11β-Hydroxysteroid Dehydrogenase in Cultured Human Vascular Cells
Possible Role in the Development of Hypertension

Haruhiko Hatakeyama, Satoru Inaba, Isamu Miyamori

Abstract—11β-Hydroxysteroid dehydrogenases (11β-HSD) interconvert cortisol, the physiological glucocorticoid, and its inactive metabolite cortisone in humans. The diminished dehydrogenase activity (cortisol to cortisone) has been demonstrated in patients with essential hypertension and in resistance vessels of genetically hypertensive rats. 11β-Hydroxysteroid dehydrogenase type 2 (11β-HSD2) catalyzes only 11β-dehydrogenation. However, a functional relationship between diminished vascular 11β-HSD2 activity and elevated blood pressure has been unclear. In this study we showed the expression and enzyme activity of 11β-HSD2 and 11β-HSD type 1 (which is mainly o xo reductase, converting cortisone to cortisol) in human vascular smooth muscle cells. Glucocorticoids and mineralocorticoids increase vascular tone by upregulating the receptors of pressor hormones such as angiotensin II. We found that physiological concentrations of cortisol-induced increase in angiotensin II binding were significantly enhanced by the inhibition of 11β-HSD2 activity with an antisense DNA complementary to 11β-HSD2 mRNA, and the enhancement was partially but significantly abolished by a selective aldosterone receptor antagonist. This may indicate that impaired 11β-HSD2 activity in vascular wall results in increased vascular tone by the contribution of cortisol, which acts as a mineralocorticoid. In congenital 11β-HSD deficiency and after administration of 11β-HSD inhibitors, suppression of 11β-HSD2 activity in the kidney has been believed to cause renal mineralocorticoid excess, resulting in sodium retention and hypertension. In the present study we provide evidence for a mechanism that could link impaired vascular 11β-HSD2 activity, increased vascular tone, and elevated blood pressure without invoking renal sodium retention. (Hypertension. 1999;33:1179-1184.)

Key Words: 11β-hydroxysteroid dehydrogenase receptors, angiotensin II cortisol vascular tone hypertension, essential

Vascular wall is a target tissue for glucocorticoids and mineralocorticoids. These steroids are essential for the maintenance of vascular tone.1 They can potentiate the vasoconstrictor action of a number of pressor hormones, including α-adrenergic agonists and angiotensin II (Ang II).2 This potentiation is postulated to be mediated by the upregulation of receptors for these pressor hormones in vascular smooth muscle cells.3 We have previously demonstrated that vascular cells per se are steroidogenic,4–8 and the locally produced aldosterone might participate in Ang II-induced vascular hypertrophy in an autocrine/intracrine manner through type 1 mineralocorticoid receptor (MR).4,8 The MR has an equal affinity for cortisol and aldosterone, despite the fact that the circulating cortisol levels are much higher than those of aldosterone.9 It has been proposed that the abundance of 11β-hydroxysteroid dehydrogenase (11β-HSD) in the kidney, which metabolizes cortisol into cortisone with very low affinity for the MR, explains how the kidney can be a mineralocorticoid target tissue.10,11 A defect of 11β-HSD activity would thus allow the MR to be occupied mostly by cortisol. In humans, two 11β-HSD isoforms have been described and cloned. The first enzyme (11β-HSD1) catalyzes both 11β-dehydrogenation and the reverse oxoreduction and is a low-affinity NADPH enzyme.12 The enzyme has been detected in a wide range of rat and human tissues including liver, lung, and testis. A second isozyme (11β-HSD2) is present in the kidney and placenta. It is a high-affinity NAD-dependent enzyme and catalyzes only 11β-dehydrogenation.13 It has been believed that in congenital 11β-HSD deficiency (apparent mineralocorticoid excess syndrome) and after administration of 11β-HSD inhibitors (licorice and carbenoxolone), the renal MR can be occupied mostly by cortisol, causing sodium retention and hypertension.14,15 Recently, it has been postulated that 11β-HSD1 does not play a significant role in conferring ligand specificity on the MR.12 Indeed, several mutations in the 11β-HSD2 gene have been identified in patients with this syndrome, but none in the 11β-HSD1 gene.16

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Since local glucocorticoids (mineralocorticoids as well) within vascular wall could directly affect vascular tone, the local metabolism of glucocorticoids mediated by 11β-HSD may be important in controlling blood pressure. Soro et al. reported that the ratio of cortisol to cortisone metabolites in the urine was significantly higher in patients with essential hypertension. We demonstrated elevated levels of 11β-HSD inhibitory substances in the urine of patients with low-renin essential hypertension. Furthermore, we reported decreased dehydrogenase activity of 11β-HSD1 in resistance vessels of genetically hypertensive rats. However, a functional relationship between diminished vascular 11β-HSD2 activity and elevated blood pressure has been unclear.

