Impaired 11-β Hydroxysteroid Dehydrogenase Type 2 Activity in Sweat Gland Ducts in Human Essential Hypertension

Brigitte Bocchi, Sabine Kenouch, Maxime Lamarre-Cliche, Martine Muffat-Joly, Michel Hubert Capron, Jean Fiet, Gilles Morineau, Michel Azizi, Jean Pierre Bonvalet, Nicolette Farman

Abstract—The enzyme 11-β hydroxysteroid dehydrogenase type 2 plays a major role in blood pressure regulation. It metabolizes glucocorticoid hormones into derivatives with low affinity for the mineralocorticoid receptor, preventing its permanent occupancy by circulating cortisol, which is 100- to 1000-fold more abundant than aldosterone in the plasma. Inactivating mutations of the enzyme result in severe hypertension, as seen in children with apparent mineralocorticoid excess syndrome. In patients with essential hypertension, however, attempts to evidence enzyme deficiency have been inconclusive. In this pilot study, its catalytic activity was measured directly in aldosterone-sensitive sweat gland ducts collected from skin biopsy samples of 10 male normotensive subjects and 10 subjects with essential hypertension (more than 140 to 90 mm Hg) with no sign of hypermineralocorticism. Isolated ducts were assayed for nicotinamide-dinucleotide-dependent dehydrogenase activity (transformation of tritiated corticosterone into tritiated-11 dehydrocorticosterone, as measured by high-pressure liquid chromatography). Hypertensive patients exhibited significantly lower 11-β hydroxysteroid dehydrogenase type 2 activity (9.7 ± 4.7 femtomoles per 3 mm length of duct and per 10 minutes incubation, median ± SD) than did normotensive subjects (15.9 ± 2.6). Such defect was undetectable using the classical urinary corticosteroid metabolism indexes, probably because of compensatory mechanisms. Relations between these findings and blood pressure levels should benefit from direct enzyme measurements in the vasculature. In conclusion, this cross-sectional study points to partial 11-β hydroxysteroid dehydrogenase type 2 deficiency as a novel feature of essential hypertension, which should stimulate search for new signaling pathways and therapeutical targets.

Key Words: mineralocorticoids ■ aldosterone ■ corticosterone ■ hypertension ■ clinical trials

Hypertension has a major impact on population morbidity, justifying sustained efforts to search for novel pathogenic pathways to improve our understanding of blood pressure regulation. The enzyme 11-β hydroxysteroid dehydrogenase type 2 (HSD2) could be a candidate in the pathogenesis of essential hypertension in humans. HSD2 metabolizes glucocorticoid hormones into 11-dehydroderivatives with low affinity for the mineralocorticoid receptor (MR), preventing permanent occupancy of MR by circulating cortisol, which is 100- to 1000-fold more abundant in the plasma than the MR ligand aldosterone.1–4 The clinical importance of HSD2 is highlighted by inactivating mutations found in the syndrome of apparent mineralocorticoid excess, a rare genetic defect characterized by early onset of severe low-renin hypokalemic hypertension.5–7 Hypertension also occurs after chronic ingestion of liquorice, whose active metabolite, glycyrrhetinic acid, inhibits HSD2 activity.8–10 More recently, it has been proposed that reduced activity of HSD2 could contribute to the pathogenesis of human essential hypertension, especially in its salt-sensitive form.11 However, several attempts to find mutations in these hypertensive patients were negative, and identification of HSD2 polymorphisms linked to essential hypertension or alterations in urinary excretion of cortisol and metabolites are controversial. Although some authors12 did not suggest that HSD2 variants contribute to hypertension, it has been shown that some polymorphisms may be associated with salt-sensitive hypertension.7,13–15 Other studies searched for functional alterations in renal HSD2 by comparison of urinary excretion of cortisol and its metabolites in normotensive and hypertensive subjects. In some cases,6,16,17 the 5β tetrahydrocortisol (THF) plus 5α tetrahydrocortisol (αTHF)/tetrahydrocortisone (THE) or urinary free cortisol (UFC)/urinary free cortisone (UFE) ratios were found slightly elevated in hypertensive
patients, while other reports indicate no significant change between normotensive and hypertensive subjects. However, 3 sets of data argue for the possibility of an impaired HSD2 activity in essential hypertension. First, Walker et al.²² showed that the half-life of cortisol (tritiated at the 11 position) was prolonged in a small series of hypertensive patients, despite normal ratios of cortisol (F) to cortisone (E) in the plasma or urine. A second observation along this line came from the evaluation of UFF in a large population of subjects with essential hypertension, which suggested an association between salt-resistant hypertension and high urine cortisol levels.²³ A third set of data relies on the notion of endogenous inhibitors of HSD2, named glycyrretinic-like factors (GALFs) by Morris;²⁴ although the nature of these inhibitors is unknown, there are some indications of an increased excretion of GALFs in hypertensive subjects.²⁵,²⁶

