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Regional Scalp Differences of the Androgenic Metabolic Pattern in Subjects Affected by Male Pattern Baldness

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Regional differences in the androgen metabolism were established in alopecic and non alopecic areas of patients affected by male pattern baldness (MPB). 5-α-reductase (5-α-R) activity was measured by the formation of dihydrotestosterone (DHT), using ^{3}H-testosterone as substrate: this activity was higher in the alopecic areas (3.4 pmol/g tissue/h) than in the non alopecic skin (1.5 pmol/g tissue/h). 3-α,β-hydroxysteroid oxoreductase (3-α,β-HO) was studied using ^{3}H-DHT as precursor and measuring the corresponding formed 3-α- and 3-β-androstanediols (αDIOL and βDIOL). The βDIOL was the predominant metabolite and total 3-α,β-HO activity was higher in alopecic skin (12.4 pmol/g tissue/h) than in non alopecic areas (8.4 pmol/g tissue/h). Also 17,β-hydroxysteroid oxoreductase was measured using either testosterone or DHT as substrates: androstenedione formed from testosterone was higher in hairy skin (12 pmol/g tissue/h) than in alopecic areas (6 pmol/g tissue/h); androstanedione formed from DHT was also higher in non alopecic areas (8.1 pmol/g tissue/h) than in alopecic skin (2.8 pmol/g tissue/h). The greater formation of βDIOL in the sebaceous glands-enriched alopecic skin supports the hypothesis for a specific role of this metabolite in the control of the sebaceous activity.

Key words: Male pattern baldness, Androgen metabolism, 5-α-reductase, 3-α,β-hydroxysteroid oxoreductase, 17,β-hydroxysteroid oxoreductase.

In most androgen-dependent tissues, testosterone (T) is converted into dihydrotestosterone (DHT), through the 5-α-reductase system (5-α-R). Thus, DHT acts as the active metabolite of T by coupling to a specific cytoplasmic receptor (1, 4, 5).

Several studies demonstrate that testosterone is converted into DHT in scalp skin and it seems that this biotransformation is high in the alopecic areas of patients suf-

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fering from male pattern baldness (MPB) (2). Hence, it has been believed that DHT would be the active form of testosterone in the sebaceous gland and that seborrhoeic disorders could be due to an excess of 5-α-R activity. Nevertheless, the relevance of both 5-α-R and DHT in the control of the sebaceous activity is controversial. COOPER *et al.* (8) postulated that intradermal DHT does not stimulate sebaceous lipogenesis and that in some individuals actually decreases it. Other authors suggested that 3-β-hydroxysteroid oxoreductase (3-β-HO), which transforms DHT into 3-β-androstanediol (βDIOL) would be involved in the localized metabolic disturbances of the androgenic hormones in some tissues. Thus, NIKKARI *et al.* (14) showed that βDIOL was a more powerful stimulator of sebum secretion in hypophysectomized female rats than DHT.

Recently we demonstrated (6) that cytosol from the scalp skin of subjects affected by MPB did not bear a specific DHT receptor. Our recent work (related with this paper) strongly suggests that there is a specific binding protein for βDIOL in the sebaceous gland of the alopecic-seborrhoeic scalp (7). The possibility that βDIOL but not DHT could be the specific-active metabolite of testosterone in the sebaceous gland and the involvement of the androgen-transforming enzymes in the etiopathogeny of MPB, led us to develop the study presented here.

Materials and Methods

Tissues. — Human scalp biopsies were obtained from patients affected by MPB, and who had been subjected to hair autotransplantation. Pieces were taken with an Orentreich punch (n = 4.5) from alopecic fronto-parietal areas and from hairy occipital-donor areas. Human prostates were obtained from patients with benign prostatic hypertrophy.

Chemicals. — 5-α-dihydro (1-α, 2-α (n)-^{3}H) testosterone (^{3}H-DHT), 56-60 Ci/mmol, 1 mCi/ml and (1, 2, 6, 7, ^{3}H) testosterone (^{3}H-T) 103 Ci/mmol, 1 mCi/ml were obtained from The Radiochemical Centre (Amersham, England). Non labeled steroids were purchased from Sigma. NADPH was obtained from Boehringer Mann. GmbH. All the reagents were of analytical grade. Aluminum oxide Type E plates (Merck) were used for the chromatographic separations.

Enzyme assay. — Tissue samples were minced with scissors and blended in a potassium phosphate buffer 0.1 M, pH 6.6 (1/10; W/V) in an Omni-Mixer (Sorvall) homogenizer cooled with crushed ice. Good performances were obtained with 4 × 30 s homogeneization periods, at the maximal speed, with 30 s pause intervals between every two periods. Homogenates were filtered through a gauze to eliminate gross particles.

