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PRODUCTION AND METABOLISM OF DIHYDROTESTOSTERONE IN PERIPHERAL TISSUES‡

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Summary—The production and metabolism of dihydrotestosterone (DHT) was studied in 10 normal men using constant infusions of [7-^{3}H]-Δ^4-androstenedione (A)/[4-^{14}C]DHT (6 men) or of [7-^{3}H]testosterone (T)/[4-^{14}C]DHT (4 men) with measurement of radioactivity as precursor and product in the brachial artery, and a superficial vein (draining primarily adipose tissue) and deep vein (draining primarily muscle) in the arm opposite to the infusion.

The metabolic clearance rates (MCR) were (mean ± SE) 560 ± 55, 1620 ± 80, and 790 ± 65, l/day for DHT, A and T respectively. The overall conversions (percent of A or T infused which was measured as DHT in arterial blood) were 2.3 ± 0.8% and 2.7 ± 0.5% for A to DHT and T to DHT.

Of the DHT entering the adipose tissue 13.8 ± 3.4% was metabolized and of that entering muscle 5.8 ± 1.8% was metabolized. On the basis of assumed blood flows, adipose tissue and muscle metabolism each carry out approx. 7–8% of the overall metabolism of DHT.

Of the A entering the forearm adipose tissue 1.9 ± 0.5% was converted to DHT. Extrapolated to the body's adipose tissue, this represents 14% of the total A converted to DHT.

Of the T entering the forearm adipose tissue 1.2 ± 0.3% was converted to DHT. Extrapolated to the body's adipose tissue, this represents 6% of the total T converted to DHT.

In three out of six subjects infused with A/DHT and in three out of four subjects infused with T/DHT small gradients indicating conversion of A and T to DHT by tissues drained by the deep vein were found. The mean value for the conversion of A to DHT across the deep vein tissues was 0.84 ± 0.51% and for T to DHT was 0.28 ± 0.12%.

Both adipose tissue and muscle metabolize DHT but only adipose tissue appears to play a role in the conversion of A and T to DHT.

INTRODUCTION

The biologically active androgen, dihydrotestosterone (17β-hydroxy-5α-androstan-3-one; DHT), arises primarily from the conversion of testosterone (T) and Δ^4-androstenedione (A) in peripheral tissues [1, 2]. While the prostate is one of the sites in men [3], studies *in vitro* have indicated that skin, especially genital skin [3, 4, 5, 6], hair follicles [7], bones [8], lung [9] and the brain [10] are also sites for these conversions in man and various animal species. Muscle, however, does not appear to be an important site and there are only limited data as to conversion in adipose tissue [3, 11, 12]. The degree to which adipose tissue might convert A and T to DHT is of potential importance since obesity in women is often associated with hirsutism, a sign of increased DHT activity. It is possible that as adipose tissue mass increases, more DHT would be formed in the adipose tissue to stimulate hair growth elsewhere.

Therefore, to explore the production and metabolism of DHT in adipose tissue as well as muscle, we carried out infusions of [^{3}H]A or [^{3}H]T and [^{14}C]DHT and measured radioactivity in veins draining these tissues.

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EXPERIMENTAL

All subjects were men over 21 years old who were in good health and who had given informed consent for the studies. For the group the mean ± SE for age was 28 ± 1 yrs, for weight was 75 ± 3 kg, for height was 178 ± 2 cm for body surface area was 1.94 ± 0.03 m^2. All studies were done with the subjects fasting and supine.

Diethyl ether was redistilled and passed through an alumina column prior to use. All other reagents used were prepared as described [6]. [7-^{3}H]Testosterone (sp. act. 25 Ci/mmol), [4-^{14}]C-Testosterone (sp. act. 57.5 mCi/mmol), [4-^{14}C]androstenedione (sp. act. 57.5 mCi/mmol) and [4-^{14}C]dihydrotestosterone (sp. act. 57.5 mCi/mmol) were obtained from New England Nuclear Corp. [7-^{3}H]Androstenedione (sp. act. 4.1 Ci/mmol) was obtained from Amersham–Searle Corp., Inc. ^{3}H- and ^{14}C-labeled androgens were purified and the purity checked as described [13]. Nonradioactive testosterone, androstenedione and dihydrotestosterone were obtained from Steraloids Co. and crystallized from

methanol prior to use. Alumina HF^{254} Basic Type E and Silica Gel HF^{254} were obtained from Brinkman Co., Westbury, L.I., N.Y. Whatman no. 2 paper was used for paper chromatography.

