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The Measurement of 11β, 17β-Dihydroxy-4-androsten-3-one (11β-Hydroxytestosterone) by Radioimmunoassay in Human Plasma

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Summary: A radioimmunoassay (RIA) is described for the measurement of 11β,17β-dihydroxy-4-androsten-3-one (11β-hydroxytestosterone) in human plasma. The reliability criteria of the new RIA were similar to those of other steroid hormone radioimmunoassays. The mean plasma 11β,17β-dihydroxy-4-androsten-3-one level for healthy young subjects was 1.31 ± 0.32 nmol/l ($\bar{x}$ ± SD) in males and 1.19 ± 0.35 nmol/l in females at 8 a. m.; during the night, there was a marked decrease, and at 11 p. m. the recorded values were 0.41 ± 0.15 nmol/l and 0.46 ± 0.14 nmol/l, respectively. During the corticotropin stimulation test, 11β,17β-dihydroxy-4-androsten-3-one increased from 1.03 ± 0.33 nmol/l to 1.31 ± 0.41 nmol/l, while in dexamethasone suppression tests a decrease from 2.04 ± 0.82 nmol/l to 0.25 ± 0.05 nmol/l was seen. In contrast, chorionic gonadotropin administration on 3 consecutive days did not influence plasma concentrations of 11β,17β-dihydroxy-4-androsten-3-one.

Radioimmunoassay zur Messung von 11β, 17β-Dihydroxy-4-androsten-3-on (11β-Hydroxytestosteron) im Plasma des Menschen

Zusammenfassung: Es wird ein Radioimmunoassay für die Messung von 11β,17β-Dihydroxy-4-androsten-3-on (11β-Hydroxytestosteron) im menschlichen Plasma beschrieben. Die Zuverlässigkeitskriterien des neu aufgebauten RIA entsprechen denen anderer Radioimmunoassays für Steroidhormone.

Die Konzentration von 11β,17β-Dihydroxy-4-androsten-3-on betrug um 8.00 Uhr im Plasma gesunder Männer 1,31 ± 0,32 nmol/l ($\bar{x}$ ± SD) und bei gesunden Frauen 1,19 ± 0,35 nmol/l. Um 23.00 Uhr fanden sich 0,41 ± 0,15 nmol/l (Männer) und 0,46 ± 0,14 nmol/l (Frauen). Im Corticotropin-Stimulationstest war ein Anstieg der Konzentration von 11β,17β-Dihydroxy-4-androsten-3-on im Plasma von 1,03 ± 0,33 nmol/l auf 1,31 ± 0,41 nmol/l zu beobachten, während sich im Dexamethasontest ein Rückgang von 2,04 ± 0,82 nmol/l auf 0,25 ± 0,05 nmol/l ergab. Nach Applikation von Choriongonadotropin wurde keine Änderung der Konzentration von 11β,17β-Dihydroxy-4-androsten-3-on gemessen.

Introduction

The adrenal androgens are quantitatively the most important steroid hormones but their physiological role remains still somewhat obscure in man. Far less information is available about the adrenal androgens that carry a functional group at C-11. 11β-Hydroxy-4-androstene-3,17-dione has been demonstrated in human plasma and might play an important role under physiological and pathological conditions (1). Recently 17β-hydroxy-4-androstene-3,11-dione and 4-androstene-3, 11,17-trione were demonstrated in human plasma (2, 3). In contrast, no information is available about 11β,17β-dihydroxy-4-androsten-3-one in human peripheral blood. The present paper describes a radioimmunoassay system for the measurement of 11β,17β-dihydroxy-4-androsten-3-one, and presents the first published values for this androgen in human plasma.

Materials and Methods

Steroids and reagents

Non-radioactive and radioactive steroids ([1,2-^{3}H]cortisol, 1.5–2.2 TBq/mol) were obtained from Sigma, Taufkirchen, and New England Nuclear, Dreieich, respectively. [1,2-^{3}H]11β,17β-Dihydroxy-4-androsten-3-one was synthesized from [1,2-^{3}H]cortisol, by a method similar to that described in a previous paper (2), following the procedure of *Appleby & Norymberski* (4). All organic solvents (Merck, Darmstadt, analytical grade) were used without further purification. Sodium phosphate buffer (0.15 mol/l, pH 7.0) containing 1 g/l gelatin served as assay buffer. Charcoal (1 g) and Dextran T70 (0.1 g) were suspended in 100 ml assay buffer. Bovine serum albumin was obtained from Merck. UV-spectra were taken with a Zeiss-spectrometer. Univsolve (Zinsser, Frankfurt) was used as scintillation mixture. Tritium radioactivity was measured in a liquid scintillation spectrometer (Packard, Frankfurt).

