Renal 11β-Hydroxysteroid Dehydrogenase in Genetically Salt-Sensitive Hypertensive Rats

Yoshiyu Takeda, Satoru Inaba, Kenji Furukawa, Isamu Miyamori

Abstract—Renal 11β-hydroxysteroid dehydrogenase II (11β-HSDII) converts glucocorticoids to inactive metabolites and plays an important role in controlling blood pressure and sodium retention. To examine whether this enzyme may be involved in the pathophysiology of salt-sensitive hypertension, we determined 11β-HSDII activity and mRNA levels in the blood vessel and kidney of Dahl Iwai salt-sensitive (DS) rats and Dahl Iwai salt-resistant (DR) rats. Urinary free corticosterone:free 11-dehydrocorticosterone ratio was measured to estimate renal 11β-HSD activity. Vascular 11β-HSDII activity was expressed as percent conversion of [3H]corticosterone to [3H]11-dehydrocorticosterone in homogenized mesenteric arteries. 11β-HSDII mRNA was estimated with the use of competitive polymerase chain reaction (PCR). Renal 11β-HSDII activity and mRNA levels were significantly decreased in 8- and 12-week-old high salt DS rats compared with DR, Sprague-Dawley (SD), or low salt DS rats of the same age. Decreased 11β-HSDII activity and mRNA levels in mesenteric arteries were observed in 8- and 12-week-old high salt DS rats. Urinary excretion of 11β-HSDII inhibitory factors was measured by inhibition of enzyme activity in microsomes from human kidney. The urinary inhibitors were significantly increased in 8- and 12-week-old high salt DS rats compared with DR, SD, or low salt DS rats of the same age. There were no significant differences in 11β-HSDII activity and mRNA levels in mesenteric arteries and kidney or in urinary inhibitors between 4-week-old DS, DR, and SD rats. These results indicate that 11β-HSDII may play a role in salt sensitivity and development of hypertension in the DS rat. (Hypertension. 1998;32:1077-1082.)

Key Words: glucocorticoids ■ mineralocorticoids ■ rats, Dahl ■ kidney ■ hypertension, essential ■ sodium

The enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD) catalyzes the conversion of glucocorticoids to their inactive metabolites. A deficiency of 11β-HSD, whether congenital or produced by inhibition of this enzyme related to the administration of licorice or carbenoxolone, leads to the activation of mineralocorticoid receptors by glucocorticoids, resulting in sodium retention and hypertension. Decreased 11β-HSD activity has been demonstrated in some patients with essential hypertension1-3 and in rats with genetic hypertension.4,5 Biochemical studies have revealed the existence of 2 isoforms of 11β-HSD: NAD+ dependent and NADP+ dependent. 11β-HSDII (the NAD+-dependent isozyme) is found in distal portions of the nephron, where it has been shown to colocalize with mineralocorticoid receptors.6 Progesterone derivatives, which are potent inhibitors of 11β-HSDII, have been shown to be active in conferring mineralocorticoid Na+-retaining activity and elevating blood pressure.7 8 We and others have previously shown that human urine contains substances that inhibit 11β-HSDII, and these substances are elevated in subgroups of hypertension.9,10 Excess sodium intake is intimately involved in the pathogenesis of hypertension. In large populations, significant correlations between the level of salt intake, blood pressure, and the frequency of hypertension have been reported. Since most people in Western countries, including Japan, ingest a high sodium diet, the fact that only about half will develop hypertension suggests a variable degree of blood pressure sensitivity to sodium, although obviously heredity and interaction with other environmental exposures may be involved.11 Dahl salt-sensitive (DS) rats are widely used to study genetic determinants of salt-sensitive hypertension. In this strain, supplemental dietary sodium increases blood pressure, whereas in the Dahl salt-resistant (DR) strain, supplemental dietary sodium has little or no effect on blood pressure. There are several reports of the abnormalities of the renin-angiotensin system,12 adrenal steroids,13 and sympathetic nerve system14 in DS rats. Recently, mutations of the gene for 11β-hydroxylase, an adrenal enzyme involved in the synthesis of 18-hydroxy-11-deoxycorticosterone, in DR rats were reported.15 These mutations may not cause hypertension in DS rats because no mutations were found in DS rats or in Sprague-Dawley (SD) rats. Cover et al16 have reported abnormalities of the aldosterone synthase gene in DR rats. These abnormalities were not found in either DS or SD rats. These findings
may not explain the cause of salt-sensitive hypertension in DS rats. To clarify the mechanism of salt-induced hypertension in DS rats, we compared 11β-HSDII activity, gene expression of 11β-HSDII in mesenteric arteries and kidneys, and urinary excretion of 11β-HSDII inhibitory factors between DS, DR, and SD rats.