Experiments were begun at 0800, and were carried out as previously described [14]. Briefly, a brachial artery was cannulated with a double lumen Lillienthal adaptor through a Cournand needle, and Evans blue dye was infused distally at 150 μg/min via the adaptor side arm. A superficial and deep vein of the same arm were catheterized retrogradely, and the catheters kept patent by a slow infusion of 0.9% NaCl. At three different times during the infusion, blood flow was calculated by a modified Fick principle [8, 9]. After the placement of these catheters a needle was inserted into a vein of the contralateral arm and a priming dose of the two androgens, one labeled with ^3H and one with ^{14}C in 10 ml of 8% ethanol in an isotonic saline solution, was injected at 0 min. An infusion of the same two labeled androgens in 15 ml of 8% ethanol in an isotonic saline solution was begun and continued for 210 min. The amounts infused were 50–60 μCi of ^3H and 2–3 μCi of ^{14}C. The priming doses were 40–50% of the amounts infused. The steroid solutions were administered using a Harvard Infusion Pump (Harvard Apparatus Co., Inc.). Aliquots of the steroid solutions were taken and counted as described [15] and the dpm of ^3H and ^{14}C were calculated [16].

Ten ml heparinized blood samples were obtained simultaneously from the arterial and both venous catheters at 150, 180 and 210 min from the start of the infusion. The samples were centrifuged and the plasma frozen and stored at $-15^{\circ}C$ until analyzed.

Prior to analysis, 20 μg each of nonradioactive androstenedione and testosterone, 200 dpm each of [4-^{14}C]testosterone and [4-^{14}C]androstenedione and 1 μg of dihydrotestosterone were added to each thawed plasma sample and the samples were then extracted with ether and methylene chloride [14, 17]. The pooled extracts were washed and the solvents removed under vacuum. The residues were taken up in CCl_4–$CHCl_3$ (5:1, v/v) and washed twice with 1.0 ml of lN NaOH, 2× with 0.5 ml H_2O and the CCl_4–$CHCl_3$ was removed under nitrogen. The dried residue was chromatographed on paper overnight in a Bush A system [13]. The areas containing A and DHT and T were located under u.v. light, eluted and the residues were dried. The residues containing T were then separately spotted on an Alumina HF_{254} thin layer plate and chromatographed in the system benzene:ethyl acetate (3:2, v/v). The testosterone area was located under u.v. light, eluted and the residue dried.

The area corresponding to A and DHT was dried and the residue was chromatographed on Silica gel HF_{254} thin layer plate in the system $CHCl_3$–MeOH (98:2, v/v) with a parallel channel containing DHT marker. The A area was located under u.v. light and the DHT marker was located by spraying that channel with polymolybdic acid (PMA). The sample areas corresponding to A and the DHT marker were then separately eluted and dried. The A residue was chromatographed on Alumina HF_{254} thin layer plate in the system benzene–ethyl acetate (60:40, v/v). The A area was located under u.v. light, eluted and dried.

The DHT residue was acetylated using 0.2 ml acetic anhydride and 0.2 ml pyridine at room temperature overnight. The DHT acetate was chromatographed on Silica gel HF_{254} thin layer plate using the system benzene–ethyl acetate (90:10, v/v) with DHT acetate as a marker in a parallel channel. The marker was located using PMA and the appropriate sample area eluted. The DHT acetate was hydrolyzed using 0.5 ml 0.15 N–NaOH in 80% methanol at room temperature in a desiccator overnight. The DHT was extracted with 5 ml methylene chloride and chromatographed on Silica gel HF_{254} on thin layer plate using the system $CHCl_3$–MeOH (98:2, v/v), with a DHT marker in a parallel channel. The marker DHT was located by spraying that channel with PMA. The sample DHT was eluted and 10% was removed for radioimmunoassay to determine losses through the procedure [17].

The T, A and DHT residues were then transferred to counting vials and the radioactivity measured using a liquid scintillation spectrometer. The amounts of radioactivity were corrected for losses through the procedure using the ^{14}C recoveries for T and A, and the RIA for DHT.

Analysis of data

The metabolism of steroids by the tissues was calculated using the approach of Chapdelaine[18] and Gurpide[19] which has been discussed by us previously [14].

From these analyses we calculate: (1) the transfer constant across a tissue which is the fraction of steroid measured in arterial blood which is measured as a different steroid in the venous blood draining a tissue ($[p]_{AM}^{A,DHT}$ and $[p]_{AAT}^{T,DHT}$; the superscripts identify the steroid: A = androstenedione, T = testosterone, DHT = dihydrotestosterone; the subscripts identify the blood sample analyzed: A = arterial, M = venous blood draining muscle, AT = venous blood draining adipose tissue).

