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EVIDENCE THAT 19-HYDROXYANDROSTENEDIONE IS SECRETED BY THE ADRENAL CORTEX AND IS UNDER THE CONTROL OF ACTH AND THE RENIN-ANGIOTENSIN SYSTEM IN MAN

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Plasma 19-hydroxyandrostenedione (19-OH-A-dione) concentrations in man were evaluated using a specific and sensitive radioimmunoassay. Plasma 19-OH-A-dione concentrations (mean ± SE) in normal subjects are 151 ± 14 pg/ml (n=13) in males and 141 ± 9 pg/ml (n=14) in females. Plasma 19-OH-A-dione (mean ± SE) rises significantly during ACTH stimulation (116 ± 25 pg/ml vs 288 ± 38 pg/ml; $P<0.01$; n=5), declines significantly during dexamethasone suppression (180 ± 30 pg/ml vs 36 ± 14 pg/ml; $P<0.01$; n=4) and rises significantly during angiotensin II infusion (89 ± 10 pg/ml vs 159 ± 27 pg/ml; $P<0.05$; n=5). Plasma 19-OH-A-dione in the adrenal vein is much higher than that in the inferior vena cava (2076-3076 pg/ml vs 115-184 pg/ml; n=2). These results demonstrate that 19-OH-A-dione is directly secreted by the adrenal cortex and is under the control of ACTH and the renin-angiotensin system.

INTRODUCTION

C19 steroid, 19-hydroxyandrostenedione (19-hydroxyandrost-4-ene-3, 17-dione, 19-OH-A-dione) has been reported as an amplifier of the action of aldosterone (1). In bioassays using adrenalectomized rats, 19-OH-A-dione does not cause any significant change in urinary Na/K ratio. However, it causes a significant decrease in urinary Na/K ratio when administered simultaneously with subthreshold doses of aldosterone. In addition, in the studies using the same experimental procedures as for DOCA-salt hypertensive rats, the 19-OH-A-dione treated rats develop hypertension, hypokalemia, suppressed plasma renin activity and low plasma aldosterone and corticosterone concentrations as the DOCA treated rats do (2). These results indicate that 19-OH-A-dione amplifies the action of endogenous aldosterone and causes the hypertensive state similar to mineralocorticoid excess.

Although the biological activities of 19-OH-A-dione have been elucidated, the presence of 19-OH-A-dione in human peripheral circulation has

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not yet been demonstrated. In the present study, we evaluate 19-OH-A-dione concentrations in human plasma using a specific and sensitive radioimmunoassay.

MATERIALS AND METHODS

Anti-19-OH-A-dione-serum

19-OH-A-dione-3-oxime-bovine serum albumin was used as antigen in developing antibodies. The details of the immunization procedure have been described previously (3). For use in radioimmunoassays, the antiserum was diluted 1:40,000 with borate buffer (0.05M, pH 7.8).

Extraction and purification procedure

After 1000 cpm of 19-OH-A-dione-19S-^{3}H (2.5 Ci/m mole) was added to plasma to correct for procedural losses, 3 ml of plasma was extracted with 10 ml of dichloromethane. The extract was evaporated to dryness and was applied to LH-20 columns (solvent, chloroform: n-heptane: methanol = 50:50: 1) and then high pressure liquid chromatography was performed (Waters Associates Inc., model M-45 with μ Bondapac C18 column, solvent, 50 % methanol). The fractions containing 19-OH-A-dione were dissolved in 1 ml of methanol. For radioimmunoassay, 0.15 and 0.3 ml aliquots were used and 0.5 ml aliquots were used for the correction of procedural losses.

Radioimmunoassay

Samples of 0, 10, 20, 50, 100, 200 and 500 pg of 19-OH-A-dione standard and 0.15 and 0.3 ml aliquots were evaporated to dryness and 0.3 ml of the diluted antiserum containing 2000 cpm of 19-OH-A-dione-19S-^{3}H, 0.05 % bovine serum albumin and 0.05 % bovine gamma globulin were added and incubated at 4 °C overnight. Then 0.3 ml of saturated ammonium sulfate was added, mixed on a vortex mixer and centrifuged for 10 minutes at 3000 rpm. Finally, 0.3 ml of the supernatant was transferred to a counting vial and counted in a scintillation spectrophotometer.

Evaluation of the radioimmunoassay

The antiserum gave a standard curve between 0 and 500 pg of 19-OH-A-dione in a dilution of 1:40,000. Blank values which were determined by measuring 3 ml of plasma obtained from patients with Addison's disease could not be differentiated from zero. To examine accuracy and precision of the assays, 500 pg of 19-OH-A-dione were added to 3 ml of blank plasma and measured. The measured amounts of 19-OH-A-dione were 487 ± 30(SD) pg (n=5). The ratios of 19-OH-A-dione measured to 19-OH-A-dione added were 0.97 and the coefficient of variation was 6.1 %. The evaluation of specificity of the antiserum revealed that it reacts only with 19-OH-A-dione and does not react with other steroids such as 19-hydroxytestosterone, 19-hydroxydehydroepiandrosterone, androstenedione, testosterone and dehydroepiandrosterone. The minimum detectable amount of 19-OH-A-dione in plasma was 40 pg/ml.

