining the overall direction of the reaction.

In the present study we consider whether the activities of 11β-HSD enzymes differ between LH and LL rats. To assess 11β-HSD activity in vivo, patterns of urinary steroid excretion were compared in LH and LL rats after injections of cortisol and cortisone. Activity in vitro was measured in liver and kidney microsomes. Urinary volume and electrolyte excretion after steroid injections were used to indicate mineralocorticoid and glucocorticoid responsiveness. Finally, 11β-HSD mRNA expression was compared in LH and LL tissues by in situ hybridization.
**Methods**

**Animals**

Male LH and LL rats were maintained in a controlled environment (21±1°C, humidity 60±10%, lighting 8 AM to 10 PM) with a standard rat chow (NA A03; Usine d’Alimentation Rationnelle) and were used at 11 weeks old. Protocols were conducted in accordance with institutional ethical guidelines. Blood pressure was measured by a tail-cuff method (Narco Biosystems).

For in vivo studies of renal function and steroid metabolism, individual rats were kept in metabolism cages with free access to food and water. Rats were injected subcutaneously on separate days with saline and then with 1 or 0.5 mg/kg of cortisone or cortisol, respectively, with an interval of 48 hours between steroid injections. Urine was collected for 24 hours after each injection. After preliminary extraction with Sep-Pak C18 cartridges, urinary free steroids were measured by modified radioimmunoassays for cortisol, cortisone, and corticosterone. Urinary sodium and potassium were measured by flame photometry (IL Photometer, model 243).

**11β-HSD mRNA Expression**

In situ hybridization techniques were used to measure mRNA expression, with liver and kidney tissues for 11-βHSD1 and kidney tissues for 11-βHSD2. Tissues for these studies were collected snap-frozen. Restriction enzymes and RNA polymerases were from Promega Corporation. A rat HSD1 cDNA was linearized with Sty I. Antisense and sense transcripts were generated with the use of T3 and T7 RNA polymerase, respectively. A rat HSD2 clone was linearized with appropriate restriction enzymes. Sense and antisense transcripts were generated from the SP6 and T7 promoter, respectively. Radioactively labeled RNA transcripts were synthesized and tissue sections were prepared for autoradiography as previously described.

Hybridization signals in the kidney were quantified in key areas of expression. Hepatic mRNA expression was quantified in both the periporal and perivenous regions of the liver. A computer-aided image analysis system (Viewsonic 17PS, Zenith Data Systems) was used to quantify mRNA expression. No significant levels of hybridization with the sense probes were observed.

**11β-HSD Enzyme Activity**

Kidneys were sectioned (50 μm) in ice-cold Krebs-Ringer solution (0.1 mol/L NaCl, 2.5 mmol/L KCl, 2.5 mmol/L CaCl₂, 1.2 mmol/L KH₂PO₄, 2.0 mmol/L MgSO₄, and 25 mmol/L NaHCO₃). For each section, cortical (including inner cortex and outer medulla) tissues were dissected from inner medullary tissue, and each region was then processed separately. Tissues were homogenized (Ystral GMbH D-7801) in 4 volumes of Ringer’s solution. Microsomal fractions were prepared by centrifugation (16000g for 20 minutes, then centrifugation of the supernatant at 106000g for 1 hour at 4°C). Dehydrogenase activity in microsomes (0.25-mL aliquots) was determined as described by Low et al by measuring the conversion of 12 nmol/L H-corticosterone (1,2,6,7-3H-corticosterone, specific activity 86 Ci/mmol; Amersham Life Sciences Ltd) to radioactive 11-dehydrocorticosterone in the presence of a range of concentrations of cold corticosterone (0 to 10 μmol/L) and either NADP or NAD as cofactor (0.2 mmol/L). After incubation (37°C, 10 minutes), reactions were terminated by adding 2 mL ethyl acetate. Steroids extracted into ethyl acetate were separated by high-performance liquid chromatography with online β-counting. The percent conversion of H-corticosterone to 11-dehydrocorticosterone with the various concentrations of cold corticosterone was used to estimate maximal velocity (Vₘₐₓ) and Kₘ values.

**Statistical Analysis**

Results are expressed as mean±SE. Urinary electrolyte and steroid values were compared by a Mann-Whitney test. In situ hybridization results and enzyme Vₘₐₓ values were compared by ANOVA.