(2) The fraction of steroid which is metabolized by the tissue, $[p]_{AM}^{DHT,0}$ [the superscript 0 is used to represent metabolism].

The metabolic clearance rates (MCR_A) were calculated as $MCR_A = r_B/X_A$ where r_B = rate of infusion into peripheral venous blood and X_A = mean concentration of radioactivity in arterial blood. The $[p]_{BA}^{PRE,PRO}$ values were calculated as $X_A PRO \times MCR_A PRO/r_B PRE$ where $X_A PRO$ is concentration in the arterial blood of radioactivity as product with the same label as the infused precursor; $MCR_A PRO$ is the MCR_A, determined from the arterial blood, of the produce; $r_B PRE$ is the rate of infusion into the peripheral venous blood of the precursor.

To determine that an isotopic steady state [20] had been reached during an infusion, the data were normalized, pooled and an estimate of variance was obtained as previously described [21].

Comparison of group means was done using Student's t-test or the Newman–Keuls Test [22]. Counting errors were determined [17] and were $<2\%$ for steroids as either precursor or product.

RESULTS

All results are expressed as mean $\pm$ SE unless specified otherwise. For the infusions, the concentrations of radioactivity as the infused free precursor or free product steroids showed no significant trends in any of the bloods sampled, and the mean slopes of the regression lines of the normalized concentrations against time were not significantly different from zero. These data are therefore compatible with attaining an isotopic steady state for precursor and product steroids by 150 min of the infusion. In all infusions blood flow, as measured by Evans blue dye, was stable.

Metabolic clearance rates (MCR_A) and $[p]_{BA}^{PRE,PRO}$ values are shown in Table 1. The mean value for MCR_A^{DHT}, 560 ± 55 l/day, was significantly ($P < 0.05$) less than the mean values for MCR^A, 1620 ± 80 l/day and for MCR_A^T, 790 ± 65 l/day. The mean values for $[p]_{BA}^{A,DHT}$ $2.41 \pm 0.93\%$ and $[p]_{BA}^{T,DHT}$ $2.97 \pm 0.38\%$ were not significantly different.

As shown in Table 2 the metabolism of DHT in adipose tissue ($[p]_{AAT}^{DHT,O}$; $14.03 \pm 3.42\%$) was significantly ($P < 0.05$) greater than the metabolism in muscle ($[p]_{AM}^{DHT,O}$; $5.80 \pm 1.84\%$). However, there was no difference ($F = 2.17$; $P < 0.05$) between the mean values for DHT, A and T metabolism in adipose tissue although the mean value for A, but not T, metabolism in muscle was greater ($P < 0.05$) than that for DHT.

The values for the conversions of A and T to DHT in adipose tissue and muscle are shown in Table 3. We were able to demonstrate a positive gradient and hence conversion by adipose tissue to [^{3}H]DHT from [^{3}H]A and [^{3}H]T in all experiments. However, we were able to show conversion to [^{3}H]DHT from [^{3}H]A by muscle in only 3 out of 6 experiments and conversion to [^{3}H]DHT from [^{3}H]T in 3 out of 4 experiments. Although the mean values for $[p]_{AAT}^{A,DHT}$ and $[p]_{AAT}^{T,DHT}$ are greater than respective values in muscle, the differences are not significant.

DISCUSSION

Our values for the MCR_A's of DHT, A, and T are similar to those previously reported [1, 2, 23]. The overall conversions of A and T to DHT ($[p]_{BA}^{A,DHT}$ and $[p]_{BA}^{T,DHT}$) are also similar to those reported by others [1, 2, 23]. Since in men the production rates of T are greater than those of A [1, 2] the similarity of the $[p]_{BA}$ values for conversion A and T to DHT indicates that in men T is a more important source of DHT than is A.

As we have noted for certain other androgens and estrogens [14, 24], the values for MCR_A^{DHT} and $[p]_{BA}^{T,DHT}$ are not different when measurements are made in arterial as opposed to venous blood.

The metabolism of DHT by adipose tissue is significantly greater than that by muscle. We have also noted greater metabolic activity of adipose tissue compared to muscle for other androgens [24] and for estrogens [14]. The metabolism of DHT by adipose tissue was similar to that for A and T, but muscle metabolism of DHT was significantly less than that of A, reflecting probably the difference in globulin binding for the two steroids. It is possible that the

Table 1. Metabolic clearance rates (MCR_A) for dihydrotestosterone (DHT), androstenedine (A) and testosterone (T) and overall conversions of A to DHT ($[p]_{BA}^{A,DHT}$) and of T to DHT ($[p]_{BA}^{T,DHT}$)

