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SIMULTANEOUS DETERMINATION OF ANDROSTENEDIONE TESTOSTERONE AND 5α-DIHYDROTESTOSTERONE IN HUMAN SPERMATIC AND PERIPHERAL VENOUS PLASMA

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SUMMARY

Androstenedione (Δ), Testosterone (T) and 5α-Dihydrotestosterone (DHT) concentrations in spermatic and peripheral venous blood obtained from twenty four men have been measured by radioimmunoassay.

The concentrations (mean ± S.D.) of 5α-dihydrotestosterone in the spermatic vein (0·42 ± 0·33 μg/100 ml) were higher than those found in the cubital vein (0·030 ± 0·012 μg/100 ml). The blood plasma levels of 5α-dihydrotestosterone correlated significantly with testosterone levels in spermatic ($P < 0·05$) as well as in peripheral venous blood plasma ($P < 0·01$); on the other hand, 5α-dihydrotestosterone did not correlate with androstenedione. The results confirm the production of 5α-dihydrotestosterone by the human testis. Since there is a significant correlation between testosterone and 5α-dihydrotestosterone both in peripheral and in spermatic venous blood and an insignificant correlation between 5α-dihydrotestosterone and androstenedione, testosterone has been confirmed to be the most important precursor of 5α-dihydrotestosterone in the testis as well as in the other androgen target tissues.

INTRODUCTION

During the past few years increased attention has been given to DHT as the result of the discovery that T is converted mainly into DHT at the site of its target tissues [1, 2, 3]; therefore it has been suggested that DHT is the active androgen in some target tissues [2, 3].

The origin of DHT in human males has been discussed at length since the published data using the traditional interconversion rate procedures showed no agreement as to whether plasma DHT is entirely derived from peripheral conversion of T and Δ [4] or whether a certain amount of DHT is produced by glandular secretion [5, 6].

Preliminary results obtained in our laboratory by measuring DHT concentration in human peripheral and spermatic venous plasma have demonstrated the production of DHT by the human testis [7]. However, the testicular production of DHT in man is not due to a direct secretion by Leydig cells. It seems that the transformation of T into DHT takes place in the seminiferous tubules.

In fact 4-ene-3-ketosteroid-5α reductase activity has been demonstrated in whole human testis [8], but only seminiferous tubules were able to synthesize DHT [9, 10].

In order to explore the origin of plasma DHT in the human male, we have measured T, Δ and DHT concentrations in spermatic and peripheral venous plasma obtained from 24 men of various ages.

EXPERIMENTAL

The subjects were admitted to our hospital for inguinal hernia operations. They were otherwise in good general health and received no medication. Varicocele was not present. When under anaesthesia during operation, samples of peripheral blood from cubital vein and spermatic blood from spermatic vein were collected almost simultaneously into heparinized syringes by needle aspiration. Blood plasma was obtained by centrifugation and stored at −20°C until analyzed for DHT, T and Δ.

Steroid analyses. Plasma concentrations of T, DHT and Δ were performed by radioimmunoassay after paper chromatography.

The details of the assay of T and DHT and their reliability have been described in detail [11, 12]. The antisera used were antitestosterone-3-oxime-RSA and antidihydrotestosterone-3-oxime-BSA respectively; therefore paper chromatography was needed to avoid aspecific interferences between T and DHT. The accuracy studies performed by recovery experiments and by studying the effects of plasma dilution showed absence of Systematic errors. The within assay coefficients of variation were 8·9% for T and 4·0% for DHT. The between assay coefficients of variation were 9·6% for T and 9·4% for DHT.

