In Vivo 11β-HSD-2 Activity
Variability, Salt-Sensitivity, and Effect of Licorice

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Abstract—Loss-of-function mutations or inhibition of 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD-2) results in overstimulation of the mineralocorticoid receptor by cortisol and causes salt-sensitive hypertension. Traditionally, 11β-HSD-2 activity has been assessed by measurement of the urinary cortisol metabolite ratio (tetrahydrocortisol [THF]/5α-THF)/tetrahydrocortisone (THE). Recently, the ratio of urinary free glucocorticoids, UFF/UFF, has been suggested to be a more reliable parameter, an aspect that has not been investigated systematically. Steroid metabolites were measured repeatedly by gas chromatography–mass spectrometry in 20 healthy subjects at baseline and after 1 week each of a 30- or 180-mmol/d of sodium diet or 500 mg/d of glycyrrhetinic acid. Intraindividual coefficients of variation from 3 random urine collections for (THF+5α-THF)/THE and UFF/UFF ratios were 11±9% and 25±14% (P<0.001). (THF+5α-THF)/THE was more sensitive than UFF/UFF for detection of glycyrrhetinic acid–induced increases higher than the upper 95% confidence interval of the coefficient of variation of the corresponding ratio. Low- or high-salt diet did not alter either ratio. Mean (THF+5α-THF)/THE but not UFF/UFF was higher in salt-sensitive than salt-resistant subjects. Absolute glycyrrhetinic acid–related increase in (THF+5α-THF)/THE but not UFF/UFF was higher in salt-sensitive than salt-resistant subjects and correlated with changes in mean BP. Intraindividual variability of (THF+5α-THF)/THE is lower than that of UFF/UFF. The UFF/UFF ratio does not appear to be more sensitive than (THF+5α-THF)/THE for detection of decreased 11β-HSD-2 activity. The (THF+5α-THF)/THE ratio better discriminates between salt-sensitive and salt-resistant subjects. Together with BP responses to glycyrrhetinic acid, these findings support a pivotal role of 11β-HSD-2 in salt sensitivity. (Hypertension. 2001;38:1330-1336.)

Key Words: hypertension, salt-sensitive ■ sodium, dietary ■ 11β-hydroxysteroid dehydrogenase ■ urine ■ glycyrrhetinic acid

The nonselective mineralocorticoid receptor (MR) has the same in vitro affinity for its physiological substrate aldosterone as for the glucocorticoids cortisol in humans and corticosterone in rodents.1 MR normally is protected from aldosterone as for the glucocorticoids cortisol in humans and overactivation of MR by cortisol.9 Mutations in the 11α-HSD-2 gene cause renal sodium retention, urinary potassium wasting, and low-renin, low-aldosterone hypertension because of excess cortisol binding to MR. Moreover, the activity of 11β-HSD-2 potently is blocked in vivo and in vitro by glycyrrhetinic acid (GA), the active compound of licorice, by 2 mechanisms, direct competitive inhibition10 and pretranslational inhibition.11 Thus, administration of high doses of GA12 and mutations in 11α-HSD-2 are phenotypically identical. These and other findings13,14 suggest that impaired 11β-HSD-2 activity may play a role in the pathogenesis of essential hypertension in some patients.15

When 11β-HSD-2 activity is decreased, urinary excretion of active cortisol (tetrahydrocortisol [THF]) to inactive cortisone (tetrahydrocortisone [THE]) metabolites is increased.9,12 Traditionally, this steroid profile has been assessed by the urinary ratio of (THF+5α-THF)/THE according to the method described by Wilson et al9 and Shackleton16 that used gas chromatography. Recent data suggest that the measurement of urinary free cortisol (UFF) and cortisone (UFE) might be more sensitive and specific for assessment of renal 11β-HSD-2 activity in vivo.17,18 However, these data have been obtained from different subjects and under different conditions, were not repeated, and were not analyzed prospectively for intraindividual variabilities. Thus, which of the urinary steroid profiles is more reliable for assessment of 11β-HSD-2 activity in humans is unclear.
Therefore, we investigated in a prospective study whether measuring UFF/UFE is superior to and more sensitive than measuring urinary (THF + 5α-THF)/THE to assess in vivo 11β-HSD-2 activity under different conditions and whether the role of the 11β-HSD-2 enzyme in human salt sensitivity could be characterized by either parameters by use of interventions that modify the enzymatic activity.

