Functional Adrenocorticotropic Hormone Receptor in Cultured Human Vascular Endothelial Cells
Possible Role in Control of Blood Pressure

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Abstract—Hypertension is a prominent feature of patients with Cushing’s disease and ectopic adrenocorticotropic hormone (ACTH) syndrome, who have elevated ACTH levels. Chronic administration of ACTH (1-24) also raises blood pressure in humans. This effect has been postulated to be due to ACTH-induced increases in cortisol secretion in the adrenal gland. It is well known that cortisol increases vascular tone by potentiating the vasoconstrictor action of a number of pressor hormones. In the present study, we show direct evidence that human aortic endothelial cells possess the ACTH receptor. 11β-Dehydrogenation, converting cortisol to its inactive metabolite, cortisone, mediated by vascular 11β-hydroxysteroid dehydrogenase type 2 is essential for the control of vascular tone, and the reduced activity may be relevant to the pathogenesis of hypertension. We found that ACTH (1-24) dose-dependently decreased the gene expression and enzyme activity of 11β-hydroxysteroid dehydrogenase type 2 in these cells, and the decrease was partially abolished by a selective ACTH receptor antagonist. This may indicate that ACTH potentiates the action of cortisol through its direct effect on the vasculature. Therefore, the present study provides important information for understanding the mechanism of ACTH-induced hypertension. (Hypertension. 2000;36:862-865.)

Key Words: adrenocorticotropic hormone ■ gene expression ■ gene regulation ■ hormones ■ hypertension, essential

Adrenocorticotropic hormone (ACTH) is the main hormone that regulates glucocorticoid synthesis and secretion in mammals by binding to specific receptors on the adrenal cortex. The ACTH receptor (ACTH-R) gene has been cloned, and the predicted amino acid sequence has demonstrated that this receptor is one of the smallest of the 7 transmembrane domain receptors identified to date.1 This receptor is expressed in the adrenal cortex but has been recently identified in extra-adrenal tissues such as human skin2 and mouse adipose tissue.3 Previous studies showed that the administration of ACTH (1-24) caused an increase in blood pressure in normotensive and hypertensive subjects, whereas no change was observed in patients with adrenal insufficiency,4 and the effect of ACTH (1-24) was mimicked by cortisol.5 Thus, the rise in pressure has been believed to be due to ACTH-induced increases in cortisol secretion in the adrenal gland.6 It has been shown that the direct effect of cortisol on vascular tone plays a significant role in the rise in pressure, because the steroid may raise pressure in the absence of any classic glucocorticoid effects (increases in plasma volume or urinary sodium retention).7 Cortisol increases vascular tone by potentiating the vasoconstrictor action of a number of pressor hormones, including α-adrenergic agonists and angiotensin II.8,9 In addition to the hormonal effect, ACTH has been suggested to have direct effects on vascular tone. In hypovolemic and hemorrhagic shock in humans, acute intravenous administration of ACTH (1-24) promptly restores blood pressure without any effect on heart rate.10 However, little is known concerning the existence of ACTH-R in the vasculature.

11β-Hydroxysteroid dehydrogenases (11β-HSDs) interconvert cortisol and its inactive metabolite, cortisone, in humans. We have demonstrated that the local glucocorticoid metabolism displayed by 11β-HSD within the vascular wall may be important in the control of vascular tone and the pathogenesis of hypertension.11 In humans, two 11β-HSD isozymes have been described and cloned. The first enzyme (11β-HSD1) mainly catalyzes oxygen reduction (cortisone to cortisol) and is a low-affinity NADP(H) enzyme.12 A second isozyme (11β-HSD2) is a high-affinity NAD-dependent enzyme and catalyzes only 11β-dehydrogenation (cortisol to cortisone).13 The diminished dehydrogenase activity was reported in patients with essential hypertension14 and in resistance vessels of genetically hypertensive rats.15 We have demonstrated that impaired 11β-HSD2 activity in the vascular wall could result in increased vascular tone by enhancing the effect of cortisol.11

The purpose of the present study was to clarify the physiological and pathophysiological significance of...
ACTH-R in human vessels. We investigated the gene expression in human aortic endothelial cells (HAECs). Furthermore, to assess its potential role in the control of blood pressure, the effect of ACTH (1-24) on vascular 11β-HSD activity was also examined.

Methods

Materials

HAECs (Clonetics Corp) were cultured according to the supplier’s instructions. [1,2,6,7-3H]Cortisol was from Amersham International plc. Cortisol and cortisone were from Sigma Chemical Co. Human ACTH (1-24) and ACTH (7-38) were from the Peptide Institute. Total RNAs from human tissues (liver, kidney, and adrenal gland) were obtained from Clontech Laboratories, Inc.