Androstenedione was measured by a radioimmunoassay after extraction with 40 ml of methylene chloride and paper chromatography on Bush A system for 8–9 h at 24°C. The antiserum used was ob-

Table 1. Specificity (*) of the androstenedione radioimmunoassay

Steroid added	Androstenedione measured (pg) (mean of two determinations)
Blank	0·5
Androstenedione (50 pg)	45
Testosterone (1 ng)	0
Dihydrotestosterone (1 ng)	0·5
Dehydroepiandrosterone (1 ng)	0
Androstane-3β-17β-diol (1 ng)	0
17-hydroxy-Pregnenolone (1 ng)	0
Pregnenolone (1 ng)	0
17-hydroxy-Progesterone (1 ng)	0
Progesterone (1 ng)	0

* 20 μl fractions of ether extracted plasma were loaded with several steroids and measured as Androstenedione by the present method.

tained by injecting androstenedione-7-carboxymethyloxime-BSA conjugate into rabbits. This antiserum was a kind gift of Dr. Lindner and its characteristics have been previously reported [13]. A charcoal-Dextran mixture was used to separate bound from free fraction. The recovery of radioactive androstenedione was 72·0% ± 8·2 (mean ± S.D.) ($N = 67$). The blank values were 0·5 ± 1 pg/tube (mean ± S.D.) ($N = 15$); therefore the theoretical sensitivity of the method was almost 3 pg [14]. However it seems unwise to measure androstenedione amounts less than 20 pg. The within assay coefficient of variation ($N = 8$) was 5·6% and the between assay coefficient of variation was 7·7% ($N = 16$). The accuracy was studied in two different ways:

(a) adding different amounts (0; 50; 100 pg) ($N = 15$) of cold androstenedione to "steroid free" plasma and measuring the androstenedione concentration by the present method. A significant correlation was observed between expected and measured values ($y = 1·095 + 1·33$).

(b) measuring the androstenedione concentration in increasing amounts of human plasma (0·03, 0·05 and 0·1 ml) ($N = 12$). A good linearity was observed ($y = 1·507 \times +6·8$).

Lastly, the capacity of the chromatographic systems to separate androstenedione from other interfering steroids was reported by Kato and Horton [15] and demonstrated by the experiment reported in Table 1.

In spite of good specificity of the methods used for DHT and Δ measurements, another experiment was performed to control the validity of the results obtained in spermatic venous plasma: 0·2 ml of "steroid free" plasma was loaded with 20 pg of 5α-dihydrotestosterone and 300 pg of testosterone, androstenedione, dehydroepiandrosterone, 5-androstene-3β,17β-diol, androsterone, etiocholanolone, 17-hydroxyprogesterone, pregnenolone and 17-hydroxypregnenolone. The value found after DHT radioimmunoassay was 19 pg (mean of two determinations). The same experiment was repeated for androstenedione radioimmunoassay adding to 0·2 ml "steroid free" plasma 100 pg androstenedione and 1 ng testosterone, dehydroepiandrosterone, 5α-dihydrotestosterone, androterone, etiocholanolone, 5-androstene-3β,17β-diol,

Table 2. T, DHT and Δ concentrations in spermatic and cubital venous plasma

Case	Age	Spermatic vein (μg/100 ml) T	DHT	Δ	Cubital vein (μg/100 ml) T	DHT	Δ
1	24	7·4	0·17	1·10	0·300	0·034	0·035
2	44	43·4	1·35	0·93	0·392	0·032	0·016
3	51	13·3	0·51	3·10	0·304	0·026	0·035
4	56	9·1	0·36	0·07	0·212	0·028	0·044
5	63	69·0	0·31	20·00	0·500	0·065	0·019
6	73	45·0	0·34	9·20	0·510	0·047	0·028
7	17	29·3	0·48	0·39	0·210	0·022	0·051
8	27	12·8	1·05	0·05	0·210	0·022	0·013
9	56	55·2	0·54	1·64	0·402	0·030	0·093
10	27	42·0	0·52	0·50	0·262	0·031	0·135
11	21	106·4	1·05	4·11	0·630	0·053	0·110
12	48	32·1	0·08	0·05	0·880	0·029	0·036
13	66	21·0	0·19	0·11	0·202	0·021	0·015
14	58	3·6	0·05	0·20	0·435	0·035	0·065
15	27	11·6	0·04	0·20	0·402	0·026	0·181
16	64	16·0	0·43	0·83	0·230	0·024	0·079
17	65	40·0	0·31	1·40	0·180	0·018	0·266
18	80	19·5	0·22	0·81	0·328	0·023	0·066
19	51	3·6	0·12	0·45	0·162	0·040	0·117
20	26	78·0	0·56	2·00	0·152	0·011	0·086
21	59	0·9	0·08	0·09	0·240	0·023	0·163
22	65	24·6	0·37	1·00	0·208	0·021	0·275
23	23	35·0	0·42	2·30	0·204	0·019	0·089
24	37	53·2	0·67	0·92	0·430	0·033	0·083
Mean ± S.D.		32·2 ±26·3	0·42 ±0·33	2·14 ±4·27	0·332 ±0·173	0·030 ±0·012	0·087 ±0·073