11β,17β-Dihydroxy-4-androsten-3-one antiserum

11β,17β-Dihydroxy-4-androsten-3-one 3-(O-carboxymethyl) oxime bovine serum albumin conjugate was prepared according to *Erlanger* et al. (5). The antigen was used to generate the antiserum in female rabbits. The working dilution of antiserum used in the radioimmunoassay was 1 : 20 000. Sera were stored at −35 °C.

Plasma extraction and chromatography

Plasma samples (1.0 ml) were mixed with labeled 11β,17β-dihydroxy-4-androsten-3-one (1500 counts/min) and allowed to equilibrate for 15 min at room temperature. Steroids were extracted with diethylether (5 ml). The solvent was removed by evaporation under a gentle stream of nitrogen and the residue was purified by thin-layer chromatography (Merck Silica 60 F 254; cyclohexane/ethyl acetate, 50 + 50 by vol.). 11β,17β-Dihydroxy-4-androsten-3-one was located from the position of chromatographed non-radioactive 11β,17β-dihydrox-4-androsten-3-one (detected by UV), and by beta-scanning of the spot.

Radioimmunoassay

The methanolic eluates of the thin-layer chromatograms, containing 11β,17β-dihydroxy-4-androsten-3-one, were evaporated under a stream of nitrogen, and the dry residues dissolved in assay buffer (0.5 ml). Duplicates of 0.10 ml were used for radioimmunoassay and a 0.20 ml sample was taken for the measurement of procedural loss after evaporation of the solvent. To each tube (unknown samples and standards) were added [1,2-^{3}H]11β,17β-dihydroxy-4-androsten-3-one (about 8000 counts/min) in 0.05 ml and diluted antiserum (0.20 ml). After incubation at 4 °C overnight, a charcoal suspension (0.5 ml) was added to each tube. The tubes were vortexed and after a 10 min incubation period at 4 °C they were centrifuged at 1500 *g* for 10 min. Aliquots of the supernatants were placed in counting vials containing 10 ml scintillation fluid. The procedures for the measurement of cortisol (6) and testosterone (7) were described previously.

Experimental conditions

11β,17β-Dihydroxy-4-androsten-3-one was measured in the plasma of healthy male (n = 21) and female (n = 21) subjects (20–40 years of age) at 8 a. m. and 11 p. m. Corticotropin stimulation of the adrenal gland was carried out by i. v. injection of 0.25 mg Synacthen (CIBA, Basel) in 9 healthy male volunteers. Blood was taken immediately before and 1 h after stimulation. Dexamethasone (3 mg, orally) was applied for adrenal suppression at 11 p. m. to 10 healthy female subjects. Blood samples were taken at 8 a. m. on the day of dexamethasone application and at 8 a. m. the day thereafter. Testicular stimulation was performed in 7 healthy male subjects as described previously (8). On the first and fourth day plasma was collected at 8 a. m. and 7 p. m., and on the first, second and third day 5000 I. U. human chorionic gonadotropin were administered i. m. at 7 p. m.

Statistics

Hormone concentrations were given as the mean ± standard deviation ($\bar{x} \pm SD$). Data were processed statistically using the *Wilcoxon* rank test.

Tab. 1. Cross reactivities of different steroids with the antiserum to 11β,17β-dihydroxy-4-androsten-3-one.