Until now, search for an alteration of HSD2 activity in humans has been hampered by the relatively low sensitivity of the detection methods relying on measurements of peripheral metabolites. Corticosteroid metabolism depends on integrated compensatory mechanisms, susceptible to mask, in plasma and urine samples, mild changes in enzyme activity, which may be important at the level of aldosterone target cells for the regulation of sodium reabsorption. To overcome this limitation, we used a very sensitive method to measure ex vivo HSD2 catalytic activity in patients with essential hypertension in isolated sweat gland ducts, which share many common properties with the renal collecting duct.²⁴,²⁵ This pilot study revealed a significant reduction in HSD2 in those patients, as compared with control subjects.

Methods

Ten white men (aged 40 to 60 years) with untreated essential hypertension in the absence of patent hypermineralocorticosis (normal plasma aldosterone [0.17 to 0.42 nmol/L] concentrations in the sitting position and with plasma renin activity below the upper limit [19 mU/L]) were selected. Volunteers to undergo skin biopsy were recruited in the Hypertension Department of the Pompidou European Organization criteria as a seated systolic blood pressure of 140 mm Hg or diastolic blood pressure of 90 mm Hg (phase V of Korotkoff sounds) measured by mercury sphygmomanometer after 5 minutes of rest. Secondary forms of hypertension were excluded during an extensive in-hospital work-up. Exclusion criteria were: liquorice derivatives or carbenoxolone ingestion, excess alcohol or tobacco consumption, and excess body weight (body mass index <15% of the superior limit of the Metropolitan Insurance Company standards). The control group consisted of 10 white men who were normotensive age-matched subjects (systolic blood pressure <130 mm Hg, diastolic blood pressure <85 mm Hg). The salt and water intake was not controlled. All subjects provided written informed consent to participate to the protocol. The protocol was approved by the Comité de Protection des Personnes se Prêtant à une Recherche Biomédicale of Aulnay-sous-Bois, France.

All 20 volunteers underwent a screening visit at the Clinical Investigation Center consisting of medical history, physical examination, measurements of plasma aldosterone and active renin, and standard laboratory examinations (including plasma creatinine, sodium, potassium, chloride, bicarbonate, glucose, uric acid, platelet counts, and proteinuria). Two weeks later, included subjects underwent 3 skin biopsies (punches, 4-mm diameter each) in the armpit after local lidocaine anesthesia. Blood was withdrawn at 8:00 AM (after 1 hour rest in the sitting position) for plasma corticosteroid hormone measurements and 24-hour urine was collected for determination of cortisol hormone measurements and 24-hour urine was collected for determination of cortisol metabolism.

Results

Isolation of Sweat Glands and Determination of HSD2 Catalytic Activity

Individual sweat gland ducts were collected (cut with scissors) from disperse-treated skin biopsy samples, presumably in their late portion, attached to epidermis (Figure 1) and measured as previously described.²⁶ Pools of sweat gland ducts (2 to 4 fragments, 3-mm total length, ie, ~1500 cells per assay) were transferred into 5-μL medium into ice-cold Eagle minimal essential medium (MEM) culture medium (5 μL) containing 10 mmol/L [1,2,6,7]-3 H corticosterone (2.22 TBq/mmol; Amersham) and 1 mmol/L NAD (Sigma) final concentrations. Three to 7 assays per subject were performed. Ducts were permeabilized by 3 freeze-thaw cycles (1 minute each) to ensure entry of NAD, followed by incubation at 37°C for 10 minutes to measure HSD2 by transformation of tritiated corticosterone (B) into 11-dehydrocorticosterone (A), and separated by HPLC as previously reported. Corticosterone was used as substrate because it is more rapidly metabolized by human HSD2 than cortisol.²⁷,²⁸ Eventual spontaneous metabolism in the incubation medium (incubation with steroids in the absence of tissue, blank value) appeared to be very low (0.5% to 2% transformation of B into A); it was deduced from each individual value. Results are expressed as femtomoles (fmol) of metabolites produced per 3-mm sweat gland ducts and per 10 minutes of incubation.