Methods
Male DS, DR, and SD rats (Eisai Supply, Eisai Animal Research Center), aged 3 to 4 weeks, were initially fed a standard chow (0.45% NaCl) purchased from Nippon Charles River. Both DS and DR rats were fed high sodium chow (7%) for 4 weeks (n = 10 in each group) and for 8 weeks (n = 10 in each group). DR rats were also fed high sodium chow (7%) for 4 weeks (n = 10) and for 8 weeks (n = 10). DS rats were fed standard chow for 8 weeks (n = 10) and for 12 weeks (n = 10). All rats were housed in metabolic cages, and daily urinary excretion was collected. The blood pressure was determined by the plethysmographic tail-cuff method, as previously reported. Blood was collected from the tail vein, as previously reported, by high-performance liquid chromatography (HPLC), as previously reported.

Rat mesenteric arteries were removed immediately after decapitation under pentobarbital anesthesia and were placed in ice-cold 0.9% NaCl. The tissues were homogenized, and the microosomal fractions were prepared as described previously and assayed for protein colorimetrically (BioRad Laboratories).

The protocol was approved by the Animal Research Committee of the School of Medicine, Kanazawa University.

Measurements of Renal 11β-HSDII Activity
Urinary free corticosterone:free 11-dehydrocorticosterone ratio was measured to estimate renal 11β-HSD activity, as previously reported. For the extraction of urinary free steroids, 1 mL of urine containing [1H]corticosterone (3000 cpm, Amersham Japan) or [1H]11-dehydrocorticosterone was passed through a prewashed (5 mL methanol, 10 mL water) Sep-Pak C18 cartridge (Waters). After the cartridge was washed with 10 mL water, steroids were eluted with 2×3 mL methanol. The combined eluates were evaporated to dryness, redissolved in 40% methanol, and chromatographed in a reversed-phase HPLC system, followed by radioimmunoassay and individual recovery measurements. [1H]11-dehydrocorticosterone was synthesized in vitro by incubation of rat kidney NRK-52E cells (Dai Nippon Seiyaku, Tokyo, Japan) with 50 μCi [1H]corticosterone for 24 hours in Dulbecco’s modified Eagle’s medium at 37°C, as previously reported. Antibodies of corticosterone and 11-dehydrocorticosterone were purchased from Cosmo Bio Corp.

Measurements of Vascular 11β-HSDII Activity
11β-HSDII activity in mesenteric arteries was determined by measuring the rate of conversion of [1H]corticosterone to [1H]11-dehydrocorticosterone at 37°C for 30 minutes. The reaction mixture contained 20 μL of microsomal fraction of mesenteric arteries (250 μg protein), 10 μL of 1.12×10^8 mol/L [1H]corticosterone, 250 μmol/L of NAD⁺, and 60 mmol/mL of corticosterone, as previously reported. The reaction was stopped by adding volumes of ethyl acetate. Metabolites of corticosterone were separated by HPLC as mentioned above.