Subjects and test procedures

Plasma 19-OH-A-dione concentrations in peripheral venous blood obtained from 13 normal males (20-49 yrs) and 14 normal females (20-46 yrs) and those in adrenal venous blood obtained from 2 normal subjects (Case 1 female 49 yrs, Case 2 female 29 yrs) were measured. ACTH stimulation was achieved in 5 normal subjects by administering 0.25 mg of α1-24 ACTH (Cortrosyn, Daiichi Pharmaceutical Company) intravenously and blood was obtained 30 minutes later. Adrenal suppression was achieved in 4 normal subjects by giving dexamethasone 0.5 mg orally every 6 hours for 2 days. Angiotensin II stimulation was performed in 5 normal subjects by infusing angiotensin II (Hypertensin, Ciba-Geigy Limited) at a rate of 0.5 ng/kg/minute for 20 minutes.

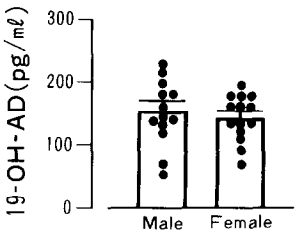

Figure 1. Plasma 19-OH-A-dione levels in normal male and female subjects. Each bar shows the mean ± SE. 19-OH-AD (19-OH-A-dione).

RESULTS

Plasma 19-OH-A-dione levels (mean ± SE) in normal subjects are shown in Figure 1. Plasma 19-OH-A-dione is 151 ± 14 pg/ml (n=13) in normal males and 141 ± 9 pg/ml (n=14) in normal females. No significant difference is found between males and females.

Plasma 19-OH-A-dione levels after the administration of ACTH, dexamethasone and angiotensin II are shown in Figure 2. Plasma 19-OH-A-dione (mean ± SE) rises significantly from 116 ± 25 pg/ml to 288 ± 38 pg/ml ($P < 0.01$; n=5) during ACTH stimulation, declines significantly from 180 ± 30 pg/ml to 36 ± 14 pg/ml ($P<0.01$; n=4) during dexamethasone suppression and rises significantly from 89 ± 10 pg/ml to 159 ± 27 pg/ml ($P<0.05$; n=5) during angiotensin II infusion.

Concentrations of 19-OH-A-dione in adrenal venous blood are compared with those in inferior vena cava blood (Figure 3). Plasma 19-OH-A-dione in the

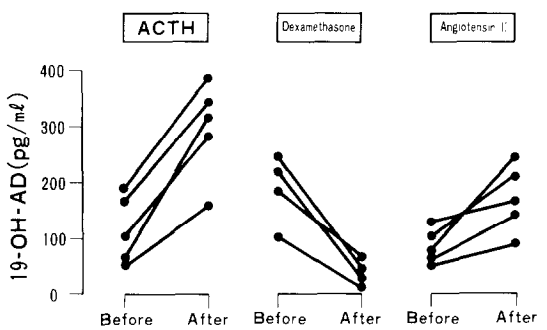

Figure 2. The changes of plasma 19-OH-A-dione levels after ACTH stimulation, dexamethasone suppression and the administration of angiotensin II. 19-OH-AD (19-OH-A-dione).

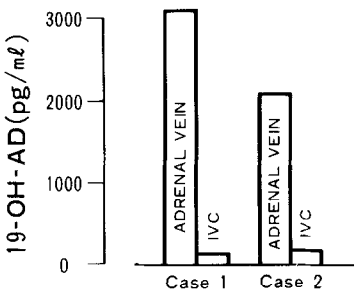

Figure 3. 19-OH-A-dione levels in adrenal venous blood as compared with simultaneous levels in mixed venous blood. 19-OH-AD (19-OH-A-dione), IVC (inferior vena cava).

adrenal vein is much higher than that in the inferior vena cava (2076 - 3076 pg/ml vs 115 - 184 pg/ml; n=2).

DISCUSSION

In the present study, plasma 19-OH-A-dione concentrations were evaluated using a specific and sensitive radioimmunoassay and 19-OH-A-dione was found to be present in human peripheral circulation. It rose with ACTH stimulation and declined with dexamethasone suppression, indicating that 19-OH-A-dione is under the control of ACTH. Plasma 19-OH-A-dione also rose with angiotensin II infusion. The results show that 19-OH-A-dione is also under the control of the renin-angiotensin system. As the concentrations of 19-OH-A-dione in the adrenal vein were much higher than those in the inferior vena cava, 19-OH-A-dione is considered to be directly secreted by the adrenal cortex.

As the action of 19-OH-A-dione is closely related to aldosterone (1, 2), it is inferred that angiotensin II, which stimulates the adrenal cortex to secrete aldosterone, also stimulates the secretion of 19-OH-A-dione. The results of the present study indicate that angiotensin II truly induces the secretion of 19-OH-A-dione. Angiotensin II is considered to stimulate the secretion of aldosterone and its amplifier at the same time.

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