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Androst-5-ene-3β,7α/β,17β-triols, their plasma levels and dependence on the hypothalamic–pituitary–adrenal axis

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ABSTRACT

Androst-5-ene-triols are metabolites of dehydroepiandrosterone, the most abundant steroid hormone in human circulation. Many observations in rodents have demonstrated the anti-inflammatory and immune modulating activity of 7β-hydroxy-androst-5-enes, and on the basis of these experiments androst-5-ene-3β,7β,17β-triol is considered as a potential agent in the treatment of autoimmune diseases. In contrast to the fairly abundant information on the levels and effects of androst-5-ene-triols in experimental animals and of their the pharmacological perspective, little is known about androst-5-ene-3β,7α/β,17β-triols circulating in human blood, their regulation by the hypothalamo–pituitary–adrenal axis, or their daily concentration variability. Here we provide some data on androst-5-ene-3β,7α/β,17β-triol concentrations under various conditions in men and women.

1. Introduction

Dehydroepiandrosterone (DHEA, 3β-hydroxy-androst-5-en-3-one) is the most abundant steroid hormone circulating in the human body. Among its metabolites are the 7-hydroxylated derivatives, some of which have attracted attention due to their immunomodulatory activity. The history of the discoveries of 7-hydroxylated derivatives of DHEA, their synthesis in mammalian tissues by specific hydroxylases, as well as early work suggesting their anti-glucocorticoid, anti-inflammatory and immune activity, was recently reviewed [1].

The first step in the biosynthesis of 7-hydroxy-androst-5-enes is the NADPH-dependent 7α-hydroxylation catalyzed by the enzyme CYP7B1, which occurs mainly in the liver and central nervous system. The precursor may be either DHEA or androst-5-ene-3β,17β-diol (AD). In addition to 7α-hydroxyderivative, its 7β-epimer is also formed; both epimers are interconvertible by 11β-hydroxysteroid dehydrogenase type1 (11β-HSD1). 7β-Epimer is not a product of CYP7B1, but of 11βHSD1 activity.

Many observations in rodents have demonstrated the anti-inflammatory and immune modulating activity of 7β-hydroxy-androst-5-enes [2–9]. Special attention has been paid to androst-5-ene-3β,7β,17β-triol (β-AET): e.g. the early works of Loria and coll. [2,3,10] showed that the regulation of interleukin-2 and interleukin-3 secretion from Con A-activated lymphocytes was potently increased by β-AET. Moreover, the classic immuno-suppressive effects of hydrocortisone on Con A-induced lymphocyte proliferation, as well as interleukine-2 and interleukine-3 production, were significantly counteracted by β-AET. β-AET potentiated the cellular response by increasing lymphocyte activation and counteracting the immuno-suppressive activity of hydrocortisone. In the series dehydroepiandrosterone ≪ androstenediol < androstenetriol, the latter is up to one hundred thousand times more potent in protecting the host from infections than the first [10]. The pharmaco-therapeutical potential of β-AET includes anti-inflammatory activity for arthritis treatment and the regulation of obesity and glucose intolerance [11]. Additionally, β-AET improves survival in a rodent model of traumatic shock [12,13]. A potential role for β-AET in obesity and metabolic syndrome has been discussed [14,15]. 7β-Hydroxyderivatives of DHEA play some role in bone remodeling [16,17] and improve insulin sensitivity [18].

The short biological half-time of β-AET was overcome by the synthesis of 17α-ethynyl-androst-5-ene-3β,7β,17β-triol (HE3286), a novel, metabolically stabilized, orally bioavailable derivative of β-AET [4,19,20]. Orally, it is 10 times more potent than β-AET in anti-inflammatory and immune-modulating properties.

In spite of many studies on rodents, information on the circulating levels of androst-5-ene-triols in humans is almost completely lacking. Only one study on 252 volunteers from the general population is available [15], reporting plasma levels of β-AET in the range from 2 to 162 pg/mL in males and from 6 to 249 pg/mL in females. The plasma concentrations of β-AET positively correlated with BMI in healthy men and women [15]. Here we try to provide additional data on the daily profile of androst-5-ene-triols in human blood, concentrations in various human diseases, and changes under ACTH or insulin-induced hypoglycaemic stimulation (insulin tolerance test, ITT) of the adrenal

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Table 1
Analytical criteria for analysis of androst-5-ene-3β,7α/β,17β-triols in serum.

Steroid	Lower limit of detection		Lower limit of quantification		R[a] (linearity)	Inter-assay CV [%] (n = 6)			Intra-assay CV [%] (n = 6)
	[pmol/L]	[fg]	[pmol/L]	[fg]		1000 pg	100 pg	10 pg	
α-AET	0.06	0.9	0.2	3	0.9999	6.4	3.3	6	2.8
β-AET	0.04	0.6	0.13	2	0.9988	4.3	5.1	4	2.2

[a] Correlation coefficients of linear regression (R).

glands.

2. Experimental

2.1. Patients

The data on steroid metabolome were measured in our laboratory in collaboration with several clinics in various groups of patients. The characterization of selected patient groups and controls, their exclusion criteria and medications are described in detail in previous studies in the following papers: schizophrenia [21,22], depression and anxiety [23–25], IgA nephropathy [26] and tinnitus [27], as well as in patients with epilepsy [28,29], multiple sclerosis [30], male and female smokers [31–34], women at delivery and with postpartum depression [35,36], in healthy women as part of a study on daily hormonal profiles [37], and in women with Alzheimeŕs disease [38]. Each study had its own control group.