Subject	MCR_A^{DHT} l/day	MCR_A^A l/day	MCR_A^T l/day	$[p]_{BA}^{A,DHT}$ %	$[p]_{BA}^{T,DHT}$ %
1	480	1460	—	1.80	
2	340	1800	—	1.65	
3	540	1360	—	1.00	
4	360	1710	—	0.99	
5	780	1840	—	6.99	
6	390	1540	—	2.03	
7	600	—	810		3.25
8	700	—	900		2.42
9	530	—	600		2.30
10	840	—	840		3.93
Mean	560	1620	790	2.41	2.97
±SE	55	80	65	0.93	0.38

Table 2. Metabolism of dihydrotestosterone ($p^{DHT,O}$) androstenedione ($p^{A,O}$) and testosterone ($p^{T,O}$) by adipose tissue (AT) and by muscle (M)

Subject	$p_{AAT}^{DHT,O}$ %	$p_{AAT}^{A,O}$ %	$p_{AAT}^{T,O}$ %	$p_{AM}^{DHT,O}$ %	$p_{AM}^{A,O}$ %	$p_{AM}^{T,O}$ %
1	17.12	27.69		6.95	19.34	
2	0.99	13.81		2.18	10.51	
3	24.74	34.36		8.62	20.37	
4	27.74	32.17		15.15	13.00	
5	3.33	11.89		6.22	5.28	
6	11.71	18.54		3.63	33.33	
7	26.40		18.41	15.22		9.50
8	21.67		3.12	0.00		3.12
9	1.28		10.67	0.00		13.88
10	5.32		12.95	0.00		7.16
Mean	14.03	23.08	11.29	5.80	16.97	8.41
±SE	3.42	3.93	3.17	1.84	3.99	2.25

Table 3. Conversion of A to DHT (A,DHT) and of T to DHT (T,DHT) by adipose tissue (AT) and by muscle (M)

Subject	$p_{AAT}^{A,DHT}$ %	$p_{AAT}^{T,DHT}$ %	$p_{AM}^{A,DHT}$ %	$p_{AM}^{T,DHT}$ %
1	0.98		0.95	
2	1.13		0.00	
3	3.52		2.33	
4	3.67		1.75	
5	1.30		0.00	
6	0.59		0.00	
7	—	2.05	—	0.61
8	—	0.80	—	0.18
9	—	1.31	—	0.35
10	—	0.76	—	0.00
Mean	1.86	1.23	0.84	0.28
±SE	0.56	0.30	0.41	0.13

similarity of A and DHT metabolism by adipose tissue and the difference by muscle is a reflection that SHBG-binding of a steroid is less important as a modulator of steroid entry into adipose cells as compared to muscle cells. However, other factors may be responsible as well.

Adipose tissue also appears to be more active in the reduction of A and T to DHT than in muscle. Studies *in vivo* and *in vitro* have indicated very low or absent levels of 5α-reductase in muscle [11, 12, 25]. It has been calculated that some 10% of the deep vein drainage comes from bone [26, 27, 28] and bone has been reported to convert T to DHT [8]. Therefore, it is possible in the subjects in whom we found a positive gradient for [^{3}H]DHT across the tissues drained by the deep vein that bone or connective tissues other than muscle were responsible for the gradient.

The tissues drained by the superficial vein include skin and skin has been shown to contain 5α-reductase activity [3, 4, 5, 6]. Most such studies have been done with genital skin [3, 4] and skin from other areas also appears to contain limited 5α-reductase activity [5, 6, 29]. Adipose tissue possesses other enzymic activity and since 90% or more of the superficial vein drains adipose tissue [26, 27, 28], it is likely that adipose tissue is responsible for most of the positive gradient for [^{3}H]DHT that we found. It has recently been reported that the stromal cells, rather than the adipocyte, contain most of the aromatase activity of adipose tissue [30]. However, we cannot say from our data which cell type contains the 5α-reductase activity.

Adipose tissue and muscle were equal in their abilities to interconvert the androgens, A and T [24] and the estrogens, estrone and estradiol [14]. Similarly, the two tissues were equal in their aromatase activities [31]. Thus, the relative lack of 5α-reductase activity in muscle as compared to adipose tissue does not reflect an overall inability of muscle to metabolize steroids.

Assuming a blood flow of 300 ml/day/m^2 through the total adipose tissue of the body [14] then some 8–10% of the metabolism of DHT would occur in that tissue as well is 16–20% of the overall conversions of A to T to DHT. For muscle the metabolism would be less but it is questionable whether that tissue is the site of significant DHT conversion from precursors.

With increasing adiposity the production of DHT in that tissue and hence the overall production would be increased and could explain, in part, the increased hirsutism noted in some obese women.

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