Methods

Subjects, Study Design, and Analytical Methods

To assess the effect of day-to-day variability, GA, or low- or high-salt diet on urinary (THF + 5α-THF)/THE and UFF/UFE as parameters of 11β-HSD-2 activity in vivo, 20 healthy volunteers (age 25±4 years; 13 men, 7 women) collected 12 separate 24-hour urine samples at different time points and under different conditions. Inclusion criteria for the participants were age between 18 and 45 years, BP 140/90 mm Hg, and normal renal and liver function. Subjects who were pregnant (as determined by β-HCG assay) were excluded. Subjects were not allowed to take any medication, including licorice and oral contraceptives. All participants gave written informed consent to the study, which was approved by the local ethical committee. At baseline, demographic data and basic hematocellular parameters, including plasma renin and aldosterone levels, were obtained. Thereafter, all volunteers underwent the following protocol.

1. To assess intraindividual day-to-day variability of (THF + 5α-THF)/THE and UFF/UFE, volunteers collected 3 samples of 24-hour urine, with each collection occurring ≥ 4 days apart.
2. While on the same diet, which contained ~150 mmol/d of sodium, volunteers ingested 500 mg/d of GA for 1 week. Again, 24-hour urine samples were collected at days 1, 3, and 7 for measurement of urinary steroids to assess whether inhibition of 11β-HSD-2 by GA is reflected better by the (THF + 5α-THF)/THE or UFF/UFE ratio. Plasma renin and aldosterone levels were also measured at day 7 of GA.
3. To assess whether urinary (THF + 5α-THF)/THE or UFF/UFE ratios change as a function of the amount of salt intake, after a 1-week washout period, all subjects received a low-salt diet for 1 week (30 mmol/d NaCl, prepared by the hospital kitchen) and a high-salt diet for another week (same diet supplemented with 150 mmol/d NaCl, 18×500-mg capsules). On days 3, 6, and 7 of each diet period, urine was collected for 24 hours for analysis of urinary steroids. Office BP changes between low- and high-salt diet at the end of each phase were used to identify salt-sensitive and salt-resistant subjects (cutoff plus 3.5 mm Hg mean office BP) as previously described (see reference 14).

Body weight and office BP were recorded at all time points. For each urine, the volume and creatinine, sodium, and potassium excretion were measured. A 24-hour ambulatory BP recording was performed once during the baseline study period and on day 7 of the GA period. Urine samples were analyzed by gas chromatography–mass spectrometry on a Hewlett-Packard gas chromatograph (model 6890) equipped with a mass selective detector (model 5973) as previously described.14,19

Statistical Analysis

Evaluation criteria consisted of the intraindividual variability of (THF + 5α-THF)/THE or UFF/UFE and the sensitivity of (THF + 5α-THF)/THE or UFF/UFE to detect changes during administration of GA or low- or high-salt diet. Differences between means were assessed by t test or ANOVA for analysis of continuous variables and by nonparametric analysis for variables that were not normally distributed. Analyses were performed by use of the Systat 9.0 (SPSS Inc) statistical software package. Values are expressed as mean±SD or median and 95% confidence interval (CI).

Results

Baseline Intraindividual Variability of Parameters of 11β-HSD-2 Activity

Values of (THF + 5α-THF)/THE or UFF/UFE for 3 separate random baseline urine collections are reported in Figure 1. When the values from days 1, 4, and 8 were pooled, the (THF + 5α-THF)/THE ratio was 0.94±0.27 and the UFF/UFE ratio was 0.41±0.13. As a measure of within-subject variability, the intraindividual coefficient of variation for the 2 ratios was derived from the values of the 3 baseline urine samples. Intraindividual variability was lower for (THF + 5α-THF)/THE (11±9%) than for UFF/UFE (25±14%, P<0.001). Urinary (THF + 5α-THF)/THE ratio correlated weakly with UFF/UFE ratio (R² 0.164; P<0.004). Intraindividual variability of total tetrahydrometabolites or total free glucocorticoids excreted in the urine was of the same magnitude (23.2±13.0% for THF, 5α-THF, and THE versus 21.0±11.6% for UFF and UFE).
Seven days of GA at a dosage level of 500 mg/d increased both (THF+5α-THF)/THE and UFF/UFE ratios to values greater than baseline (Figure 2A). Increment of the ratios from baseline at days 1, 3, and 7 was 66%, 93%, and 85%, respectively, for (THF+5α-THF)/THE and 103%, 167%, and 209%, respectively, for UFF/UFE. The skew in the distribution of ratios during GA was due to 1 subject, whose (THF+5α-THF)/THE and UFF/UFE ratios increased to 4.29 and 2.27, respectively after GA (Figure 2A). A value higher than the upper 95% CI of the coefficient of variation for random urine samples at baseline was considered to be a significant GA-induced increase in either ratio. At this cutoff, only 2 subjects were below the limit (change plus 15.5%) for (THF+5α-THF)/THE, whereas 7 subjects were below the limit (change plus 31.6%) for UFF/UFE (Figure 2B). During GA administration, urinary (THF+5α-THF)/THE correlated positively with UFF/UFE (R² = 0.223; P < 0.0001).