We have also focused on the physiological response of both triols, specifically the course of their concentrations during the daytime and reactions to ACTH and hypoglycemia [40,41].

While in some of these publications the levels of both triols were presented as a part of steroid metabolome, in others they were measured but not included in the published paper.

2.2. Steroid analysis

The method for the measurement of β-AET and of androst-5-ene-3β,7α,17β-triol (α-AET) largely followed that developed previously for analysis of the steroid metabolome [39]. Details on the method and its validation are described elsewhere [42].

2.2.1. Sample preparation

Steroids were extracted from 1 mL of plasma with diethyl-ether (3 mL). This diethyl-ether extract was dried in a block heater at 37 °C. The lipids in the dry residue of the diethyl-ether extract were then separated by partitioning between a mixture of ethanol–water 4:1 (1 mL) and pentane (1 mL). The pentane phase was discarded and the polar phase was dried in the vacuum centrifuge at 60 °C (2h). The dry residue from the polar phase was first derivatized with 2% methoxylamine hydrochloride solution in pyridine (60 °C, 1 h). This step was included due to the steroids containing oxo-groups and did not affect androstenetriols, which do not contain the oxo-group. After the first derivatization the mixture was dried in a flow of nitrogen and the dry residue was treated with the reagent Sylon B (99% of bis(trimethylsilyl)-trifluoroacetamide and 1% of trimethylchlorosilane), forming trimethylsilyl derivatives on hydroxy-groups (90 °C, 1 h). Finally, after the second derivatization step the mixture was dried in a flow of nitrogen, the dry residue was dissolved in 20 µL of isooctane, and 1 µL of the solution was used for GC–MS/MS analysis.

Prior to further processing, the original samples were spiked with 17α-estradiol (as an internal standard) at a concentration of 1 ng/mL. The internal standard was recorded at effective masses m/z = 231, 285 and 416. The addition of this internal standard to the body fluid before sample preparation assured that losses during sample processing did not affect steroid quantification.

2.2.2. Gas chromatography coupled to mass spectrometry

2.2.2.1. Operating system. The GCMS-QP2010 Plus system from Shimadzu (Kyoto, Japan) consisted of a gas chromatograph equipped with automatic flow control, AOC-20s autosampler and a single quadrupole detector with an adjustable electron voltage of 10–195 V. A capillary column with medium polarity RESTEK Rxi (diameter 0.25 mm, length 15 m, film thickness 0.1 µm) was used for analyses. Electron-impact ionization with electron voltage fixed at 70 V and emission current set to 160 µA was used for the measurements. The temperatures of the injection port, ion source and interface were maintained at 220, 300, and 310 °C, respectively. Analyses were carried out in the splitless mode with a constant linear velocity of the carrier gas (He), which was maintained at 60 cm/s. The septum purge flow was set to 3 mL/min. The samples were injected using the high pressure mode, which was applied at 200 kPa, and this pressure was maintained for 1 min. The detector voltage was set to 1.4 kV.

Temperature and pressure gradients for the GC–MS/MS analysis of trimethylsilyl derivatives were as follows: 1 min delay at 80 °C, increase to 190 °C (40 °C/min) increase to 210 °C (4 °C/min) increase to 300 °C (20 °C/min), 5 min delay at 300 °C, initial pressure 34 kPa, injector temperature 220 °C, analysis duration 18.25 min. The retention times (± SD) for α-AET and β-AET were 8.41 ± 0.016 min and 9.72 ± 0.012 min, respectively. Both steroids were recorded on effective masses 208, 327, and 432 Da and quantified at the dominant effective mass 432 Da. Molecular weight after derivatisation for the analytes α-AET, β-AET and the internal standards was 522 Da.

2.2.2.2. Analytical criteria. Analytical criteria for β-AET and α-AET in plasma are shown in Table 1. More detailed data on inter- and intra-assay, recovery and validation of the method are published elsewhere [42].

2.3. Dynamic testing – the ACTH and ITT tests

Testing of the hypothalamus–pituitary–adrenal axis (HPA axis) is based on two models: activation of the adrenal glands is initiated either in the hypothalamus (e.g. by hypoglycaemia induced by insulin) or in the pituitary gland (by the adrenocorticotrophic hormone ACTH).

The gold standard for evaluating the HPA axis is considered to be the insulin tolerance test (ITT), but this test has several limitations and is not used in many countries. Another common test is the Synacthen (tetracosactide, ACTH1-24) test (or ACTH test), but various doses of the tetracosactide given have been proposed (most commonly 1 µg, 10 µg or 250 µg) and there is no consensus on which dose is diagnostically most accurate and sensitive; however, the most preferable seems to be 10 µg [43].

Thirteen healthy subjects participated in our study on the changes of androst-5-ene-triols under ACTH stimulation or the insulin tolerance test: seven healthy women (with mean/median 42.88/41.5 age (SD ± 5.98) years, and mean/median 24.6/23.84 BMI (SD ± 3.02) kg/m^2) and six healthy men (with mean/median 49.83/53 age (SD ± 8.6) years, and mean/median 26.1/26.27 BMI (SD ± 2.38) kg/m^2). None of the subjects took any medications or had a history of taking either corticosteroids or estrogens. All of them signed informed consent prior to the study commencement. The study was