**Results**

**Blood Pressure, Urinary Volume, and Electrolyte Excretion**

At 10 weeks of age (1 week before steroid treatments were started), systolic blood pressure in LH rats reached a plateau (160±5 mm Hg) that was 40 mm Hg greater than that in LL rats (119±2 mm Hg). The effects of cortisol and cortisone injection on urinary volume and sodium and potassium excretion by LH and LL strains at 4 and 24 hours are shown in Figure 1. Baseline urinary volumes and sodium and potassium excretion were not different between strains except that at 24 hours, urinary volume appeared slightly elevated in LL rats. As expected, cortisol injection caused an increase in urinary volume, with natriuresis and kaliuresis at 4 hours. At 24 hours only a natriuretic response was seen, which was less in LL than in LH rats. Cortisone markedly increased urinary volume in LL but not in LH rats at 24 hours and did not affect electrolyte excretion.

**Urinary Steroid Excretion**

Corticosterone but not cortisol is the major endogenous glucocorticoid hormone in rodents. LH and LL rats were injected with cortisol and cortisone to assess 11β-HSD activities independent of changes in endogenous steroid metabolism. The possibility that injected steroids might either decrease urinary corticosterone by negative feedback control of corticosterone synthesis or increase urinary corticosterone...
excretion by acting as competitive inhibitors at sites of endogenous steroid metabolism was also considered.

Urinary corticosterone values were generally greater for LH than for LL rats, particularly for samples collected after control and cortisol injections (Figure 2). Cortisol and cortisone increased both LL and LH urinary corticosterone values \( (P, 0.01) \), which suggests that, at the doses given, both steroids act as competitive inhibitors of endogenous steroid metabolism. Cortisol values in LH and LL samples after saline injections were \( \approx 1 \% \) of those of corticosterone. Cortisol injection increased cortisol excretion \( \approx 20 \)-fold in both LH and LL rats. The high ratio of urinary cortisone:cortisol after control and cortisol injections reflects greater dehydrogenase activity, which might be interpreted as higher \( \beta \)HSD2 than \( \beta \)HSD1 activity. After cortisol injection, there were no strain differences of urinary cortisone:cortisol ratios or cortisone excretion, implying that \( \beta \)HSD2 activities are similar in LL and LH rats.

Increased urinary cortisol after cortisone injection indicates reductase activity, the direction of metabolism favored by \( \beta \)HSD1 in vivo, although, overall, the high ratio of cortisone:cortisol confirms that \( \beta \)HSD2 activity is greater than \( \beta \)HSD1 activity. However, after cortisone treatment, the ratio was less in LH than in LL rats, suggesting reduced \( \beta \)HSD1 activity in LH rats. This is supported by evidence of 2-fold greater levels of cortisone in the urine of LH than LL rats, although this difference was not statistically significant.

**11β-HSD Enzyme Assays**

11β-HSD activities were measured in microsomes of liver and of kidney cortex with NADP as cofactor. \( V_{\text{max}} \) values were lower in LH than LL samples (Figure 3), but \( K_m \) values (\( \mu \)mol/L) were not different (LL liver, 5.3±0.8; LL kidney, 7.2±1.1; LH liver, 5.8±0.4; LH kidney, 6.4±1.5). No strain difference of 11β-HSD activity \( (V_{\text{max}}) \) was seen in microsomes of the kidney medulla when NAD was added (LL, 1.36±0.06; LH, 1.84±0.22 pmol corticosterone per milligram protein per hour; \( n = 7 \); \( K_m \) values for corticosterone also were not different (LL, 0.14±0.01; LH, 0.17±0.01 \( \mu \)mol/L).

**11β-HSD mRNA Expression**

In situ hybridization studies confirmed that 11β-HSD1 mRNA is expressed throughout the liver, with no differences between periportal and perivenous regions in either strain. Expression of 11β-HSD1 mRNA appeared less in LH than in LL tissues (Table), although the difference was not significant \( (P, 0.1) \).

