Measurement of Urinary Free 20α-Dihydrocortisol in Biochemical Diagnosis of Chronic Hypercorticoidism

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Using liquid chromatography, we estimated the urinary excretion of 20α-dihydrocortisol (20-DH) and urinary free cortisol (UFC) in normal subjects and in 40 patients with Cushing's syndrome of different etiologies. The median normal excretion rate (nmol/24 h) was 174 for 20-DH and 68 for UFC, the 20-DH/UFC ratio thus being 2.55. For patients with Cushing's syndrome, the excretion rate was 1798 for 20-DH and 296 for UFC, the ratio 6.03. We evaluated the effect of acute stimulation of adrenal secretion on 20-DH and UFC by administering corticotropin to six normal subjects. After such stimulation, the excretion rate was 566 for 20-DH and 1238 for UFC (ratio 0.45). Whereas 20-DH excretion rate exceeded the normal range in all patients, six patients had normal or even below-normal values for UFC excretion. Evidently, measurement of urinary 20-DH is a better test for chronic hypercorticoidism than is measurement of urinary UFC, and chronic hypercorticoidism can be differentiated from the acute state by the 20-DH/UFC ratio.

Additional Keyphrases: steroids · urine · chromatography, reversed-phase · Cushing's syndrome · adrenal disorders · acute and chronic hypercorticoidism differentiated · free-cortisol excretion compared

Urinary free cortisol (UFC) is considered to reliably reflect the concentration of free cortisol in plasma, useful for the biochemical diagnosis of adrenal disorders involving glucocorticoids (1). However, Streeten et al. (2) found the UFC excretion rate to be normal in several patients who showed overwhelming collateral evidence of hypercortisolism. Voccia et al. (3), evaluating the diagnostic significance of 6α-hydroxy-cortisol excretion in hypercortisolemic states, reported a normal excretion of UFC but an above-normal excretion of 6α-hydroxycortisol in a patient with Cushing's syndrome. In urine from a hypercortisolemic but hypocortisolic patient with Cushing's disease, we recently found large amounts of a cortisol-immunoreactive compound, which was identified as 20α-dihydrocortisol (20-DH) by mass spectrometry (4).

Here, we have measured the excretion of 20-DH and compared it with that of UFC in chronic and acute states of hypercorticoidism. We studied chronic hypercorticoidism in patients with Cushing's syndrome of different etiologies, and acute hypercorticoidism in normal subjects after intravenous administration of corticotropin (19-24). Thus, we have evaluated the potential utility of 20-DH measurements for the biochemical diagnosis of hypercorticoidism and for differentiating the chronic from the acute form.

Materials and Methods

Subjects

Patients. Included in the study were 40 patients with clinically, biochemically, and pathologically proven Cushing's syndrome: 20 women and 12 men with Cushing's disease (bilateral adrenal hyperplasia), six women with adrenal adenoma, and two men with the ectopic-corticotropin syndrome. They had no clinical symptoms or biochemical signs of other organic diseases known to influence steroid excretion.

Six of the patients had normal or below-normal excretion of UFC. There were no clinical features nor pathological findings that we could consider specific for these patients. The biochemical diagnoses of hypercorticoidism were based on pathological results for dexamethasone suppression tests, loss of circadian rhythmicity of cortisol secretion, or both. In five of these patients, urinary excretion rates of 15 non-metabolized steroids had been studied (5). Except for dehydroepiandrosterone, which was slightly increased in one patient, all other steroids were excreted at normal or below-normal rates. In one patient, UFC was determined daily during a four-day dexamethasone test at 2 mg/day dosage and a following four-day test at 8 mg/day dosage. Whereas above-normal cortisol concentrations in serum decreased slightly under the 8-mg dosage only, values for UFC were below normal during the whole test period.

Controls. For comparison, we investigated 22 normal, non-obese students without evidence of any metabolic, endocrine, renal, or hepatic disease.

Samples

We collected 24-h urine specimens from the patients and the controls, storing the urine at 4 °C during the period of collection, with no preservatives. From the collected urine, we took aliquots of about 10 mL and stored them frozen until analysis. Completeness of collecting was roughly checked by measuring the urinary creatinine excreted. From the controls, we determined one urine specimen, from the patients one or two.

Procedures

Adrenal stimulation. We studied the effect of acute stimulation of adrenal secretion in six normal subjects, two men and four women. After collecting fresh urines at 08:00 hours to determine baseline concentrations of 20-DH and UFC, we injected 250 μg of Synacthen® (corticotropin 19-24) into the subjects intravenously, and again collected urine specimens 4 h later.

Analytical procedures. To estimate the concentrations of urinary free steroids in 24-h urine specimens, we used

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automated liquid chromatography, following, in principle, the same procedure described for UFC quantification (6). In brief, the procedure is as follows. A 1-mL urine sample is concentrated on a reversed-phase precolumn. The bulk of impurities is removed with alkaline, acid, and organic washes. After selective elution with a methanol/water mixture, the steroid-containing eluate is "polarized" by admiring water in such a way that the steroids are focused on the top of a second reversed-phase precolumn. From this precolumn, steroids are desorbed by backflush, separated from the related compounds remaining by "high performance" liquid chromatography, with detection of ultraviolet absorbance at 254 nm (for procedural details see ref. 6). The present method differs from that of UFC determination (6) in the use of 120 mL of acetonitrile per liter mixture instead of 150 mL of acetonitrile in the step of washing with organic solvent. Because losses of UFC and 20-DH throughout the total procedure are negligible, external calibration is feasible for quantification. Coefficients of variation were 4.1% for interassay variability (n=10), 2.6% for intra-assay variability (n=14). As little as 15 nmol of steroid per liter is reliably detectable.