**Effect of Salt Depletion and Salt Load on Parameters of 11β-HSD-2 Activity**

One week of low- or high-salt diet did not affect the (THF+5α-THF)/THE or UFF/UFE ratio (Table). Average values of urinary (THF+5α-THF)/THE ratio tended to be higher in men than in women, whereas average UFF/UFE ratio tended to be higher in women than in men, although gender differences were not significant.

**BP, Body Weight, Urinary and Plasma Electrolytes, and Other Hormones**

Office systolic BP increased (F ratio = 3.547; P = 0.018 by ANOVA), whereas diastolic BP remained unchanged during GA administration (Figure 3). Ambulatory BP was higher at day 7 of GA than at baseline (126/77 ± 10/7 versus 115/73 ± 6 mm Hg; P < 0.001 for systolic and P < 0.05 for diastolic). A small but not significant increase occurred in body weight after 7 days of GA (from 70.3 ± 6.7 to 72.1 ± 6.7 kg). Urinary sodium excretion and sodium/potassium ratio tended to be lower at days 1 and 3 of GA versus corresponding values at baseline and increased again at day 7 of GA, which suggests mineralocorticoid escape (Figure 3). Urinary aldosterone/potassium ratios decreased from 0.25 ± 0.17 at baseline to 0.11 ± 0.07 μg/mmol at day 7 of GA (F ratio = 5.564; *P* < 0.002). Plasma sodium tended to increase from 136.5 ± 0.6 to 138.7 ± 0.7 mmol/L (F ratio = 3.095; *P* = 0.05) and potassium decreased from 4.1 ± 0.1 to 3.7 ± 0.1 mmol/L (F ratio = 29.6; *P* < 0.0001) during GA administration. Compared with baseline, a significant decrease occurred in plasma renin (14.3 ± 10.9 versus 8.2 ± 6.4 ng/L; *P* < 0.05) and aldosterone (354 ± 179 versus 210 ± 132 pmol/L; *P* < 0.01) at day 7 of GA.

**Parameters of 11β-HSD-2 Activity in Salt-Sensitive and Salt-Resistant Subjects**

Difference in mean office BP between high and low salt identified 6 salt-sensitive (7.5 ± 2.8 mm Hg) and 14 salt-re-
sistant \(-1.1 \pm 3.7 \text{ mm Hg}\) subjects. Gender distribution and body habitus were comparable in these 2 groups. At baseline, (THF+5α-THF)/THE ratio was higher in salt-sensitive than in salt-resistant subjects \((1.18 \pm 0.31 \text{ versus } 0.83 \pm 0.18, P<0.05)\), whereas UFF/UFE ratio was not significantly different between groups \((0.36 \pm 0.07 \text{ versus } 0.42 \pm 0.14)\). After GA, a more pronounced increase in (THF+5α-THF)/THE was observed in salt-sensitive than salt-resistant subjects, whereas no such difference was observed when the UFF/UFE ratio was considered. A stronger increase occurred in office mean BP in salt-sensitive \(6 \pm 7, 7 \pm 5, \text{ and } 9 \pm 5 \text{ mm Hg at days } 1, 3, \text{ and } 7\) than in salt-resistant subjects \(3 \pm 4, 5 \pm 2, \text{ and } 6 \pm 3 \text{ mm Hg at days } 1, 3, \text{ and } 7\), respectively; \(F \text{ ratio } 6.814; P=0.012\). The increase in 24-hour mean BP was more marked in salt-sensitive than in salt-resistant subjects \(15 \pm 6 \text{ versus } 9 \pm 4 \text{ mm Hg}; P<0.05\). GA intake increased both BP and urinary steroid ratios, which reflected \(11\beta\)-HSD-2 activity. The increase in body weight at day 7 of GA also tended to be more pronounced in salt-sensitive \(1.7 \pm 1.2 \text{ kg}\) than salt-resistant \(0.8 \pm 1.2 \text{ kg}\) subjects.