Sites of 11β-HSD1 and 11β-HSD2 mRNA expression in the kidney are mutually exclusive (Figure 4). 11β-HSD1 is localized to the outer medulla/inner cortex, whereas 11β-HSD2 expression is in an inner band of the medulla; neither is highly expressed in the outer cortex or the papilla. Both 11β-HSD1 and 11β-HSD2 mRNA appeared lower in LH kidney.

**In Situ Hybridization Studies of 11β-HSD1 mRNA Expression (Optical Density/Unit Area) in Liver and Kidney Cortex and 11β-HSD2 mRNA Expression in Kidney Medulla of LH and LL Rats**

<table>
<thead>
<tr>
<th>Tissue (mRNA)</th>
<th>LH ((n=7))</th>
<th>LL ((n=7))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver (11β-HSD1)</td>
<td>0.239±0.028</td>
<td>0.291±0.015</td>
</tr>
<tr>
<td>Kidney (11β-HSD1)</td>
<td>0.170±0.006*</td>
<td>0.194±0.007</td>
</tr>
<tr>
<td>Kidney (11β-HSD2)</td>
<td>0.184±0.006*</td>
<td>0.208±0.007</td>
</tr>
</tbody>
</table>

*\( P < 0.05 \) compared with LL.
tissues of LL rats are impaired in LH rats compared with LL rats. First, in vitro cortisol to cortisol after injection of cortisone was higher in LH than in LL urine. Because, in vivo, 11\(\beta\)-HSD1 mRNA expression levels. The association between high corticosterone from 11-dehydrocorticosterone in glucocorticoid target tissues. While this latter study shows that inhibition of either glucocorticoid receptors or mineralocorticoid receptors in tissues unprotected by 11\(\beta\)-HSD2, could be a contributory factor when acting in concert with other genetic factors. Differences in 11\(\beta\)-HSD activity have been noted in mesenteric arteries and kidney and liver fractions of spontaneously hypertensive rats compared with Wistar-Kyoto rats. Although a clear distinction between 11\(\beta\)-HSD1 and 11\(\beta\)-HSD2 was not made in these studies, both indicated that reductase rather than oxidase activity is favored in spontaneously hypertensive rats. Again, it is possible that the 11\(\beta\)-HSD phenotype is associated with increased adrenocortical activity. The functional significance of 11\(\beta\)-HSD activity in vascular tissues is interesting. A bidirectional 11\(\beta\)-HSD1 appears to be expressed in vascular smooth muscle, whereas 11\(\beta\)-HSD1 and 11\(\beta\)-HSD2 are expressed in vascular endothelial cells. Glucocorticoids are known to control vascular reactivity, and there are reports that inhibition of dehydrogenase activity potentiates the effects of corticosterone on noradrenaline-induced vasoconstriction in aortic tissues.
rings. However, it is not clear whether 11β-HSD1 or 11β-HSD2 regulates corticosterone activity in vascular tissues or even whether this process is involved in blood pressure control. The recent studies of Takeda et al. have suggested that decreased expression and activity of 11β-HSD2 in Dahl salt-sensitive rats is a factor in blood pressure determination, although, in other respects, this model of hypertension has little in common with the syndrome of apparent mineralocorticoid excess.

One possibility that should be considered is that altered 11β-HSD activity is a consequence of hypertension or some other common phenotype. It is perhaps significant that a number of strains of rat, including LH, Milan hypertensive, obese Zucker, Dahl salt-sensitive, and certain strains of spontaneously hypertensive rats, all exhibit evidence of elevated plasma insulin levels in relation to glucose as well as evidence of impaired 11β-HSD activity. Insulin has been shown to downregulate 11β-HSD mRNA expression and activity.

In summary, we have clear evidence that 11β-HSD1 activity is lower in LH than in LL rats, whereas 11β-HSD2 is relatively unaffected. Raised plasma insulin may be the cause of lower 11β-HSD activity. Increased urinary corticosterone excretion in LH rats may reflect compensatory increases in adrenocortical steroidogenesis. It is unlikely that reduced 11β-HSD1 in LH rats directly causes hypertension, but it may be of secondary importance. Raised corticosterone secretion could potentiate responsiveness to vasoconstrictors in vascular tissues. Alternatively, if 11β-HSD1 were to act as a dehydrogenase rather than as a reductase in some tissues, then lower 11β-HSD1 might potentiate corticosterone actions.

Acknowledgment

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References


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