RT-PCR Method

Oligonucleotide primers (Table) for reverse transcriptase (RT)–polymerase chain reaction (PCR) were synthesized with an Applied Biosystems model 392 DNA synthesizer and purified with oligonucleotide purification columns. RT-PCR was performed as described previously.16 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. Each experiment was repeated 3 times, and the data shown are representative of those obtained in 3 experiments.

Northern Blot Analysis

Total RNAs (30 μg) isolated from confluent culture of HAECs were electrophoresed on 1.5% agarose gel containing 1.1 mol/L formaldehyde, blotted onto Hybond N+ nylon membrane, and hybridized at 42°C for 20 hours in 50% formamide, 0.5× Denhardt’s solution, 0.5% SDS, and 0.4 mg/mL salmon sperm DNA containing 5 ng/mL human ACTH-R cDNA, which had been labeled with [α-32P]dCTP to a specific activity of ~1×108 cpm/μg. The cDNA probe (631 bp) was originally obtained by RT-PCR with adrenal gland total RNA as described above. The sequence was verified by the dieoxy chain termination method. After hybridization, the membrane was washed twice with 2× standard saline citrate and 0.1% SDS at room temperature and then twice with 0.1× standard saline citrate and 0.1% SDS at 42°C and autoradiographed. The hybridized signals were analyzed with a BAS 1500 Bioimaging Analyzer.

Assay of 11β-HSD Activity

11β-HSD activity was measured by a radiometric conversion assay as previously described.17 In brief, confluent HAECs were incubated in a hydrocortisone-free and serum-free medium containing 10 nmol/L [1,2,6,7-3H]cortisol 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 activity was calculated as follows: counts per minute for cortisone/(counts per minute for cortisol+counts per minute for cortisone)100.

Competitive PCR Method

Single-stranded cDNA was prepared with total RNA (1 μg) from HAECs as described previously.16 The single-stranded cDNA was used in competitive PCR. The sequences of sense and antisense primers for 11β-HSD2 were the same as in the Table. The competitive templates were made with a PCR MIMIC Construction Kit (Clontech Laboratories, Inc). After quantification, a series dilution was used as an internal standard for competitive PCR. Competitive PCR was performed with 20 μL of the reverse-transcribed DNA, 2 μL of different concentrations of the competitive template, 0.5 μmol/L each of sense and antisense primers, and 0.5 U of Taq DNA polymerase (TaKaRa) in 50 μL of 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 2 mmol/L MgCl2, and 0.2 mmol/L of each deoxy-NTP. The reactions were performed for 30 seconds at 94°C, 30 seconds at 69°C, and 1 minute at 72°C for 35 cycles. Aliquots of 10-μL amplification products were electrophoresed on a 2% agarose gel. The gel was stained with ethidium bromide and photographed. The signal intensity was quantified by computerized densitometry with the use of the BIO-PROFIL BIO-1D system (Compak). The intensities of the products from cDNA and from competitive templates were plotted as a function of the known amounts of the competitive templates. The intra-assay and interassay variabilities of the competitive PCR method were 13.5% and 17.3%, respectively.

Statistical Analysis

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

Results

Human ACTH-R Exists in HAECs

We examined human ACTH-R expression in HAECs. As shown in Figure 1, 631-bp amplified products corresponding to the transcripts were clearly detected in the HAEC and adrenal cortex lanes. Cloning and sequence analysis of the PCR products demonstrated that both bands had the known coding sequences of human ACTH-R mRNA (data not shown). Northern blot analysis with the use of a probe that contained the coding sequence of human ACTH-R revealed predominant transcripts at 3.8 kb in the HAEC and human umbilical vein endothelial cell lanes. However, the adrenal gland expressed a predominant band of 1.8 kb as well as a less intense band of 3.8 kb (Figure 2).
Next, we examined the expression of 11β-HSD1 and 11β-HSD2 genes in HAECs. With the use of the RT-PCR method, amplified products corresponding to transcripts of the 11β-HSD2 gene were detected (Figure 3B). However, the HAECs showed no detectable 11β-HSD1 mRNA with the conditions used (Figure 3A). Cloning and sequence analysis of the PCR products demonstrated that these bands had the known sequences of human 11β-HSD mRNAs (data not shown).