The purpose of this study was to clarify the physiological and pathophysiological significance of 11β-HSD2 activity in human resistance vessels. We investigated the activity and gene expression of the enzyme in human coronary artery smooth muscle cells (HCASMC). Furthermore, to assess its potential role as a modulator of vascular tone, a functional relationship between the vascular 11β-HSD2 activity and the effect of physiological concentrations of cortisone on Ang II receptor regulation was also tested by manipulating 11β-HSD2 gene expression with an antisense DNA.

**Methods**

**Materials**

HCASMC (Clonetics Corp) were cultured according to the supplier’s instruction. 1 Sar, [125I]Tyr, Ile-Ang II was purchased from NEN-DuPont. [1,2,6,7-3H]Cortisol was from Amersham International plc. Cortisol, cortisone, spironolactone, and RU38486 were from Sigma. Ang II was from Peptide Institute. DuP 753 and PD123319 were from Yamanouchi Pharmaceutical Co.

**Cell Culture**

HCASMC were cultured to confluence in Dulbecco’s modified Eagle’s medium with 5% FCS plus 4 μg/mL of gentamicin under 5% CO2/95% air at 37°C. Cells at 5 to 8 passages were used for the experiments. >95% of the cells were identified as smooth muscle cells by their typical “hill-and-valley” morphology and by immunofluorescence with the use of a monoclonal antibody against human α-smooth muscle actin.

**Detection of 11β-HSD mRNA**

Oligonucleotide primers for reverse transcription–polymerase chain reaction (RT-PCR) were synthesized with an Applied Biosystems model 392 DNA synthesizer and purified with an oligonucleotide purification column. The sequences of sense and antisense primers were 5′-TCGAGTTGCTGATTCTTTATG-3′ and 5′-ACTTGGTGCAGAATAGG-3′ for detecting 11β-HSD1 mRNA.12,13 The sequences of sense and antisense primers were 5′-ACCGTATTGGAGTTGAACG-3′ and 5′-TCATTGCACTGTCTGTTTGAAGC-3′ for detecting 11β-HSD2 mRNA.12,13 RT-PCR experiments were performed to amplify the ubiquitously expressed α1 subunit of human Na,K-ATPase using the sense (5′-ATATGGAACAGACTTGGACCC-3′) and antisense (5′-GCGAATTTCTCCATCATGACT-3′) primers.22 RT-PCR was performed as described previously.4 A 10-μL aliquot of each RT-PCR reaction mixture was electrophoresed on a 2% agarose gel. The gel was stained with ethidium bromide and photographed.

**Assay of 11β-HSD Activity**

The apparent $K_m$ values for the dehydrogenase reaction and the reductase reaction in vascular smooth muscle cells are ~100 and ~300 nmol/L, respectively.23 11β-HSD activities were measured by a radiometric conversion assay, as previously described.24 In brief, confluent HCASMC were incubated in a hydrocortisone-free and serum-free medium containing 100 nmol/L [1,2,6,7-3H]cortisol or [1,2,6,7-3H]cortisone for 8 hours, after which steroids were extracted with chloroform and were resolved by thin-layer chromatography. Radioactivities corresponding to cortisol and cortisone were determined. Dehydrogenase and reductase activities were calculated as counts per minute for cortisone/(counts per minute for cortisol+counts per minute for cortisone)×100 and as counts per minute for cortisol/(counts per minute for cortisol+counts per minute for cortisone)×100, respectively. [1,2,6,7-3H]Cortisone was prepared from [1,2,6,7-3H]cortisol as described previously.25 Briefly, the labeled cortisol (10 μCi) was incubated in 1 mL of 50% aqueous acetic acid containing 1% chromium trioxide at room temperature for 20 minutes. The residue from the dichloromethane extract of the reaction products was chromatographed by thin-layer chromatography with the use of chloroform-methanol (9:1) as solvent and nonradioactive cortisol and cortisone as reference markers. The cortisone-containing resin was scraped off and eluted with ethyl acetate.