Homogenate aliquots were incubated with radioactive steroids according to the method described by MOORE and WILSON (11). In the kinetic study, the radioactive isotopes were conveniently diluted with non labeled steroids to obtain a range of final concentrations between 5 and 100 × 10^{-6} M. In the comparative studies (alopecic and hairy areas) only radioactive steroids at 4 × 10^{-9} M final concentration were used.

Steroids were dissolved in chloroform, introduced into the incubation tubes and the solvent was evaporated to dryness by means of a nitrogen stream. In the same tube, were added: 250 µl of NADPH in potassium phosphate buffer to obtain a final concentration of 0.5 × 10^{-3} M (previous analysis with NADPH ranging from 0.1 to 1 × 10^{-3} M demonstrated that this concentration is high enough to maintain the enzyme kinetics during the incubation time); 500 µl of the homogenate (0.2 mg protein in routine experiments); 250 µl of buffer. Finally, a stream of O_2/CO_2 (95/

5 %) was bubbled for 30 s and each tube was immediately closed with a hermetic cap. The tubes were then introduced in a thermostated shaking incubator at 37 °C for different periods of time. The reaction was stopped by adding 5 ml of a chloroform:methanol (2:1) mixture.

In some of our experiments the protein concentrations varied from 0 to 0.4 mg/tube. At the end of the incubation time, the tubes were centrifuged at 5,000 × g for 10 min. The supernatant and the interphase were discarded. The underlying organic phase was transferred to a smaller tube and evaporated to dryness by a stream of N_2. Residues were dissolved in 60 µl of a chloroformic solution containing the carrier steroids (T, βDIOL, 3-α-androstanediol (αDIOL), androstenedione (AEDION), androstanedione (AADION) and DHT) in a final concentration of 0.25 mg/ml each to perform the thin-layer chromatographic separation.

Thin-Layer Chromatography. — From each sample 50 µl were applied to aluminum oxide plates. The eluting phase was a mixture of bencene:ethanol (96:4). Each plate was run twice in the same eluting phase, to achieve a good separation of both androstanediols (12). At the end of the second run, the plates were stained with iodine vapors to visualize the steroids.

A good qualitative/quantitative analysis of the radioactive steroids was obtained with the aid of a Berthold LB 2832 Radiochromatograph. This equipment has a resolution power higher than 0.25 mm, which permits an optimal identification of each steroid. Keeping exactly the same working parameters in the different experiments and using appropriate radioactive standards, the DPM of each steroid were calculated from the arbitrary counts of the radiochromatograph. Finally, DPM can easily be transformed into moles of steroid.

Previous controls of purity by recristallization had been done to confirm the soundness of the detection of the radiochromatograph.

Protein assay. — Proteins were quantified by the method of Lowry.

Statistical analysis.— For paired results, the unilateral Wilcoxon test was used; for non paired results the bilateral Mann-Whitney «U» test was employed.

Results

Human prostate was used to check the reliability of this method; no complete kinetic studies were done on this tissue. At 2×10^{-9} M, testosterone is converted into DHT and then after into αDIOL and βDIOL. In fig. 1 the complete separation of the steroids can be seen.

Testosterone metabolism

Kinetic Studies of 5-α-R. — In scalp homogenates coming from the alopecic

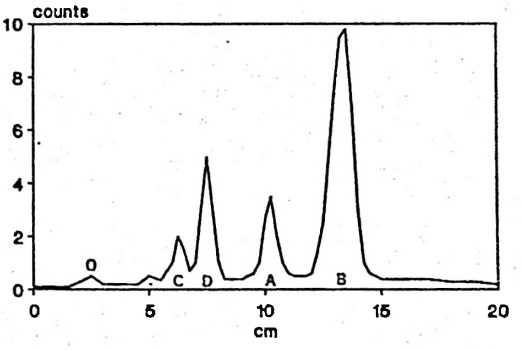

Fig. 1. *Radiochromatograph of testosterone-derived metabolites.*
^{3}H-T (2 nM) was incubated with human prostate homogenate. Thin-layer chromatography quantified by a Berthold LB 2832 Radiochromatograph. In abscissae: length of run; in ordinate: arbitrary counts. At the end of the incubation time, most of the testosterone is consumed. A: testosterone; B: dihydrotestosterone; C: 3-β-androstanediol; D: 3-α-androstanediol; O: origin.

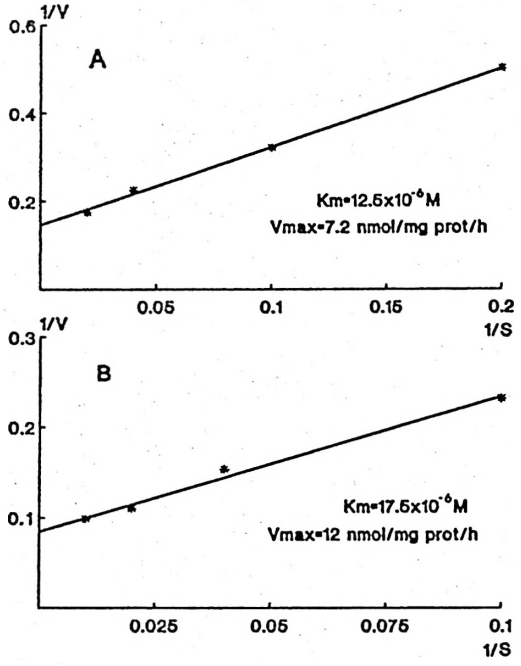

Fig. 2. *Lineweaver-Burk plots of the testosterone and dihydrotestosterone (DHT) biotransformation.*