**Discussion**

Impaired \(11\beta\)-HSD-2 activity causes hypertension and has been related to salt sensitivity.\(^{14,15,20}\) To investigate \(11\beta\)-HSD-2 activity in disease states, correct measurement of enzyme activity in vivo is imperative. Traditionally, \(11\beta\)-HSD-2 activity has been assessed by gas-chromatographic assay of the urinary (THF+5α-THF)/THE ratio.\(^6\) Recent data suggest that the measurement of UFF and UFE is more sensitive and specific for assessment of renal \(11\beta\)-HSD-2 activity in vivo.\(^{17,18}\) The present study indicates (1) that intraindividual variability of (THF+5α-THF)/THE is substantially lower than that of UFF/UFE, (2) that the UFF/UFE ratio is not superior for detection of reduced \(11\beta\)-HSD-2 activity, (3) that the (THF+5α-THF)/THE ratio better discriminates between salt-sensitive and salt-resistant subjects than the UFF/UFE ratio, and (4) that \(11\beta\)-HSD-2 activity as assessed by (THF+5α-THF)/THE but not UFF/UFE correlates with changes in BP, which indicates biological relevance of the urinary (THF+5α-THF)/THE ratio.

The present investigation demonstrates a higher intraindividual variability of UFF/UFE \(25 \pm 14\%\) than (THF+5α-
cluded that the UFF/UFE ratio is a more sensitive index of congenital 11β-HSD-2 deficiency normalized UFF/UFE but not (THF+5α-THF)/THE. However, interpretation of these findings is not unequivocal, because cortisol metabolites were measured after a dosage of 15 mg of exogenous cortisol in addition to the synthetic glucocorticoids, which act as competitive inhibitors of 11β-HSD-2.

The weak positive correlation between urinary (THF+5α-THF)/THE and UFF/UFE (R²=0.164; P<0.004) suggests that factors other than renal 11β-HSD-2 account for the difference between the 2 ratios. Because no direct measurement of 11β-HSD-2 in renal tissue can be performed in humans, ascertaining the deviation of either ratio from true renal 11β-HSD-2 activity is not possible. Best and Walker tried to dissect 11β-HSD-1 and 11β-HSD-2 activities by sequential treatment with carbenoxolone and GA, assuming differential inhibition of 11β-HSD-1 and 11β-HSD-2 by the 2 compounds. They found that the effect of carbenoxolone was readily observed by measurement of urinary UFF/UFE but was not detectable by measurement of urinary (THF+5α-THF)/THE. Because carbenoxolone, like GA, inhibits 11β-HSD-2 activity both in vitro and in vivo, the findings by Best and Walker cannot be interpreted as proof that the UFF/UFE ratio is superior to the (THF+5α-THF)/THE ratio for assessment of in vivo 11β-HSD-2 activity. Thus, the assumption that (THF+5α-THF)/THE is an index for both 11β-HSD-1 and 11β-HSD-2 activity, although UFF/UFE is a more accurate parameter of 11β-HSD-2 activity, seems questionable. This is corroborated by the observation of the largely unaltered urinary ratio of corticosterone to dehydrocorticosterone metabolites in 11β-HSD-1 knockout mice.

In the present study, GA was given for 1 week at a dosage that produced a significant mineralocorticoid effect, as demonstrated by an increase in mean 24-hour BP and plasma sodium as well as decreased levels of plasma potassium, plasma renin, and aldosterone and a decreased urinary aldosterone/potassium ratio. In terms of inhibition of 11β-HSD-2 activity, this regimen resulted in an ~200% increment of the UFF/UFE ratio and a 90% increment of the (THF+5α-THF)/THE ratio after 7 days. Only 1 previous study reported both GA-induced mineralocorticoid effects and changes in urinary A-ring–reduced glucocorticoids. In 30 healthy volunteers, ingestion of 100 g of licorice (∼270 mg of GA) caused a moderate and (after withdrawal of the licorice) reversible rise in systolic BP (6.5 mm Hg) and a fall in plasma potassium (∼0.24 mmol/L), whereas the mean (THF+5α-THF)/THE ratio increased by 55%. These 2 observations taken together indicate a dose-response relationship between dosage of GA and changes in urinary steroids, BP, and serum potassium. Thus, a clinically significant GA-induced mineralocorticoid response seems to be readily reflected by the increase in (THF+5α-THF)/THE as a measure for in vivo 11β-HSD-2. Not all individuals challenged with GA responded...
with significant inhibition of 11β-HSD-2 activity according to selected cutoff criteria. This occurrence could be due to differences in compliance of the subjects or bioavailability of the compound associated with the composition of the diet. For instance, bioavailability of GA was found to be increased by the hydrophilic but not the lipophilic components of glycyrrhiza extract in rats.26