Normal reference intervals were evaluated according to a logarithmic distribution (7).

Results

Table 1 summarizes our data on excretion of UFC and 20-DH by normal subjects, by patients with Cushing's syndrome, and by normal subjects undergoing adrenal stimulation with corticotropin. Figure 1 illustrates the rates for UFC and 20-DH excretion by normal subjects and by patients with Cushing's syndrome. Among the patients, the range of excretion rates, both for UFC and 20-DH, was very broad. Extremely high values were measured in the two patients with ectopic corticotropin syndrome: 2200 and 11 955 nmol/24 h for UFC, and 22 285 and 81 238 nmol/24 h for 20-DH. They significantly exceeded (p = 0.001) those measured in patients with Cushing's disease and adrenal adenoma. Whereas all patients excreted more 20-DH than the normal subjects did, only 34 of the 40 patients excreted more UFC, and three patients excreted even less, than did the normal subjects.

The mean mass ratio of 20-DH to UFC in the control group was 2.55; it was 5.0 for the patients with Cushing's syndrome. There was no significant difference in ratios for patients with different etiologies of Cushing's syndrome.

Adrenal stimulation. Excretion of both 20-DH and UFC markedly increased in the urine of six normal individuals after corticotropin stimulation (Figure 2). In each subject, however, UFC increased substantially more than 20-DH.

| Table 1. Urinary Excretion of 20-DH and UFC by Normal Subjects, before and after Stimulation with Corticotropin, and by Patients with Cushing's Syndrome |
|-----------------|-----------------|-----------------|
|                  | 20-DH           | UFC             | 20-DH/UFC ratio |
| Normal subjects  | Excretion rate, nmol/24 h |                |                |
| (22)             | 124–174–243 a  | 25–68–125       | 2.55           |
| Patients with Cushing's syndrome (40) | 576–1798–5612 | 79–298–1117 | 6.03 |
| Conc. in urine, nmol/L |
| Before corticotropin stimulation | 56–172–521 | 53–99–183 | 1.72 |
| After corticotropin stimulation | 135–566–2373 | 570–1238–2686 | 0.45 |

*Lower limit–median–upper limit of values calculated according to a logarithmic distribution. No. of subjects is in parentheses.
Thus acute adrenal stimulation resulted in a decrease in the 20-DH/UFC ratio (Table 1).

Discussion

The present findings on the excretion rates of UFC in patients with chronic hypercorticism agree with those of other reports, demonstrating that the diagnostic sensitivity of UFC is not as high as generally assumed (1, 2, 5). No data in the literature so far plausibly explain why some patients with hypercorticism have normal excretion of UFC.

However, 20-DH does show a clear-cut difference between our hypercortoid patients and normal controls. As to reference-interval values, it must be mentioned that if control subjects such as non-endocrine inpatients or patients under intensive care were to be included, the reference interval for 20-DH—like that for UFC (6)—slightly shifts to higher values.

Except for our previous observation of increased excretion of 20-DH by a hypercortisolemic but hypocortisolic patient with Cushing’s disease (4), no data bearing on the diagnostic potency of this analyte have been presented hitherto. Likewise, information is scanty on the role of 20-DH in human physiology and on its origin in the human organism. Probably its origin is multiple, its formation having been demonstrated in human kidney (8), liver (9), and adrenal cortex (10).

It is well known that clearance rates of steroids derived from the peripheral catabolism of cortisol are distinctly higher than that of cortisol itself (11). This phenomenon is mainly ascribable to a decreased binding to plasma proteins and to impaired renal reabsorption. The fact that the concentrations of 20-DH measured in serum are far lower than those of cortisol (unpublished data) therefore suggests that 20-DH in urine is due more to peripheral metabolism than to adrenal secretion.

The 20-DH/UFC ratio of 5.0 in patients with Cushing’s syndrome (vs 2.55 in normals) indicates a shift of cortisol metabolism towards the reduction of the 20-keto group under chronic hypercortisoid conditions. The underlying mechanisms seem to resemble those that lead to an above-normal 6β-hydroxycortisol/UFC ratio in patients with Cushing’s syndrome (3, 12). Voccia et al. (3) suggested that the trophic effect of cortisol on its own hepatic microsomal hydroxylation may be responsible for this metabolic shift.

Under conditions of a rapid, exaggerated increase of cortisol in serum, as occurs after corticotropin administration, the binding capacity of transcortin is exceeded and a disproportionately large amount of unbound (free) secreted cortisol appears in the urine. This may account for the low 20-DH/UFC ratio of 0.45 in the corticotropin-stimulated subjects (Table 1).

From the present observations we conclude that cortisol induction of peripheral 20-oxidereductase occurs in chronic hypercortisolemia only, whereas in acute hypercortisolemia the cortisol is eliminated from the circulation by increased renal excretion of unmetabolized cortisol before it can undergo peripheral metabolism. Thus, measurement of 20-DH excretion appears to be a rapid and valuable diagnostic tool for chronic hypercortisoid states, such as various forms of Cushing’s syndrome. By additionally evaluating the 20-DH/UFC ratio, one can differentiate a chronic hypercortisoidism from an acute state. In clinical practice, such differentiation sometimes is helpful in evaluation of hypercortisoid states that are secondary to acute “stress” situations rather than to pathological alterations.

However, a shift of cortisol metabolism towards the C-20 reduction may be induced also by other endogenous steroids or by drugs, a phenomenon well known and studied for the microsomal 6β-hydroxylation of cortisol (13). Before the diagnostic relevance of 20-DH excretion in urine is finally evaluated, such effects remain to be elucidated.

References