Blood and Urine Steroids Analyses

Plasma aldosterone and active renin were measured by radioimmunoassay (Behring GmbH and Pasteur Diagnostics France kits, respectively) as well as plasma DOC, corticosterone, cortisol and cortisone, and UFF and cortisone UFE, after extraction followed by a chromatographic step, as previously described. Urinary cortisol and cortisone metabolites (tetrahydrocortisol THF, as 5αTHF and 5βTHF, tetrahydrocortisone TFE) were quantified as described by Gourmelon et al. as gas–liquid chromatography.

Statistical Analysis

Plasma and urine values are means ± SD. For HSD2 activity, median values for measurements per patient were used as individual values. Comparisons between groups were made using the nonparametric Mann Whitney U test, and P = 0.05 was considered significant. An eventual relation between HDS2 activity and other biological parameters was tested using the Spearman-rank method. Tests have been
Results

Characteristics of the Two Groups

Hypertensive patients did not significantly differ from normotensive subjects in terms of age, creatinine, uric acid, sodium, potassium, and bicarbonate concentrations, urine Na and K excretion, and Na/K ratios (Table 1). All these parameters were within the normal range in the 2 groups. Daily diuresis was reduced in hypertensive patients. Plasma renin and aldosterone concentrations were comparable in the 2 groups and were within the normal values. Thus, there was no sign of hypermineralocorticism, as expected from the inclusion criteria. Heart rate was slightly (but not significantly) higher in hypertensive patients than in normotensive subjects. Body mass index and plasma glucose concentration were significantly higher in hypertensive patients than in normotensive subjects.

Measurements of HSD2 Activity in Sweat Gland Ducts

In contrast with the peripheral measurements (see later), direct evaluation of the catalytic activity of the enzyme HSD2 in the aldosterone–target sweat gland duct using a very sensitive assay yielded significant differences between the 2 groups. Figure 2 shows the sweat gland duct HSD2 activity of normotensive subjects and hypertensive patients expressed in fmol of $^3$H-corticosterone transformed into $^3$H-11 dehydrocorticosterone per 10 minutes and per 3 mm of permeabilized ducts in the presence of the cofactor NAD. The mean value of HSD2 catalytic activity was significantly lower in the hypertensive patient group (9.7±4.7 fmol/3 mm per 10 minutes versus 15.9±2.6 in NT group, $P=0.0052$). An overlap between the hypertensive patients and the normotensive subjects was observed, probably because the defect in HSD2 activity is not present in all hypertensive patients, or that individual defects may be of variable magnitude. Of note, there was a significant negative correlation between plasma glucose and HSD2 activity in the global population (Figure 3), which was not significant, however, within each group; no correlation appeared between body mass index and HSD2.

Plasma and Urine Cortisol and Cortisone and Their Metabolites

Most corticosteroid hormone concentrations in plasma and urine, as well as their main metabolites, were within the range of the normal values (Table 1). Although remaining within

### Table 1. Characteristics of the Two Groups of Patients

<table>
<thead>
<tr>
<th>Clinical and Biological Features</th>
<th>NT</th>
<th>HT</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>46.7±6.8</td>
<td>47.8±8.0</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.7±1.7</td>
<td>26.5±2.8*</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>121±12</td>
<td>161±18‡</td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>73±7</td>
<td>100±8‡</td>
<td></td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>62±5</td>
<td>74±17</td>
<td></td>
</tr>
<tr>
<td><strong>Plasma</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na (mmol/L)</td>
<td>142±1</td>
<td>139±2</td>
<td></td>
</tr>
<tr>
<td>K (mmol/L)</td>
<td>4.24±0.38</td>
<td>4.06±0.40</td>
<td></td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>88±11</td>
<td>82±12</td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.86±0.60</td>
<td>5.50±0.65*</td>
<td></td>
</tr>
<tr>
<td>Aldosterone (nmol/L)</td>
<td>0.23±0.07</td>
<td>0.22±0.07</td>
<td>0.17–0.42</td>
</tr>
<tr>
<td>Active renin (mU/L)</td>
<td>17.9±7.7</td>
<td>15.0±7.4</td>
<td>3.5–19</td>
</tr>
<tr>
<td>Cortisol F (nmol/L)</td>
<td>417±119</td>
<td>282±50*</td>
<td>193–634</td>
</tr>
<tr>
<td>Cortisone E (nmol/L)</td>
<td>54±9</td>
<td>85±29*</td>
<td>14–61</td>
</tr>
<tr>
<td>F/E (nmol/nmol)</td>
<td>7.9±2.2</td>
<td>3.8±1.9†</td>
<td>5–16</td>
</tr>
<tr>
<td>Corticosterone (nmol/L)</td>
<td>12.8±9.1</td>
<td>6.6±3.1</td>
<td>1.4–43.3</td>
</tr>
<tr>
<td>DOC (nmol/L)</td>
<td>0.63±0.13</td>
<td>0.64±0.25</td>
<td>0.2–1.13</td>
</tr>
<tr>
<td><strong>Urine</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary flow rate (L/24 hours)</td>
<td>1.81±0.52</td>
<td>1.02±0.29†</td>
<td></td>
</tr>
<tr>
<td>Na (mmol/24 h)</td>
<td>150±61</td>
<td>155±62</td>
<td></td>
</tr>
<tr>
<td>K (mmol/24 h)</td>
<td>66±38</td>
<td>61±15</td>
<td></td>
</tr>
<tr>
<td>Na/K (mmol/mmol)</td>
<td>2.60±0.91</td>
<td>2.52±0.72</td>
<td></td>
</tr>
<tr>
<td>UFF/UFE</td>
<td>0.44±0.17</td>
<td>0.30±0.08*</td>
<td>0.12–0.91</td>
</tr>
<tr>
<td>(THF+αTHF)/THE</td>
<td>1.44±0.47</td>
<td>0.79±0.18*</td>
<td>0.58–2.65</td>
</tr>
</tbody>
</table>