Because the increase in BP associated with decreased 11β-HSD-2 activity is salt dependent, assessment of whether changes in dietary salt consumption affect activity of the enzyme is important. To date, the urinary steroid profile under low salt or high salt has been systematically studied only in rats,27,28 not in humans. By use of a standardized low-sodium diet for 2 weeks supplemented by salt capsules during the second week to increase sodium intake from 30 to 180 mmol/d, no effect on in vivo 11β-HSD-2 activity was identified either by the UFF/UFE or (THF+5α-THF)/THE ratios in our healthy volunteers (Table 1). That dietary sodium may alter 11β-HSD-2 in humans of a different ethnic background, as suggested by animal experiments, cannot be ruled out. For instance, in Wistar rats, dietary sodium did not affect 11β-HSD-2 mRNA expression in collecting tubules of the medulla, but tissue 11β-HSD-2 activity was lower after a high-sodium diet.28 In contrast, in Dahl salt-sensitive rats, renal 11β-HSD-2 activity and mRNA expression were reduced with high but not low salt versus Dahl salt-resistant or Sprague-Dawley rats.29 Recently, we described decreased 11β-HSD-2 activity in salt-sensitive subjects based on measures of the urinary (THF+5α-THF)/THE ratio.14 The present data confirm this previous observation in a different group of subjects and also indicate that, at baseline, urinary (THF+5α-THF)/THE is superior to UFF/UFE for discriminating between salt-sensitive and resistant subjects. Moreover, the GA-induced increase in both BP and (THF+5α-THF)/THE is more pronounced in salt-sensitive than in salt-resistant subjects (Figure 4). Several reasons for the difference in the GA-related effects on urinary steroids and BP in salt sensitivity need to be considered. That salt-sensitive subjects might have had higher GA concentrations seems unlikely, given that the 2 groups who ingested a constant amount of the inhibitor were matched for body habitus. Increased affinity of the 11β-HSD-2 enzyme for GA can be expected in the presence of a mutant enzyme with modified amino acid sequence. However, although a genetic basis for salt sensitivity has been documented,1,2,4,14,20 decreased expression of the enzyme rather than an enzyme with altered amino acid sequence can be expected in salt-sensitive subjects, because the frequency of homozygosity for mutated alleles of the HSD11B2 gene is estimated to be <1/250 000 in whites.29 The increase in urinary (THF+5α-THF)/THE on GA was smaller in salt-resistant than in salt-sensitive subjects, although the latter also had higher baseline values for this ratio. This occurrence suggests that the effect of inhibition may depend on the sensitivity range of the enzyme kinetics, in which the same amount of GA can produce a more marked increase in the ratio when the enzyme is already largely saturated. For instance, in humans, prednisolone/prednisone ratios were significantly higher during high versus low dose steady-state prednisolone infusion, and 200 mg of GA produced a 1.5-fold higher increase in this ratio during high-dose prednisolone infusion,30 which suggests enzyme saturation. In the present study, the total amount of tetrahydrodrometabolites as a measure of cortisol secretion did not differ between the 2 groups either at baseline or during GA. Thus, the differences in 11β-HSD-2 activity between salt-sensitive and salt-resistant subjects can be explained by a reduced amount of an enzyme with normal substrate affinity. This view is in line with the hypothesis of genetically determined decreased expression of normal 11β-HSD-2 enzyme in some salt-sensitive subjects.14,20

In contrast to previous reports that suggested a clear advantage of UFF/UFE measurements over (THF+5α-THF)/THE, we conclude that the urinary (THF+5α-THF)/THE ratio is equivalent and may even be superior to the UFF/UFE ratio for detection of clinically significant changes in the activity of the renal 11β-HSD-2 in vivo. The GA-related increases in (THF+5α-THF)/THE and BP support the importance of 11β-HSD-2 in salt sensitivity.

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References


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