Steroid	Reactivity (%)
11β,17β-Dihydroxy-4-androsten-3-one (11β-hydroxytestosterone)	100.00
17β-Hydroxy-5α-androstan-3-one (5α-dihydrotestosterone)	0.44
17β-Hydroxy-4-androstene-3,11-dione (11-oxotestosterone)	0.34
17β-Hydroxy-4-androsten-3-one (testosterone)	0.20
17α,21-Dihydroxy-4-pregnene-3,11,20-trione (cortisone)	0.15
11β,17α,21-Trihydroxy-4-pregnene-3,20-dione (cortisol)	0.14
11β-Hydroxy-4-androstene-3,17-dione (11β-hydroxyandrostendione)	< 0.10
4-Androstene-3,11,17-trione (11-oxoandrostendione)	< 0.10
4-Androstene-3,17-dione (androstendione)	< 0.10
3α-Hydroxy-5α-androstan-17-one (androsterone)	< 0.10
4-Pregnene-3,20-dione (progesterone)	< 0.10
17-Hydroxy-4-pregnene-3,20-dione (17-hydroxyprogesterone)	< 0.10
3β-Hydroxy-5-pregnen-20-one (pregnenolone)	< 0.10
3-Hydroxy-1,3,5(10)-oestratrien-17-one (oestrone)	< 0.10
1,3,5(10)-Oestratriene-3,16α,17β-triol (oestriol)	< 0.10
1,3,5(10)-Oestratriene-3,17β-diol (oestradiol-17β)	< 0.10
3,20-Dioxo-11β-21-dihydroxy-4-pregnen-18-al (aldosterone)	< 0.10
9-Fluoro-16α-methyl-11β,17,21-trihydroxy-1,4-pregnadiene-3,20-dione (dexamethasone)	< 0.10

Results

Specificity

The specificity of the antiserum was determined by incubation of constant amounts of antiserum and labelled antigen with varying amounts of cross-reacting steroids (tab. 1). The highest cross-reaction was 0.44%.

Imprecision

The intra-assay coefficient of variation was 7.2% (n = 25) and the inter-assay coefficient of variation was 9.6% (n = 25).

Sensitivity

The lower assay detection limit was estimated using the formula of *Abraham* (9). The blank values for 11β,17β-dihydroxy-4-androsten-3-one were 0.010 pmol per tube. Significant differences from zero values were obtained starting with 0.016 pmol. After semilogarithmic transformation the standard curves showed linearity from 0.033 to 0.657 pmol.

Recovery

The average recovery of added [1,2-^{3}H]11β,17β-dihydroxy-4-androsten-3-one from 92 plasma samples was 78.5 ± 4.1%. All hormone concentrations given are corrected for procedural loss using an internal standard for each sample. Aliquots (1 ml) of pooled male plasma were heated for 1 h at 70 °C, and cleared of endogenous steroids by charcoal adsorption. To these plasma samples 0.045, 0.15 or 0.30 pmol 11β,17β-dihydroxy-4-androsten-3-one were added. The samples were processed as usual. Comparison of the expected amounts determined in the described radioimmunoassay gave a correlation coefficient of r = 0.993. Furthermore, the accuracy of the RIA was checked against gas chromatography/mass spectrometry. The difference between 11β,17β-dihydroxy-4-androsten-3-one concentrations in a given sample of pool plasma measured by RIA (0.295 nmol/l) or by the physico-chemical method (0.309 nmol/l) was about 5%.

Plasma concentrations of 11β,17β-dihydroxy-4-androsten-3-one

The mean 11β,17β-dihydroxy-4-androsten-3-one serum concentration was found to be 1.31 ± 0.32 nmol/l in male and 1.19 ± 0.35 nmol/l in female young subjects at 8 a. m. Concomitantly with cortisol, 11β,17β-dihydroxy-4-androsten-3-one decreased during the night, giving at 11 p. m. 0.41 ± 0.15 nmol/l in males and 0.46 ± 0.14 nmol/l in females (tab. 2). Sex differences in plasma concentrations of 11β,17β-dihydroxy-4-androsten-3-one were not significant. During adrenal cortex stimulation by corticotropin, cortisol increased 3.2-fold 1 h after corticotropin administration (tab. 3), while 11β,17β-dihydroxy-4-androsten-3-one increased 1.3-fold 1 h after corticotropin administration. Dexamethasone suppression of

Tab. 2. Diurnal variation of plasma cortisol and 11β,17β-dihydroxy-4-androsten-3-one (nmol/l) in healthy subjects, 20–40 years of age.

	Males (n = 21)			Females (n = 21)		
	8 a.m.	11 p.m.	p	8 a.m.	11 p.m.	p
Cortisol	277.95 ± 59.23	61.20 ± 59.82	<0.01	429.97 ± 190.99	112.64 ± 91.77	<0.01
11β,17β-Dihydroxy-4-androsten-3-one	1.31 ± 0.32	0.41 ± 0.15	<0.01	1.19 ± 0.35	0.46 ± 0.14	<0.01

To obtain [ng/ml] multiply by 0.362 (cortisol) or 0.304 (11β,17β-dihydroxy-4-androsten-3-one).

Tab. 3. Plasma cortisol and 11β,17β-dihydroxy-4-androsten-3-one (nmol/l) before and after corticotropin stimulation in healthy males, 20–40 years of age. Blood was drawn at 8 a.m. and 1 h after injection of 0.25 mg synacthen.