Measurements of Urinary 11β-HSDII Inhibitory Factors
For determination of the retention time of 11β-HSDII inhibitory factors, urine extracts were diluted with methanol to a final concentration of 30% methanol and chromatographed on a C18 Ultrasphere ODS column (5 μm, Beckman Instruments). Components were eluted with a methanol gradient beginning with 30% aqueous methanol that increased linearly to 100% methanol by 60 minutes at a flow rate of 1 mL/min. Each fraction was evaporated under nitrogen gas and assayed for inhibitory activity in 11β-HSDII radioenzymatic assays. Radioenzymatic assay of urinary 11β-HSDII inhibitory activity was performed by a procedure based on previously described methods. Briefly, human renal cortex microsomes (250 μg protein) were incubated at 37°C for 30 minutes with 1.2×10^7 mol/L [1H]corticosterone and 250 μmol/L of NAD⁺ in 50 mmol/L Tris-HCl buffer (pH 8.5) in a total volume of 0.25 mL. For the assay, an aliquot of either water (control), urine sample (methanol-hydrolyzed) that were major peak separated by HPLC, or 0.5% aqueous ethanol solution of glycyrrhetinic acid (GA) was added. The reaction was terminated by the addition of 4 mL of ethyl acetate. Metabolites of corticosterone were separated by HPLC as mentioned above, and the percentage of conversion of corticosterone to 11-dehydrocorticosterone was calculated. The percent inhibition was calculated relative to picomoles of GA (GA equivalence units) with the appropriate GA standard curve.

Competitive Polymerase Chain Reaction Assay of Renal 11β-HSDII mRNA
Rat kidneys and mesenteric arteries were removed immediately after decapitation with the animals under pentobarbital anesthesia and were frozen in liquid nitrogen and stored at −80°C before use. Total RNA from rat renal cortex and mesenteric arteries was isolated with guanidine thiocyanate, followed by centrifugation in a cesium chloride solution. One microgram of total RNA was incubated at 42°C for 60 minutes with 2.5 U M-MLV reverse transcriptase (RT) (Perkin-Elmer Japan) in a 20-μL reaction mixture containing random hexanucleotide primers. After incubation for 5 minutes at 99°C, the single-stranded cDNA in the 20-μL reaction mixture was amplified with a polymerase chain reaction (PCR) mixture containing 0.2 mmol/L of each dNTP. The reaction was followed by incubation at 92°C for 3 minutes and 30 cycles of the following sequential steps: 92°C for 1 minute, 60°C for 1 minute, and 72°C for 2 minutes.

The sequences of sense and antisense primers for 11β-HSDII were 5′-AATCCGTTGCGC-3′ and 5′-TTCAGTCTACCA-CACAG-3′, respectively, as previously described. The sense and antisense primers for 11β-HSDII correspond to nucleotides 1208 to 1227 and 1503 to 1522, respectively, of the complementary DNAs. The competitive templates for 11β-HSDII were made with the use of the PCR MIMIC Construction Kit (Clontech), as previously reported. After quantification, a set of serial dilutions was used as an internal standard for competitive PCR. Competitive PCR was performed with 2.5 μL of the reverse-transcribed DNA, 2 μL of different concentrations of the competitive template, 0.5 μmol/L of each of sense and antisense primers, and 0.5 U of Taq DNA polymerase (Perkin-Elmer Japan) in 50 μL of 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 2 mol/L MgCl₂, and 0.2 mmol/L of each dNTP. The reactions were performed for 1 minute at 94°C, 1 minute at 95°C, and 2 minutes at 72°C for 30 cycles. Ten-microliter aliquots of amplification products were electrophoresed on a 3.0% agarose gel. The gel was stained with ethidium bromide and photographed.

The signal intensity was quantified by computerized densitometry with the BIO-PROFIL BIO-1D system (Compak). The intensities of each product from cDNA and from competitive templates were plotted as a function of the known amounts of the competitive templates. To test the yield and the efficiency of the reverse transcriptase reaction, 1 μg of total RNA was subjected to reverse transcription as above, with 5 μmol/L of radioactively labeled [32P]dCTP (New England Nuclear) added to the reaction. The total volume of the RT reaction was increased to 30 μL. Before the addition of enzyme, 1 μL of the reaction was removed for the determination of trichloroacetic acid (TCA)–precipitable counts (background). After 1 hour of incubation at 42°C, 1 μL of the reverse transcription was taken out for measurement of incorporated labeled dCTP. Samples were precipitated in cold 5% TCA and filtered on Whatman GF/C fiberglass filters (Whatman Inc) under a slight vacuum. Filters were dried and placed in scintillation vials. After addition of scintillation fluid, samples were counted in a β-counter.