NT indicates normotensive subjects; HT, hypertensive subjects; UFF, urinary free cortisol; UFE, urinary free cortisone; THF, β-tetrahydrocortisol; αTHF, a tetrahydrocortisol; THE, tetrahydrocortisone.

Values are means±SD.

* $P<0.05$; † $P<0.01$; ‡ $P<0.001$ vs NT group (Mann-Whitney U test).

performed using Statview 4.5 statistical software (Abascus Concept, Calif).
the normal range, plasma cortisol was significantly lower in the hypertensive patient group, whereas plasma cortisone was significantly higher in hypertensive patient than in the normotensive subject group. Plasma corticosterone and DOC concentrations were similar in the 2 groups. The UFF/UFE and THF plus αTHF/THE ratios were significantly lower in the hypertensive than in the normotensive group but remained within the normal range. The excretion of free urinary cortisol and cortisone were comparable in normotensive subjects and hypertensive patients, despite differences in plasma cortisone and cortisol between the 2 groups. There was no significant correlation between HSD2 activity and blood pressure, plasma renin or aldosterone, or plasma or urine corticosterone within each group, as evaluated by Spearman rank method.

Discussion

The main limitation of all human studies investigating the functional implication of HSD2 in the pathogenesis of essential hypertension arises from the difficulty to assess HSD2 activity in humans in vivo in a complex environment. To bypass these difficulties, we have measured tissue HSD2 activity directly on sweat gland ducts as a model of aldosterone-sensitive cells, because of their easier access than renal collecting duct and colonic cells and because they share many physiological properties with the renal collecting tubule. Sweat gland ducts exert aldosterone-regulated sodium reabsorption and they express both the MR and HSD2 (ie, essentially NAD-dependent HSD activity) in a way comparable to human collecting tubules. In a preceding study, we have shown that HSD2 activity in nonpermeabilized sweat gland ducts from nonselected subjects was ~5 fmol/3 mm per 10 minutes (in the presence of 10 nmol/L 3 HB as substrate); permeabilization in the presence of the cofactor NAD increased this activity by a factor of 3, thus reaching values close to 15 fmol/3 mm per 10 minutes, which are quite similar to those found in this study. In another study, we had access to human kidney and cortical collecting ducts could be microdissected and assayed for HSD2 activity (using the same protocol as for human sweat gland ducts); it was found to be ~10 fmol/3 mm and per 10 minutes (nonpermeabilized tubules from 4 different human kidneys), ie, values approximately twice those of nonpermeabilized human sweat gland ducts. These results led us to consider the sweat gland duct as an appropriate epithelium to evaluate HSD2 activity in humans. We also tried to quantify HSD2 activity in circulating human lymphocytes using the same methodology; they exhibited a very low rate of conversion of 3H-corticosterone in vitro, which was not influenced by the presence of carbenoxolone (inhibitor of HSD2) added to the medium (NAD alone: 0.37±0.11 fmol/106 cells per 10 minutes versus NAD plus carbenoxolone: 0.31±0.13 fmol/106 cells per 10 minutes). Therefore, lymphocytes appeared not suitable for measuring the catalytic activity of HSD2.

