**11β-Hydroxysteroid 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**—Adrenocorticosteroid activity in Lyon hypertensive (LH) and low blood pressure (LL) rat strains differ in several respects. Abnormal activity of 11β-hydroxysteroid 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 cortisone:cortisol 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 directly 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 cortisone, respectively.3,4 Two distinct isozymes of 11β-hydroxysteroid 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.5,6

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 cortisol or cortisosterone, 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 and cortisosterone. 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 11β-HSD1 and kidney tissues for 11β-HSD2. Tissues for these studies were collected snap-frozen. Restriction enzymes and RNA polymerases were from Promega Corporation. A rat HSD1 cDNA15 was linearized with Sty I. Antisense and sense transcripts were generated with the use of T3 and T7 RNA polymerase, respectively. A rat HSD2 clone14 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.15

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 mol/L KCl, 2.5 mol/L CaCl₂, 1.2 mol/L KH₂PO₄, 1.2 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 (16 000 g for 20 minutes, then centrifugation of the supernatant at 106 000g for 1 hour at 4°C). Dehydrogenase activity in microsomes (0.25-mL aliquots) was determined as described by Low et al16 by measuring the conversion of 12 mmol/L H-corticosterone (1,2,6,7-H-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 mol/mL). 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 ($V_{max}$) and $K_m$ 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 $V_{max}$ values were compared by ANOVA.
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 corticosterone 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 $<1\%$ 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 $11\beta$-HSD2 than $11\beta$-HSD1 activity. After cortisol injection, there were no strain differences of urinary cortisone:cortisol ratios or cortisone excretion, implying that $11\beta$-HSD2 activities are similar in LL and LH rats.

Increased urinary cortisol after cortisone injection indicates reductase activity, the direction of metabolism favored by $11\beta$-HSD1 in vivo, although, overall, the high ratio of cortisone:cortisol confirms that $11\beta$-HSD2 activity is greater than $11\beta$-HSD1 activity. However, after cortisone treatment, the ratio was less in LL than in LH rats, suggesting reduced $11\beta$-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.

**$11\beta$-HSD Enzyme Assays**

$11\beta$-HSD activities were measured in microsomes of liver and of kidney cortex with NADP as cofactor. $V_{max}$ 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 $11\beta$-HSD activity ($V_{max}$) 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).

**$11\beta$-HSD mRNA Expression**

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

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

**In Situ Hybridization Studies of $11\beta$-HSD1 mRNA Expression (Optical Density/Unit Area) in Liver and Kidney Cortex and $11\beta$-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 ($11\beta$-HSD1)</td>
<td>0.239±0.028</td>
<td>0.291±0.015</td>
</tr>
<tr>
<td>Kidney ($11\beta$-HSD1)</td>
<td>0.170±0.006*</td>
<td>0.194±0.007</td>
</tr>
<tr>
<td>Kidney ($11\beta$-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 11
b-cortisone to cortisol after injection of cortisone was higher in LH than in LL urine. Because, in vivo, 11-b-cortisone metabolism, the reciprocal effect of endogenous steroid levels on the metabolism of exogenous cortisol and cortisone should also be considered. However, it is difficult to show whether higher endogenous steroids or intrinsic differences in steroid metabolic pathways are primary or secondary causes of increased excretion of unmetabolized cortisol and cortisone by LH rats. Third, 11-b-HSD1 activity would account for differences in renal responses between strains. Cortisone only caused a diuresis in LL rats, implying that LH rats were not exposed to active glucocorticoid hormone. Fourth, expression of 11-b-HSD1 mRNA in liver and kidney cortex is reduced in LH compared with LL tissues.

There appeared to be little or no difference in 11-b-HSD2 activities between strains. Neither the urinary excretion of cortisol and cortisone (after cortisol injection) nor the renal activity of 11-b-HSD2 differed between strains. Evidence of a difference in the pattern of electrolyte excretion after cortisol injection was inconclusive. Conventionally, mineralocorticoid hormones cause an antinatriuresis and kaliuresis, whereas glucocorticoids, as found in the present study, cause a natriuresis and kaliuresis,17,18 A smaller natriuretic response was observed in LL rats at 24 hours, but there was no difference in potassium excretion. The relative natriuresis seen in LH rats may relate to inherent differences of blood pressure between strains rather than altered 11-b-HSD activity. Expression of 11-b-HSD2 mRNA in the renal medulla of LH rats was 10% less than that of LL. Because patients with the syndrome of apparent mineralocorticoid excess with a complete lack of 11-b-HSD2 activity exhibit marked sodium retention, the slightly greater natriuresis in LH rats after cortisol injection is incompatible with reduced 11-b-HSD2 mRNA expression.

Other studies of 11-b-HSD enzymes in rat models of genetically determined hypertension have been reported. Increased corticosterone levels in Milan hypertensive rats were associated with decreased hepatic 11-b-HSD1 activity and mRNA.19 There were no differences in renal activities or expression levels. The association between high corticosterone levels and 11-b-HSD1 deficiency is not unexpected. It has been argued that adrenocortical secretory activity and plasma corticosterone concentrations are elevated in 11-b-HSD1 knockout mice to compensate for reduced regeneration of corticosterone from 11-dehydrocorticosterone in glucocorticoid target tissues.5 While this latter study shows that 11-b-HSD1 deficiency alone does not cause hypertension, one might speculate that raised corticosterone, acting through either glucocorticoid receptors or mineralocorticoid receptors in tissues unprotected by 11-b-HSD2, could be a contributory factor when acting in concert with other genetic factors.

Differences in 11-b-HSD activity have been noted in mesenteric arteries20 and kidney and liver fractions of spontaneously hypertensive rats compared with Wistar-Kyoto rats.21 Although a clear distinction between 11-b-HSD1 and 11-b-HSD2 was not made in these studies, both indicated that reductase activity is favored in spontaneously hypertensive rats.22 The functional significance of 11-b-HSD activity in vascular tissues is interesting. A bidirectional 11-b-HSD phenotype is associated with increased adrenocortical activity.23 The functional significance of 11-b-HSD activity in vascular tissues is interesting. A bidirectional 11-b-HSD phenotype is associated with increased adrenocortical activity.23 The functional significance of 11-b-HSD activity in vascular tissues is interesting. A bidirectional 11-b-HSD phenotype is associated with increased adrenocortical activity.23 The functional significance of 11-b-HSD activity in vascular tissues is interesting. A bidirectional 11-b-HSD phenotype is associated with increased adrenocortical activity.23 The functional significance of 11-b-HSD activity in vascular tissues is interesting. A bidirectional 11-b-HSD phenotype is associated with increased adrenocortical activity.23
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. Since 11β-HSD1 acts as a reductase in many tissues, the conversion of inactive 11-dehydrocorticosterone to active corticosterone is impaired, thereby reducing glucocorticoid activity including, perhaps, negative feedback control of hypothalamic-pituitary-adrenal 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.

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References


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