**Ang II Binding**

Confluent HCASMC were washed 3 times with saline and incubated with Sar, [125I]Tyr, Ile-Ang II for 60 minutes at room temperature. Assay buffer consisted of 500 mMol/L Tris (pH 7.4), 100 mMol/L NaCl, 5 mMol/L MgCl2, 0.25% BSA, and 0.5 mg/mL bacitracin. At the end of incubation, the cells were washed with saline 4 times, solubilized in 1% sodium dodecyl sulfate, and counted with a γ-counter. Saturation binding assays were performed with increasing concentrations of [125I]Ang II (50 to 700 nmol/L) in the presence (nonspecific binding) or absence (total binding) of 1 μmol/L unlabeled Ang II and processed as explained above. Competition binding assays were performed with 200 n mole/L of [125I]Ang II in the presence of increasing concentrations of unlabeled Ang II, nonpeptide Ang II type 1 receptor antagonist DuP 753, and type 2 receptor antagonist PD123319.

**Antisense Oligonucleotides**

A 24-mer phosphorothioate antisense oligonucleotide (AS) complementary of the 5′ region of human 11β-HSD2 mRNA containing the initiator AUG codon and, as a control, a nonsense oligonucleotide (NS) containing the same base composition but in a random, scrambled order were synthesized with an Applied Biosystems model 392 DNA synthesizer. Sequences of AS and NS were 5′-CGACGGCCAGGCCAGGCTTCCAT-3′ and 5′-TCACGCACGCGCCACAGGAGAT-3′, respectively.

**Statistical Analysis**

Data are expressed as mean±SEM. The significance of differences was assessed by 1-way ANOVA and multiple comparison test. Values of $P<0.05$ were accepted as statistically significant.

**Results**

**11β-HSD Expression in HCASMC**

We examined the expression of 11β-HSD1 and 11β-HSD2 genes in HCASMC. With the use of the RT-PCR method, amplified products corresponding to transcripts of both genes were detected (Figure 1). Cloning and sequence analysis of the PCR products demonstrated that both bands had the known sequences of the human 11β-HSD1 and 11β-HSD2 mRNA, respectively (data not shown). Although the amount of mRNA of 11β-HSD2 seemed lower than that of 11β-HSD1, more exact quantification of the comparative levels of 11β-HSD1 and 11β-HSD2 was not attempted because of possible differences in amplification efficiency with the various sets of primers.
To assess $11\beta$-HSD activities, labeled cortisol or its metabolite cortisone was added to each medium. Incubation with cortisol resulted in moderate (18 ± 2%) conversion to cortisone after 8 hours, whereas incubation with cortisone resulted in more conversion (42 ± 5%) to cortisol (Figure 2). Thus, HCASMC were capable of performing the dehydrogenase as well as the reverse oxoreductase phase of the reaction, and under physiological conditions these cells favored the latter phase.

**Ang II Receptor in HCASMC**

Before we investigated the cortisol effect on Ang II binding, HCASMC were tested for their ability to bind to $^1$Sar, $[^{125}\text{I}]^4$Tyr, $^8$Ile-Ang II. A saturation binding study demonstrated the specific binding of $[^{125}\text{I}]$Ang II at the concentration of 50 to 700 pmol/L in HCASMC. Scatchard analysis of the binding data revealed a single class of high-affinity (0.31 ± 0.06 nmol/L) and low-capacity (10.8 ± 0.5 fmol/mg protein, 468 ± 21 sites per cell) binding sites (Figure 3A). A competition binding study showed that radioligand binding was potentially inhibited by unlabeled Ang II and the Ang II type 1 receptor selective antagonist DuP 753; however, PD123319, an Ang II type 2 receptor selective antagonist, had no effect on the binding of $[^{125}\text{I}]$Ang II at doses as high as 1 µmol/L (Figure 3B). This indicated that HCASMC exhibited high-affinity Ang II type 1 receptor, the predominant Ang II receptor subtype in vascular smooth muscle cells.27

**Effect of Cortisol on Ang II Binding**

Next we examined the effect of cortisol on Ang II binding in these cells. Incubation of HCASMC for 24 hours with cortisol resulted in concentration-dependent increases in Ang II binding, with a maximal increase (98 ± 10%) at 1 µmol/L cortisol (Figure 4A). Lower concentrations of cortisol (1 to 10 pmol/L) had no effect on Ang II binding (data not shown). The competition binding data and the Scatchard analysis of the binding data from control cells and cells treated for 24 hours with cortisol demonstrated that Ang II receptors upregulated by cortisol (as well as basal Ang II receptors) were of the type 1 receptor, and the affinity was not significantly changed (data not shown). Upregulation of Ang II binding was completely inhibited by RU38486, a specific antagonist for glucocorticoid receptors (GR), but not by spironolactone, a selective antagonist for MR, indicating that the regulation...
was mediated through GR (Figure 4B). RU38486 or spironolactone alone did not alter Ang II binding (data not shown).