	Before corticotropin (n = 9)	After corticotropin (n = 9)		
	B	A	A/B	p
Cortisol	201.94 ± 43.24	658.37 ± 89.38	3.26	<0.01
11β,17β-Dihydroxy-4-androsten-3-one	1.03 ± 0.33	1.31 ± 0.41	1.27	<0.01

Tab. 4. Plasma cortisol and 11β,17β-dihydroxy-4-androsten-3-one (nmol/l) before and after adrenal suppression by dexamethasone in healthy females, 20–40 years of age. Blood was drawn at 8 a.m. before and at 8 a.m. after dexamethasone application (at 11 p.m., orally).

	Before dexamethasone (n = 10)	After dexamethasone (n = 10)		
	B	A	A/B	p
Cortisol	279.39 ± 59.39	6.82 ± 3.77	0.024	<0.01
11β,17β-Dihydroxy-4-androsten-3-one	2.04 ± 0.82	0.25 ± 0.05	0.122	<0.01

Tab. 5. Plasma testosterone and 11β,17β-dihydroxy-4-androsten-3-one (nmol/l) before and after chorionic gonadotropin stimulation in 7 healthy male subjects. 5000 I.U. chorionic gonadotropin were given i.m. on 3 consecutive days at 7 p.m. Blood was drawn on the first and the fourth day. Data represent the mean of the two samples of each day.

	Before chorionic gonadotropin (n = 7)	After chorionic gonadotropin (n = 7)		
	B	A	A/B	p
Testosterone	18.92 ± 6.78	37.95 ± 10.87	2.01	<0.02
11β,17β-Dihydroxy-4-androsten-3-one	1.23 ± 0.34	1.14 ± 0.26	0.26	n.s.

To obtain [ng/ml] multiply by 0.288 (testosterone) or 0.304 (11β,17β-dihydroxy-4-androsten-3-one).

the adrenal gland caused substantial decreases in the plasma concentrations of cortisol and 11β,17β-dihydroxy-4-androsten-3-one (tab. 4). 11β,17β-Dihydroxy-4-androsten-3-one decreased by 88% (from 2.04 ± 0.82 nmol/l to 0.25 ± 0.05 nmol/l). Human chorionic gonadotropin stimulation of the testes over a period of 3 days in 7 male volunteers resulted in a 2-fold increase in testosterone. Plasma 11β,17β-dihydroxy-4-androsten-3-one, however, remained unaffected (tab. 5).

Discussion

In the present paper, a radioimmunoassay (RIA) for the determination of 11β,17β-dihydroxy-4-androsten-3-one in human plasma is described. The antiserum was of high specificity (tab. 1); the maximal cross-reaction with other steroids was less than 0.5%. Because we were the first to measure 11β,17β-dihydroxy-4-androsten-3-one in human plasma, we used thin layer chromatography to minimize unspecific effects, and we monitored the RIA by gas chromatography/mass spectrometry. The two methods gave nearly identical results when measuring plasma concentrations of a plasma pool. Our data demonstrate that 11β,17β-dihydroxy-4-androsten-3-one is present in the plasma of male and female subjects. The range of plasma concentration (about 1.25 nmol/l) is comparable with that of 17β-hydroxy-5α-androstan-3-one (10). However, in contrast to other C_{19} steroids, no significant sex difference could be found in the 11β,17β-dihydroxy-4-androsten-3-one plasma concentration. Furthermore, it was demonstrated that the 11β,17β-dihydroxy-4-androsten-3-one concentration is substantially higher in the morning (tab. 2) than in the evening, suggesting a circadian rhythm. Adrenal stimulation by corticotropin resulted in a small but significant increase in 11β,17β-dihydroxy-4-androsten-3-one (tab. 3), whereas gonadal stimulation by human chorionic gonadotropin had no effect (tab. 5). Moreover, it was shown that dexamethasone suppression of the adrenal gland lowered 11β,17β-dihydroxy-4-androsten-3-one levels significantly. Thus from these experiments it appears most likely that 11β,17β,dihydroxy-4-androsten-3-one is mainly a secretory product of the adrenal gland, and that this steroid is a specific marker of adrenal function. Furthermore, 11β,17β-dihydroxy-4-androsten-3-one might be of interest in regard to the autoregulation of steroidogenesis in the adrenal gland by modifying enzyme activities (11, 12).

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