The amount of DNA synthesized was calculated by multiplying the fraction of total dCTP incorporated into TCA-precipitated counts per
minute by the number of nanomoles of each dNTP in the reaction and the average weight of all 4 dNTPs. The intra-assay and interassay variabilities of the competitive PCR were 11.5% and 14.8%, respectively. The concentration of 11β-HSDII mRNAs was expressed as attomoles per 100 ng of RNA.

The RT-PCR products in 10-μl aliquots were electrophoresed on a 3% agarose gel and transferred to nylon membranes. The membranes were prehybridized in 50% formamide, 5× SSC (1× SSC: 0.15 mol/L NaCl, 0.015 mol/L sodium citrate), 5× Denhardt’s reagent, 1% SDS, and 0.5 g/L salmon sperm DNA at 50°C for 6 hours. They were then hybridized in the same buffer at 50°C for 15 hours with the specific oligoprobes for 11β-HSDII (5’-GCCATCATGATGCACTGCT-3’) that had been end-labeled with [32P]ATP (6000 Ci/mmol, New England Nuclear) with a 5’-end oligonucleotide labeling kit. Next, the membrane was washed twice in 2× SSC/0.1% SDS at room temperature for 20 minutes and twice in 0.1× SSC/0.1% SDS at 50°C for 20 minutes in preparation for autoradiography.

Data are expressed as mean±SEM. The significance of differences was assessed by 1-way ANOVA and a multiple comparison test. Statistical significance was accepted for P<0.05.

Results

Renal 11β-HSDII inhibitory activity, as determined by HPLC analysis, peaked at 25 to 29 minutes (Figure 1). The standards for cortisol, cortisone, corticosterone, 11-dehydrocorticosterone, deoxycorticosterone, and progesterone were eluted at the different retention times of this peak.

The Table shows body weight, systolic blood pressure, heart rate, plasma sodium and potassium, and plasma corticosterone and aldosterone concentrations of DS, DR, and SD rats. The blood pressure of 8- and 12-week-old DS rats on a high salt diet was significantly higher than that of DR, SD, or DS rats on a low salt diet of the same age (P<0.05). The plasma potassium and sodium concentrations did not differ between experimental groups. Plasma aldosterone concentrations were significantly lower in DS rats on a high salt diet than in DR, SD, or DS rats on a low salt diet (P<0.05). Plasma corticosterone concentrations did not show any significant differences between groups. Urinary free corticosterone/free 11-dehydrocorticosterone ratio was significantly higher in 8- and 12-week-old DS rats on a high salt diet than in SD, DR, or DS rats on a low salt diet of the same age (P<0.05) (Figure 2). There were no significant differences in these parameters between 4-week-old DS, DR, and SD rats. 11β-HSDII activity in mesenteric arteries of 8- and 12-week-old DS rats on a high salt diet was significantly decreased compared with DR, SD, or DS rats on a low salt diet of the same age (P<0.05) (Figure 3). Specific mRNA for 11β-HSDII could be detected in rat renal cortex and mesenteric artery by PCR analysis. Figure 4 shows that increasing concentrations of each competitive template for 11β-HSDII from 0 to 80×10⁻³ attomoles per microliter increasingly inhibited the amplification of endogenous 11β-HSDII in kidney. Renal 11β-HSDII mRNA levels in 8- and 12-week-old DS rats on a high salt diet were significantly lower than those in SD, DR, or DS rats on a low salt diet of the same age (P<0.05) (Figure 2). There were no significant differences in 11β-HSDII mRNA levels between 4-week-old SD, DS, and

![HPLC profile shows renal 11β-HSDII inhibitory factors (11β-HSDII).](http://hyper.ahajournals.org/)

