Original Article

11 β -Hydroxysteroid Dehydrogenase Activity in Human Aortic Smooth Muscle Cells

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11 β -Hydroxysteroid dehydrogenases (11 β -HSD) interconvert cortisol, the physiological glucocorticoid, and its inactive metabolite cortisone in humans. There are two isoforms. The type 1 isoform (11 β -HSD1) catalyzes both 11 β -dehydrogenation (cortisol to cortisone) and the reverse oxoreduction (cortisone to cortisol), but the type 2 isoform (11 β -HSD2) catalyzes only 11 β -dehydrogenation. The diminished dehydrogenase activity has been demonstrated in resistance vessels of genetically hypertensive rats. However, the isoform(s) that plays a significant role in conferring the dehydrogenase activity on vasculature has not been determined. We investigated 11 β -HSD activities in human vascular smooth muscle cells by manipulating 11 β -HSD expressions with antisense oligonucleotides. The results showed that 11 β -HSD2 dominates functioning in the dehydrogenase mode in these cells. This indicates that impairment of 11 β -HSD2 activity in vascular wall may be related to the pathogenesis of hypertension. (*Hypertens Res* 2001; 24: 33-37)

Key Words: 11 β -hydroxysteroid dehydrogenases (11 β -HSD), antisense oligonucleotide, human aortic smooth muscle cells (HASMC), hypertension

Introduction

11 β -Hydroxysteroid dehydrogenases (11 β -HSD) interconvert cortisol, the physiological glucocorticoid, and its inactive metabolite cortisone in humans. Two forms of the enzyme, belonging to the protein superfamily of short-chain dehydrogenases/reductases, have been structurally and functionally characterized. The type 1 isoform (11 β -HSD1) catalyzes both 11 β -dehydrogenation (cortisol to cortisone) and 11 β -oxoreduction (cortisone to cortisol), and is a low affinity NADP(H)-enzyme (1). The type 2 isoform (11 β -HSD2) is a high affinity NAD-dependent enzyme and catalyzes only 11 β -dehydrogenation (2). Since glucocorticoids increase vascular tone by potentiating the vasoconstrictor action of a number of pressor hormones, including α -adrenergic agonists and angiotensin II (3, 4), local cortisol metabolism mediated by 11 β -HSD

within vascular wall may be important for the control of vascular tone. Brem *et al.* reported that inhibition of the reductase activity decreased contractile responses of rat aortic rings to vasoconstricting hormones (5).

Cortisol has an affinity to type 1 mineralocorticoid receptor (MR) and thus induces mineralocorticoid effects. 11 β -Dehydrogenase activity is crucial for prevention of mineralocorticoid activity of cortisol (6, 7). The diminished dehydrogenase activity has been demonstrated in patients with essential hypertension (8) and in resistance vessels of genetically hypertensive rats (9). Recently, we have shown that suppression of 11 β -HSD2 expression in vascular wall could result in increased vascular tone by the contribution of cortisol, which acts as a mineralocorticoid (10). This indicates that 11 β -HSD2 plays a significant role in conferring the dehydrogenase activity on human vasculature. However, the functional role of 11 β -HSD1 in the vascular dehydrogenase activity remains uncertain.

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This work has been supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

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Received June 2, 2000; Accepted in revised form September 1, 2000.

The purpose of this study was to clarify the physiological significance of 11 β -HSD activities in human vascular wall. We investigated the activities in human aortic smooth muscle cells (HASMC).

Methods

Materials

HASMC were obtained from Clonetics Corp. (Walkersville, USA) and cultured according to the supplier's instruction. [1,2,6,7-³H]cortisol was from Amersham International PLC (Arlington Heights, USA). Cortisol and cortisone were from Sigma (St. Louis, USA).

Cell Culture

HASMC were cultured to confluence in Dulbecco's modified Eagle's medium with 5% FCS plus 4 μ g/ml of gentamicin under 5% CO₂-95% air at 37°C. Cells at 5-8 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 using a monoclonal antibody against human α -smooth muscle actin.