Effect of ACTH (1-24) on 11β-HSD2 Gene Expression

To investigate the effect of ACTH (1-24) on 11β-HSD2 gene expression, we conducted a competitive PCR analysis. Representative results for the 11β-HSD2 expression are shown in Figure 4. The signals of endogenous 11β-HSD2 cDNA increased with the serial dilution of the competitive template. For ACTH (10 nmol/L)–treated HAECs, the point of equivalence of signals was at the 10-fold diluted mimic. The result indicates that ACTH (10 nmol/L) caused an ∼10-fold decrease in the amount of 11β-HSD2 mRNA level. More exact quantification in the presence of 2-fold serial diluted mimics revealed that ACTH (1 nmol/L) reduced ∼4-fold the level of 11β-HSD2 mRNA (data not shown). Because the amount of 11β-HSD1 mRNA in HAECs was below the detectable level, we did not attempt to examine the effect on 11β-HSD1 expression.

Effect of ACTH (1-24) on 11β-HSD Activity

Incubation with cortisol resulted in moderate (30±3%) conversion to cortisone in HAECs (Figure 5). Next, we investigated the effect of ACTH (1-24) on 11β-HSD activity. The tested ACTH concentrations included the physiologically and pathophysiologically relevant ranges. As shown in Figure 5, ACTH (1-24) induced dose-dependent decreases in the dehydrogenase activity of HAECs, with a maximal decrease (78±6%) at 10 nmol/L. The inhibitory effect of ACTH (1-24) on the dehydrogenase activity was dose-dependently reduced in the presence of ACTH (7-38), a selective ACTH-R antagonist. This result indicates that ACTH (1-24) reduces the dehydrogenase activity of HAECs through an interaction with its own receptor.

Discussion

In the present study, we showed for the first time that human vascular endothelial cells possess functional ACTH-Rs. Although cardiovascular actions of ACTH have been demonstrated, the existence of the receptor has been unknown. Northern blot analysis showed that HAECs contain transcripts that are different from those of the adrenal gland. Because the ACTH-R gene has a single exon in the coding region and because the probe we used contained the complete coding sequence, the presence of different transcripts may result from different promoter usage and/or different polyadenylation usage.
ACTH regulates cortisol synthesis in the adrenal cortex. The cortisol is important in the control of vascular tone. The cortisol action may be modified by vascular 11β-HSD activities. We have demonstrated that the dehydrogenase activity (cortisol to cortisone) in human vascular cells is mediated by 11β-HSD2 and that the reduced activity may be relevant to the pathogenesis of hypertension. The present study clarified that ACTH downregulates the expression of 11β-HSD2, suggesting that this peptide plays a significant role in the control of vascular tone through an interaction with the vascular receptor. This may be compatible with the fact that peripheral conversion of cortisol to cortisone in the kidney is inhibited by ACTH and that the marked increase of cortisol/cortisone ratio is observed in urine and plasma during ACTH infusion but not after hydrocortisone infusion in humans.

Diederich et al. reported that 11β-HSD activity in human kidney slices was not influenced by incubation with increasing doses of physiological ACTH (1-39) for 1 hour. Because ACTH-R is not expressed in the kidney and because the authors examined a fast effect of ACTH on renal 11β-HSD activity, their study may not be contradictory to our results.

Brem et al. reported that two 11β-HSD isozymes were expressed in rat aortic endothelial cells and that these cells possessed a predominant oxygen reductase activity. Our results demonstrated that HAECs expressed no detectable 11β-HSD1 mRNA. The difference of species might account for the observed differences. Because dehydrogenase activity has been demonstrated to play a significant role in conferring the mineralocorticoid specificity on the type 1 mineralocorticoid receptor, the predominant dehydrogenase activity in the present study may be related to the presence of much higher levels of the receptor in human blood vessels. Comparative levels of the receptor in various vessels are to be examined in further investigations.

The blood pressure–raising effect of ACTH has been known since its introduction into clinical practice. Indeed, hypertension is a crucial feature of patients with Cushing’s disease and ectopic ACTH syndrome. Exogenous ACTH (1 mg/d) administered to normotensive subjects can also raise their blood pressures by ~20 mm Hg over the treatment period. When ACTH is given by constant intravenous infusion, rates as low as 50 mcg/d raise pressure. ACTH concentrations rose with the infusion but remained within the normal range, suggesting that concentrations achieved under physiological conditions could be sufficient to raise blood pressure in humans. The effect of ACTH has been postulated to be caused by ACTH-induced increases in cortisol production in the adrenal cortex. Our results indicate that ACTH could enhance blood pressure not only by regulating the production of cortisol in the adrenal cortex but also by enhancing the effect of cortisol on vascular tone through the adrenally independent mechanism. The present study may provide the starting point for a novel understanding of the molecular basis of hypertension.

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References
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