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

Brigite Bocci, 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-nucleotide-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. 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. A third set of data relies on the notion of endogenous inhibitors of HSD2, named glycyrrhetinic-like factors (GALFs) by Morris; although the nature of these inhibitors is unknown, there are some indications of an increased excretion of GALFs in hypertensive subjects.

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. 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. 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 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 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 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).

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 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 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 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 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 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).

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 cortisol metabolites (tetrahydrocortisol THF, as 5αTHF and 5βTHF, tetrahydrocortisone THE) were quantified as described by Gourmelon et al. by 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 HSD2 activity and other biological parameters was tested using the Spearman-rank method. Tests have been
performed using Statview 4.5 statistical software (Abacus Concept, Calif).

### 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 normal range, plasma aldosterone was significantly lower in hypertensive patients than in normotensive subjects. A significant negative correlation between plasma glucose and aldosterone was also observed in the global population (Figure 3).

<table>
<thead>
<tr>
<th>Table 1. Characteristics of the Two Groups of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical and Biological Features</strong></td>
</tr>
<tr>
<td><strong>NT</strong></td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td><strong>Age (y)</strong></td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
</tr>
<tr>
<td><strong>Systolic blood pressure (mm Hg)</strong></td>
</tr>
<tr>
<td><strong>Diastolic blood pressure (mm Hg)</strong></td>
</tr>
<tr>
<td><strong>Heart rate (bpm)</strong></td>
</tr>
<tr>
<td><strong>Plasma</strong></td>
</tr>
<tr>
<td><strong>Na (mmol/L)</strong></td>
</tr>
<tr>
<td><strong>K (mmol/L)</strong></td>
</tr>
<tr>
<td><strong>Creatinine (μmol/L)</strong></td>
</tr>
<tr>
<td><strong>Glucose (mmol/L)</strong></td>
</tr>
<tr>
<td><strong>Aldosterone (nmol/L)</strong></td>
</tr>
<tr>
<td><strong>Active renin (mU/L)</strong></td>
</tr>
<tr>
<td><strong>Cortisol F (nmol/L)</strong></td>
</tr>
<tr>
<td><strong>Cortisone E (nmol/L)</strong></td>
</tr>
<tr>
<td><strong>F/E (nmol/nmol)</strong></td>
</tr>
<tr>
<td><strong>Corticosterone (nmol/L)</strong></td>
</tr>
<tr>
<td><strong>DOC (nmol/L)</strong></td>
</tr>
<tr>
<td><strong>Urine</strong></td>
</tr>
<tr>
<td><strong>Urinary flow rate (L/24 hours)</strong></td>
</tr>
<tr>
<td><strong>Na (mmol/24 h)</strong></td>
</tr>
<tr>
<td><strong>K (mmol/24 h)</strong></td>
</tr>
<tr>
<td><strong>Na/K (mmol/mmol)</strong></td>
</tr>
<tr>
<td><strong>UFF/UFE</strong></td>
</tr>
<tr>
<td><strong>(THF+αTHF)/THE</strong></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).
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 corticosteroids within each group, as evaluated by Spearman rank test.

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 (i.e., 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 3HB 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), i.e., 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 defect 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

![Figure 2](image.png)

**Figure 2.** HSD2 catalytic activity in sweat gland ducts of normotensive (NT) and hypertensive (HT) subjects. Median values per subject are indicated by white circles. Black circles are the mean of the individual medians in NT and HT; SDs are represented. The box plots show the median and the interquartile range of the HSD2 values of each group. The difference between NT and HT is highly significant: *P*=0.0052 (Mann-Whitney U test).

![Figure 3](image.png)

**Figure 3.** Correlation between plasma glucose concentrations and HSD2 activity. The plasma glucose level is negatively correlated with HSD2 catalytic activity, measured in isolated sweat gland ducts from normotensive (white circles) and hypertensive (black squares) subjects. Correlation test: *r* = −0.0684, *P*=0.0009; Spearman-rank non-parametric test: *P*=0.0024.
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, and mice with HSD2 gene knockout exhibit vascular wall endothelial dysfunction. 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). 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. 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 phenomonens 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.

**Acknowledgments**

This study has been supported by INSERM and by the Fondation Searle pour la Recherche. B. Bocchi was supported by the Fondation Searle pour la Recherche, by the Société Française de Néphrologie, and by the Fondation pour la Recherche Médicale. We thank Pr Joël Ménard, Dr Maria-Christina Zennaro, and Dr Marc Lombès for critical reading of this manuscript.

**References**


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 and Nicolette Farman

Hypertension. 2004;43:803-808; originally published online February 23, 2004;
doi: 10.1161/01.HYP.0000121362.64182.ad

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 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/43/4/803

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/