Detection of 11 β -HSD mRNA

Oligonucleotide primers for reverse transcription-polymerase chain reaction (RT-PCR) were synthesized with an Applied Biosystems Model 392 DNA synthesizer (PE Biosystems, Foster City, USA) and purified with an oligonucleotide purification columns. The sequences of sense and antisense primers for detecting 11 β -HSD1 mRNA were 5'-CTCGAGTCGGATGGCTTTTATG-3' and 5'-ACTTGCTTGCAAGATAGG-3' (1). The sequences of the sense and antisense primers for detecting 11 β -HSD2 mRNA were 5'-ACCGTATTGGAGTTGAACAGC-3' and 5'-TCACTGACTCTGTCTTGAAGC-3' (2). RT-PCR was performed as described previously (11). A 10- μ l 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 11 β -HSD Activity

11 β -HSD activities were measured by a radiometric conversion assay as previously described (12). In brief, confluent HASMC 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 h, after which steroids were extracted with chloroform and resolved by thin-layer chromatography (TLC). Radioactivities corresponding to cortisol and cortisone were determined. Dehydrogenase and reductase activities were calculated

as cpm for cortisone/(cpm for cortisol+cpm for cortisone) $\times$ 100 and cpm for cortisol/(cpm for cortisol+cpm for cortisone) $\times$ 100, respectively. [1,2,6,7-³H]cortisone was prepared from [1,2,6,7-³H]cortisol as described previously (13). Briefly, the labelled cortisol (10 μ Ci) was incubated in 1 ml of 50% aqueous acetic acid containing 1% chromium trioxide at room temperature for 20 min. The residue from the dichloromethane extract of the reaction products was chromatographed by TLC using 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.

Antisense Oligonucleotides

24-mer phosphorothioate antisense oligonucleotides (AS) complement of the 5'-region of human 11 β -HSD mRNAs containing the initiator AUG codon and, as controls, nonsense oligonucleotides (NS) containing the same base composition but in a random, scrambled order, were synthesized with an Applied Biosystems Model 392 DNA synthesizer. Sequences of 11 β -HSD1 AS and NS were 5'-GAGATATTTTTCATAAAAGCCAT-3' and 5'-AATCTGATGTAATGCTTCATAATA-3', respectively. Sequences of 11 β -HSD2 AS and NS were 5'-CGACGGCC AAGGCCAGCGCTCCAT-3' and 5'-TCACGCACGCG CCAACCGCGGAGT-3', respectively.

Statistics

Data are expressed as mean $\pm$ SEM. The significance of differences was assessed by 1-way ANOVA followed by Student-Newman-Keuls Multiple Comparison Test. *P* values less than 0.05 were considered to indicate statistical significance.

Results

11 β -HSD Expression in HASMC

We examined the expression of 11 β -HSD1 and 11 β -HSD2 genes in HASMC. Amplified products corresponding to transcripts of the 11 β -HSD2 gene were detected by RT-PCR. However, HASMC showed no detectable 11 β -HSD1 mRNA under the conditions employed (Fig. 1). Cloning and sequence analysis of the PCR products demonstrated that these bands corresponded to known sequences of human 11 β -HSD mRNAs (data not shown).

11 β -HSD Activity in HASMC

In order to assess 11 β -HSD activities, labelled cortisol or its metabolite cortisone was added to each medium. Incubation with cortisol resulted in moderate (20 $\pm$ 4%) con-

Fig. 1. 11 β -HSD expression in HASMC. One hundred nanograms of poly (A)⁺ RNAs from the cells were amplified by RT-PCR for the detection of 11 β -HSD1 and 11 β -HSD2 mRNAs as described under Methods. The liver (L) and kidney (K) lanes were used as positive controls. EC, human aortic endothelial cells; SMC, HASMC.

Fig. 2. 11 β -HSD activity in HASMC. Dehydrogenase and oxoreductase activities were calculated as described under Methods. Open columns represent data from experimental blanks (n=6).

version to cortisone after 8 h, whereas incubation with cortisone resulted in higher rate of conversion ($26 \pm 5\%$) to cortisol (Fig. 2). Thus, HASMC were capable of performing the dehydrogenase as well as the reverse oxoreductase phase of the reaction.