**Body Weight, Systolic Blood Pressure, Heart Rate, Serum Electrolytes, and Plasma Aldosterone and Corticosterone Concentrations in Experimental Rats**

<table>
<thead>
<tr>
<th></th>
<th>Body Weight, g</th>
<th>SBP, mm Hg</th>
<th>HR, bpm</th>
<th>Plasma Na, mmol/L</th>
<th>Plasma K, mmol/L</th>
<th>Plasma Aldosterone, pmol/L</th>
<th>Plasma Corticosterone, nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD (n=10)</td>
<td>122±5</td>
<td>100±4</td>
<td>418±8</td>
<td>146±1.6</td>
<td>4.3±0.2</td>
<td>380±50</td>
<td>41±6</td>
</tr>
<tr>
<td>DR (n=10)</td>
<td>118±3</td>
<td>97±16</td>
<td>384±16</td>
<td>146±1.4</td>
<td>4.4±0.1</td>
<td>360±60</td>
<td>43±9</td>
</tr>
<tr>
<td>DS (n=10)</td>
<td>128±3</td>
<td>108±3</td>
<td>424±7</td>
<td>142±0.9</td>
<td>4.0±0.1</td>
<td>290±80*</td>
<td>36±8</td>
</tr>
<tr>
<td>8 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD (n=10)</td>
<td>266±13</td>
<td>108±3</td>
<td>420±9</td>
<td>145±1.1</td>
<td>4.4±0.1</td>
<td>150±30</td>
<td>45±5</td>
</tr>
<tr>
<td>DR (n=10)</td>
<td>265±15</td>
<td>106±4</td>
<td>412±13</td>
<td>145±1.0</td>
<td>4.2±0.1</td>
<td>180±20</td>
<td>50±6</td>
</tr>
<tr>
<td>DS (n=10)</td>
<td>281±17</td>
<td>228±17*</td>
<td>385±18</td>
<td>145±1.0</td>
<td>3.9±0.2</td>
<td>110±20*</td>
<td>51±9</td>
</tr>
<tr>
<td>Low salt DS (n=10)</td>
<td>275±20</td>
<td>120±6</td>
<td>408±11</td>
<td>145±0.2</td>
<td>4.0±0.1</td>
<td>145±18</td>
<td>48±8</td>
</tr>
<tr>
<td>12 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD (n=10)</td>
<td>367±16</td>
<td>110±9</td>
<td>407±10</td>
<td>143±1.2</td>
<td>4.3±0.2</td>
<td>149±30</td>
<td>43±6</td>
</tr>
<tr>
<td>DR (n=10)</td>
<td>355±18</td>
<td>115±11</td>
<td>410±15</td>
<td>146±0.9</td>
<td>4.2±0.1</td>
<td>167±25</td>
<td>48±8</td>
</tr>
<tr>
<td>DS (n=10)</td>
<td>348±14</td>
<td>235±25*</td>
<td>378±21</td>
<td>148±1.1</td>
<td>3.8±0.3</td>
<td>106±20*</td>
<td>49±8</td>
</tr>
<tr>
<td>Low salt DS (n=10)</td>
<td>360±21</td>
<td>130±14</td>
<td>386±25</td>
<td>144±1.2</td>
<td>4.2±0.2</td>
<td>140±22</td>
<td>45±6</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

SBP indicates systolic blood pressure; HR, heart rate; bpm, beats per minute; and Low salt DS, DS rats on a low salt diet.

*P<0.05 vs DR or SD rats.
The concentration of 11β-HSDII mRNA in mesenteric arteries of 8- and 12-week-old DS rats on a high salt diet was significantly lowered compared with DR, SD, or DS rats on a low salt diet of the same age (P<0.05) (Figure 3). The urinary excretion of the endogenous 11β-HSDII inhibitory factor(s) was significantly increased in 8- and 12-week-old DS, DR, and DS rats on a low salt diet compared with SD, DR, or DS rats on a low salt diet of the same age (P<0.05) (Figure 5). The urinary excretion of the inhibitory factor(s) did not differ between 4-week-old DS, DR, and SD rats. We measured urinary excretion of 11β-HSDII inhibitory factor(s) using whole urine extracts, as previously reported.10 The urinary excretion of the inhibitory factor(s) from whole urine extracts was also increased in DS rats on a high salt diet (data not shown).