Table 3. Values of the correlation coefficient (r) between T, DHT and Δ levels in cubital and in spermatic venous plasma

Steroids		Correlation coefficient (r)	Level of significance
T and DHT	Spermatic vein	+0·5098	$P < 0·05$
T and Δ	Spermatic vein	+0·4614	$P < 0·05$
DHT and Δ	Spermatic vein	+0·0095	P No sign.
T and DHT	Cubital vein	+0·5585	$P < 0·01$
T and Δ	Cubital vein	−0·2830	P No sign.
DHT and Δ	Cubital vein	−0·2666	P No sign.

17-hydroxyprogesterone, progesterone, 17-hydroxypregnenolone and pregnenolone. After androstenedione radioimmunoassay, the value found was 105 pg (mean of two determinations).

RESULTS

Values for T, DHT and Δ in peripheral and spermatic venous plasma are listed in Table 2. The DHT concentration in the spermatic vein (0·42 μg/100 ml) was decisively higher than that found in the cubital vein (0·030 μg/100 ml). The blood plasma levels of DHT were significantly correlated (see Table 3) with T levels in spermatic as well as in peripheral venous blood plasma; the levels of DHT did not correlate with Δ levels; the T levels correlated with Δ levels in spermatic venous blood plasma but did not correlate in peripheral venous blood plasma.

DISCUSSION

The present results confirm our preliminary study using a competitive protein binding method for DHT measurement and demonstrated the production of DHT by the human testis.

Utilizing the mean blood production rate of T for normal adult males (5·5 mg/day in relation to the mean age of our subjects) and the experimentally determinated T and DHT gradients from our data, we estimate, according to the method proposed by Weinstein *et al.* [16], that testicular DHT secretion is 67·3 μg/day.

A comparison of this estimate with total production rate of DHT (300–390 μg/day) [4, 5, 6, 17] indicates that testicular secretion of DHT is about 20% of total DHT production.

The significant correlation between T and DHT levels both in peripheral and spermatic venous plasma indicates that T may be the most important precursor of DHT in the testis as well as in other androgen target tissues connected with peripheral blood.

On the other hand, there was no correlation between Δ and DHT levels in agreement with the findings of Saez *et al.* [6] who calculate that only 10 μg/day of DHT come from peripheral transformation of Δ.

The significant correlation between T and Δ levels in spermatic venous plasma may depend on the role of Δ in testicular steroidogenesis since Δ is a direct precursor of testicular T [18].

Otherwise the absence of a significant correlation between T and Δ levels in peripheral venous plasma confirms the previous results on Δ production in men: the greatest amount of plasma Δ comes from adrenocortical secretion [19], while plasma T comes from the testis [18].

Although the method used to prove the DHT testicular secretion and the DHT relationships with T and Δ seems valid, unqualified extrapolation of these data to the normal states is unwarranted because the effects of anaesthesia on blood flow and steroidogenesis of the human testis are unclear [20]. Moreover Baird and Fraser [21] have recently demonstrated significant variations of metabolic clearance rates of estrone and estradiol-17β during anaesthesia.

Some of our cases present low plasma value of steroids, especially androstenedione. These low androstenedione values may be partly due to body position: in fact measurements of Δ in our laboratory in subjects recumbent in bed demonstrated a reduction of about 20% in comparison to upright position values. The episodic secretion of steroids can also provoke important variations of the plasma levels of these hormones [22].

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