Effect of Antisense Oligonucleotides on 11 β -HSD Activities in HASMC

The reductase activity was decreased by approximately 40% but the dehydrogenase activity was unaffected with

an antisense oligonucleotide (10 μ mol/l) complementary to 11 β -HSD1 mRNA (Fig. 3A). We also conducted this assay using an additional antisense oligonucleotide (10 μ mol/l) that was raised against nucleotides 46-69 relative to the first letter of the initiator codon of 11 β -HSD1 mRNA at +1 (data not shown). Since this additional experiment resulted in the similar inhibition of reductase activity in HASMC, the possibility of a non-specific effect on the activity was considered unlikely. When HASMC were grown with an 11 β -HSD2 antisense oligonucleotide (10 μ mol/l), the dehydrogenase activity was reduced by approximately 80% but the reductase activity was unchanged (Fig. 3B).

Discussion

We demonstrated that the type 2 isoform dominates functioning in the dehydrogenase mode in HASMC. Glucocorticoids can potentiate the vasoconstrictor action of a number of pressor hormones, including α -adrenergic agonists and angiotensin II. This potentiation is postulated to be mediated by the upregulation of receptors for these pressor hormones in vascular smooth muscle cells (4). 11 β -Reductase regenerates active glucocorticoids from circulating inactive 11-keto forms, and modulates the access of glucocorticoids to glucocorticoid receptors in vascular wall. An increase in available glucocorticoids could make vascular cells more responsive to circulatory vasoconstricting hormones. Brem *et al.* reported that inhibition of reductase activity decreased the contractile responses of rat aortic rings to vasoconstricting hormones (5). In the present study, HASMC expressed no detectable 11 β -HSD1 mRNA but these cells were capable of performing oxoreductase activity, and the effect of 11 β -HSD1 antisense oligonucleotides on the reductase activity was only partial. Brem *et al.* also demonstrated the incomplete effect of 11 β -HSD1 antisense oligonucleotides

Fig. 3. Effect of antisense oligonucleotides on 11 β -HSD activities in HASMC. After confluent HASMC had been incubated in the presence of the indicated concentrations of antisense oligonucleotide (AS) or nonsense oligonucleotide (NS) for 24 h, dehydrogenase (open columns) and oxoreductase (crosshatched columns) activities were determined (n=6). *p<0.05; **p<0.01, compared with cells cultured without oligonucleotides.

on the activity in rat aortic endothelial cells (14). These findings indicate that there might be other 11 β -reductase(s) which shares a high degree of sequence identity with human 11 β -HSD1. The existence of such enzymes is a subject worthy of further investigation.

Type 1 mineralocorticoid receptor (MR) has an equal affinity for cortisol and aldosterone, despite the fact that the circulating cortisol levels are much higher than those of aldosterone. Dehydrogenase activity has been demonstrated to play a significant role in conferring the mineralocorticoid specificity on MR. The defect of dehydrogenase activity would thus allow the MR to be occupied mostly by cortisol (15). We previously demonstrated that diminished vascular dehydrogenase activity enhances the effect of cortisol on angiotensin II receptor regulation partly by the activation of MR (10). Since local glucocorticoids (mineralocorticoids) within vascular wall could directly affect vascular tone, the local metabolism of glucocorticoids mediated by 11 β -HSD2 may be important in controlling blood pressure. Soro *et al.* reported that the ratio of cortisol to cortisone metabolites in urine was significantly higher in patients with essential hypertension (8). We previously demonstrated elevated levels of 11 β -HSD2 inhibitory substances in the urine of patients with low renin essential hypertension (16). Furthermore, we have reported decreased dehydrogenase activity in resistance vessels of genetically hypertensive rats (9). The present study suggests that the vascular dehydrogenation may be mediated by 11 β -HSD2 alone, indicating that impairment of 11 β -HSD2 activity in vascular wall could be relevant to the pathogenesis of hypertension.

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