Discussion

The effect of aldosterone on sodium absorption has been studied in classic mineralocorticoid-sensitive tissues such as kidney, colon, and salivary glands. Mineralocorticoid receptors and 11β-HSDII mRNA are similarly distributed in human and rat kidney.1 The crucial physiological role of 11β-HSDII in conferring aldosterone selectivity on mineralocorticoid receptor in epithelial tissues has been amply demonstrated by the recent finding that patients with the syndrome of apparent mineralocorticoid excess (AME) have point mutations or deletions in the 11β-HSDII gene, resulting in diminished or absent function.25,26 Urinary free cortisol:free cortisone ratio is reported to be a sensitive index of renal 11β-HSDII activity in humans.20 We estimated this ratio as renal 11β-HSDII activity in rat. Our study showed that urinary free corticosterone:free 11-dehydrocorticosterone ratio in DS rats was increased compared with DR or SD rats. The levels of 11β-HSDII mRNA in the kidney were lower in DS rats than in DR or SD rats. These results indicate that renal 11β-HSDII activity is decreased in DS rats. Decreased renal 11β-HSDII activity may play a role in salt sensitivity and development of hypertension in DS rats. However, in rats 11β-HSDII in kidney is more abundant than 11β-HSDII.27 Brem et al28 reported that a high sodium diet increases 11β-HSDII activity and levels of mRNA in normal

11β-HSDII cDNA

Endogenous →
Mimic →

Competitor (10^3 attomol/µL)

Figure 4. Analysis of relative changes in 11β-HSDII mRNA concentrations by competitive PCR. Increasing the concentration of competitive template for 11β-HSDII from 0 to 80×10^3 attomoles per microtiter increasingly inhibited the amplification of endogenous 11β-HSDII cDNA in the kidney or mesenteric artery.
dog kidney but does not change 11β-HSDII activity and levels of mRNA. Franco-Saenz et al\(^29\) demonstrated that hypertensive DS rats (aged 10 weeks) had lower kidney 11β-HSD. In our data, plasma potassium concentrations in DS rats did not differ from those in DR or SD rats. Patients with AME show hypokalemia; however, concentrations of plasma potassium in AME range from 0.9 to 3.8 mmol/L and do not correlate with the activity of 11β-HSD.\(^23\) Recently, normokalemic AME with abnormal 11β-HSDII gene was reported.\(^30\) Normokalemic primary aldosteronism has also been reported.\(^31,32\) Walker et al\(^2\) reported that half-time periods of 11β-
[α-H\(^3\)]cortisol were prolonged in a subgroup of hypertensive patients who did not show hypokalemia. Thus, a mineralocorticoid excess state does not always show hypokalemia. In our data, plasma aldosterone levels were reduced in DS rats. Patients with AME also show low aldosterone levels.\(^22\) Micropuncture studies examining segmental NaCl transport in Dahl rats have demonstrated no differences in NaCl transport beyond the loop segment between S and R strains. Kudo et al\(^33\) reported that cultures from the cortical collecting duct of DS rats show no transport abnormalities compared with cultures from DR rats. DS rats with a low sodium diet did not show hypertension and had no decreased renal 11β-HSDII activity and mRNA levels. Taken together, there is a possibility that reduced 11β-HSDII activity in kidney of DS rats may be a consequence rather than a cause of hypertension. However, 11β-HSDII activity and mRNA levels in blood vessels were decreased in hypertensive DS rats. Smith et al\(^4\) reported the presence of 11β-HSDII in vascular smooth muscle cells by immunohistochemistry. We detected the expression of 11β-HSDII mRNA in cultured vascular smooth muscle cells using RT-PCR methods (data not shown).