Effect of 11β-HSD2 Antisense Oligonucleotides

We next tested whether vascular 11β-HSD2 activity could be functionally related to the cortisol effect on Ang II binding. For this, a 24-mer phosphorothioate AS and, as a control, an NS containing the same base composition but in a random, scrambled order were administered to the culture medium in which HCASMC were grown. No visible signs of toxicity were observed. As shown in Figure 5, incubation of HCASMC for 24 hours with the AS induced dose-dependent decreases in the dehydrogenase activity, with a maximal decrease (78±6%) at 10 μmol/L AS, but the oxoreductase activity was unaffected. The NS altered neither activity. Since the oxoreductase activity was unaffected, the present results indicate that the inhibitory effect of AS is due to a specific decrease of 11β-HSD2 activity. The possibility of the effect on 11β-HSD1 activity would be very unlikely.

Effect of 11β-HSD2 Activity on Ang II binding

After confluent HCASMC had been incubated with the AS for 24 hours, the cells were further incubated with 0.5 μmol/L cortisol for 24 hours. The AS induced dose-dependent increases in Ang II binding, with a maximal increase (48±5%) at 10 μmol/L AS (Figure 6A). The NS did not alter the Ang II bindings. We then investigated whether the upregulation was mediated through GR or MR. HCASMC were exposed to RU38486 or spironolactone and then assayed for Ang II binding under the presence of 0.5 μmol/L cortisol and 10 μmol/L AS (Figure 6B). The upregulated Ang II binding in the cells was significantly inhibited by the presence of spironolactone or RU38486. The effects of both spironolactone and RU38486 were dose dependent, with a maximal inhibition (24±3%) at 1 μmol/L spironolactone (72±6% inhibition with 1 μmol/L RU38486). Concomitant administration of both spironolactone and RU38486 completely inhibited the upregulation of Ang II binding. Mineralocorticoids are known to increase the Ang II receptor number through its action on MR.28 Our results indicate that diminished vascular 11β-HSD2 activity enhances the effect of cortisol, and the enhancement is mediated through both GR activation by cortisol as a glucocorticoid and MR activation by cortisol as a mineralocorticoid, suggesting that 11β-HSD2 plays a significant role in conferring the ligand specificity on MR in HCASMC.

Discussion

We demonstrated for the first time the gene expression and the bidirectional activity of 11β-HSD in vascular smooth muscle cells cultivated from a human resistance vessel. Previous studies indicated bidirectional activity, favoring the oxoreductase reaction 4-fold over the dehydrogenase reaction, in rat aortic vascular smooth muscle cells23 and greater dehydrogenase reaction in rat resistance vessels than aorta.29 Since dehydrogenase activity has been demonstrated to play a significant role in conferring the mineralocorticoid specificity on MR, the greater dehydrogenase activity in the present study may be related to the presence of much higher levels of MR in resistance vessels. Comparative levels of MR in various vessels are to be examined for further investigation. Glucocorticoids (and mineralocorticoids as well) in-
It has been suggested that the rise in blood pressure, which is not associated with renal mineralocorticoid target tissues such as the kidney contain 11β-HSD activity. In congenital or acquired 11β-HSD-deficient states, suppression of 11β-HSD2 activity in the kidney has been believed to cause renal mineralocorticoid excess, resulting in sodium retention and hypertension. However, after administration of 11β-HSD inhibitors, there is a discrepancy between sodium retention (which occurs in the first few days) and elevated blood pressure (which occurs only after chronic administration). Therefore, the rise in blood pressure may be independent of renal mineralocorticoid excess. In administration of glucocorticoids to rats, increased vascular responses to pressor hormones precede the rise in blood pressure, which is not associated with renal MR. The present study has shown that 11β-HSD could modulate the access of glucocorticoids to vascular receptors and influence vascular tone. We propose that vascular 11β-HSD2 activity could influence blood pressure by this mechanism without invoking renal sodium retention.

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