Our results indicate that patients with essential hypertension and no sign of hyperaldosteronism have an overall significant 39% reduction in ex vivo HSD2 catalytic activity, as measured directly by a very sensitive assay on aldosterone–target sweat gland duct cells, as compared with normotensive subjects. Reduced HSD2 activity was associated with increased plasma glucose, raising questions about unknown putative relationships between HSD2 and diabetes. Our protocol did not include determinations of plasma vasopressin levels; of note, diuresis was reduced in HT, despite the increase in blood pressure. Some reports have shown a positive correlation between vasopressin levels and blood pressure (contrasting, however, with higher diuresis). Although in vitro vasopressin addition to rat collecting tubules increases transiently HSD2 activity, it is unknown whether long-term in vivo exposure to vasopressin may affect HSD2 activity. This would be interesting to determine. It should be kept in mind, however, that complex regulatory (compensatory) mechanisms occur in vivo, often precluding mechanistic interpretations and extrapolations from in vitro findings. Providing deficit in HSD2 also occurs in the renal collecting duct, it would indicate that HSD2 activity may be partly deficient in patients with normal-renin, normal-aldosterone essential hypertension; although of limited magnitude (and irrespective of its cause) it might then contribute to elevate the blood pressure levels of the hypertensive patients by
enhancing renal tubular sodium reabsorption in the distal nephron. Furthermore, in addition to its well-documented role in renal collecting duct cells, HSD2 is also expressed in non-epithelial cells, where its functional impairment may also contribute to the pathogenesis of hypertension. Abnormal HSD2 activity in smooth muscle cells of blood vessels has been recently shown to be associated with hypertension.\textsuperscript{36–38} and mice with HSD2 gene knockout exhibit vascular wall endothelial dysfunction.\textsuperscript{39} Direct assessment of HSD2 in the vasculature was not included in our protocol; future studies should address this point to establish links with blood pressure levels.

Reduction in HSD2 activity was undetectable using the classical corticosteroid metabolism indexes (UFF/UFE) and (THF plus αTHF/THE). This is in variance with observations made in apparent mineralocorticoid excess, in which these ratios are very high (10 to 30 or even higher).\textsuperscript{5,40} In fact, despite the deficiency in sweat gland HSD2 observed here, significantly lower plasma cortisol concentrations and higher plasma cortisone levels were unexpectedly observed in hypertensive subjects as compared with normotensive. This paradox has been previously reported.\textsuperscript{17} Such differences in plasma cortisol/cortisone levels likely influence the amount of filtered steroids susceptible to undergo metabolism by HSD2. As an attempt to evaluate urinary corticosteroid excretion independent of their plasma concentrations, the urinary ratios can be divided by F/E for each subject. As shown in Table 2, such “normalized” corticosteroid metabolism indexes appear somewhat higher in hypertensive patients than in normotensive subjects; this may be indicative of a subtle alteration in HSD2 activity in HT, masked by other adaptive phenomena modifying corticosteroid status in hypertensive patients. As a matter of fact, it is likely that multiple compensatory mechanisms of the cortisol/cortisone pathway occur in hypertensive patients. The concentrations of corticosteroids and their metabolites in plasma or urine depend on several factors, including circadian rhythms of secretion rates and binding to plasma proteins; urinary excretion of metabolites results from the dehydrogenase activity of HSD2 but is also influenced by the opposite reductase activity driven by HSD1. In addition, it is not known whether hypertension alters the level of expression of the MR or the glucocorticoid receptor. Any of these parameters may be subjected to different regulations in normotensive and hypertensive subjects. Thus, it is conceivable that classical hormonal markers are not sensitive enough to assess subtle changes in tissue enzyme activity, because they depend on the global functioning and adaptations of the glucocorticoid pathways.

In conclusion, in this cross-sectional controlled study, we show that patients with essential hypertension and without any biological evidence of aldosterone excess have an overall significant 39% reduction in ex vivo HSD2 catalytic activity in sweat gland ducts, as compared with normotensive subjects.

Perspectives

HSD2 activity appears to be partly deficient in sweat glands of subjects with essential hypertension. It would be of interest to evaluate whether such defect also occurs in the blood vessels, thus contributing to increase the high blood pressure levels. Strategies to correct HSD2 impairment could improve therapeutical control of hypertension. Search for genetic or functional alterations of networks of genes or signaling cascades (mostly unknown at the present time) that regulate HSD2 activity now appears of major interest to propose new pathophysiological hypotheses and possibly new candidate genes in hypertension.

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References


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