There has been increasing evidence that mineralocorticoids, acting on peripheral vascular tissue, cause hypertension.\(^35,36\) Tobian and Redleaf\(^37\) have proposed that aldosterone affects salt and water balance in vascular cells and thereby influences vessel lumen size. We have reported that vascular 11β-HSDI and mRNA were decreased in DS rats.\(^4\) Franco-Saenz et al\(^29\) also reported decreased 11β-HSDI activity in the kidney of DS rats. Not only 11β-HSDII but also 11β-HSDI may play a role in salt sensitivity and development of hypertension in the DS rats. The decreased 11β-HSDII activity in 8- or 12-week-old DS rats was not improved after treatment of hypertension with a calcium channel blocker (data not shown). This change in 11β-HSDII activity in DS rats does not seem to be merely secondary to hypertension.

The excretion of endogenous 11β-HSD inhibitory factor(s) has been reported in human urine.\(^9,38\) Glycyrrhetinic acid (GA), the active agent in licorice root, markedly inhibits 11β-HSD when incubated with this enzyme. Morris et al\(^38\) quantified this 11β-HSD inhibitory factor(s) (glycyrrhetinic acid–like factors [GALFs]) using rat liver microsome and reported increased excretion in pregnancy. Walker et al\(^39\) reported that concentrations of GALFs do not show diurnal rhythm and are unaffected by dexamethasone treatment in patients with low corticotropin or in patients with ectopic corticotropin secretion. They also reported that in hypertensive patients with impaired 11β-HSD activity, GALF concentrations do not correlate with blood pressure, and they concluded that GALFs are unlikely to be involved in the pathophysiology of hypertension.\(^40\) However, Semafuko et al\(^41\) demonstrated that urinary GALF was increased in patients with congestive heart failure. It was hypothesized that 11β-HSDII inhibitory factors would serve to cause glucocorticoids, and possibly other steroids, to elicit Na\(^+\) retention by mineralocorticoid-mediated mechanisms and therefore augment, either naturally or in disease states, the Na\(^+\)-retaining actions of aldosterone. We have reported that 11β-HSDII inhibitory factors exist in human urine, and urinary excretion of these factors is increased in subgroups of hypertensive patients.\(^9\) Souness et al\(^7\) reported that 11α- and 11β-hydroxyprogesterone are potent inhibitors of 11β-HSDII and are extremely active in conferring mineralocorticoid Na\(^+\)-retaining activity on corticosterone in vivo in a rat bioassay. They also recently reported the hypertensinogenic activity of these progesterone metabolites in the rat.\(^8\) In this experiment, urinary excretion of 11β-HSDII inhibitory factors was increased in DS rats compared with DR or SD rats. Lo et al\(^10\) reported that kidney 11β-HSDII is inhibited by urinary 11β-HSDII inhibitory factors extracted and partially purified from human urine. There is a possibility that increased 11β-HSDII inhibitory factors may directly or indirectly decrease the 11β-HSDII activity in kidney or blood vessel in hypertensive DS rats. Decreased 11β-HSD mRNA levels might suggest that these factors operate at the transcriptional level. Further studies are needed to determine not only the chemical structures of the renal 11β-HSDII inhibitory factor(s) but to reveal the source and their pathophysiological roles.

References
3. Soro A, Ingram MC, Tonolo G, Glorioso N, Fraser R. Evidence of coexisting changes in 11β-hydroxysteroid dehydrogenase and...
19. Stewart PM, Murry BA, Mason JJ. Human kidney 11β-hydroxysteroid dehydrogenase is a high affinity nicotinamide adenine dinucleotide-dependent enzyme and differs from the cloned type I isozyme. J Clin Endocrinol Metab. 1994;79:480–484.
Renal 11β-Hydroxysteroid Dehydrogenase in Genetically Salt-Sensitive Hypertensive Rats
Yoshiyu Takeda, Satoru Inaba, Kenji Furukawa and Isamu Miyamori

Hypertension. 1998;32:1077-1082
doi: 10.1161/01.HYP.32.6.1077

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://hyper.ahajournals.org/content/32/6/1077

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/