A) 5-α-reductase activity was calculated by using testosterone as substrate (5-100 × 10^{-6} M), after the incubation with alopecic skin homogenates during 30 min. B) 3-α,β-hydroxysteroid oxoreductase was assayed by using DHT as substrate (10-100 × 10^{-6} M) during 60 min, also with alopecic skin homogenates. Data at saturation are not shown.

areas, since the amount of tissue was large enough, complete kinetic analyses were performed. However, it was very difficult (for evident ethical reasons) to obtain samples from the occipital donor areas, comparative studies being only performed with these samples.

Testosterone is converted mainly into DHT in the alopecic areas, the formation of AEDION being also observed in some experiments. The 5-α-R activity is lineal during the first 30 min of the incubation period, which was the time used for the experiments. The formation of DHT correlates well with the amount of proteins in the range used here (0.1 to 0.4 mg/tube). The enzyme parameters were calculated from the results obtained by varying the testosterone concentration from 5 to 100 × 10^{-6} M. Equilibrium occurs at 50 × 10^{-6} M. Figure 2 depicts a Lineweaver-Burk plot of a typical experiment.

Complete analysis was developed in ten patients. Mean values for V_{max} = 7.5 ± 2.4 nmol/mg prot/h and K_m = 18.8 ± 12 × 10^{-6} M were obtained for 5-α-R. In these experiments the amount of the transformed testosterone was less than 5 %.

Comparative regional studies. — To compare the 5-α-R activity in both alopecic and non alopecic areas ^{3}H-T at a final concentration of 4 × 10^{-9} M was incubated with homogenates coming from seven patients in which pieces from both areas were obtained. Due to the small amount of tissue, only one duplicate point was obtained in each case. The DHT formed allowed us to calculate a 5-α-R activity of 0.4 ± 0.12 pmol/mg prot/h or 3.4 ± 0.8 pmol/g tissue/h in the alopecic areas. In the hairy areas these values were 0.22 ± 0.1 pmol/mg prot/h or 1.5 ± 0.6 pmol/g tissue/h, the amount being zero in two cases. Using the values expressed in g of tissue, these differences are statistically significant for $p < 0.05$.

In the present experimental conditions it was also possible to detect the activity of the 17,β-hydroxysteroid oxoreductase (17,β-HO) system by studying the conversion of testosterone into AEDION in some patients. In the same seven samples in which the activity of 5-α-R was studied, only in three the formation of AEDION in the hairy area was detected, whereas this metabolite was absent in all patients in the alopecic areas. In samples coming from eight different patients, the formation of AEDION from testosterone in the alopecic area was demonstrated. The mean values obtained in the hairy area were 12 ± 0.7 pmol/g tissue/h and 6 ± 2.2

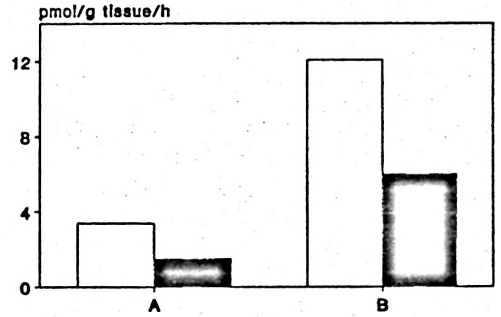

Fig. 3. *Regional differences in testosterone metabolism.*
^{3}H-testosterone (4 nM) was incubated with scalp skin homogenates during 30 min. 5-α-reductase activity was evaluated by the formation of dihydrotestosterone (A). 17,β-hydroxysteroid oxoreductase activity was evaluated by the androstenedione formation (B). Filled bars: hairy tissue; empty bars: alopecic tissue.

pmol/g tissue/h in the alopecic area. These differences are statistically significant for $p < 0.05$. Figure 3 shows these results. The amount of testosterone transformed in these experiments (table I) is greater than that obtained in the kinetic studies, where higher concentrations of testosterone were used.

Table I. *Metabolism of testosterone (T) and DHT in hairy and alopecic scalp skin biopsies.*
Starting substrate concentration was 4 nM. Values are expressed as percent of metabolized precursor at the end of the incubation time.

	Metabolites			
Substrate	Alopecic		Hairy	
	DHT	AEDION	DHT	AEDION
^{3}H-T	2.18±0.47	3.75±1.25	1.03±0.43	7.5±0.37
	n = 7	n = 8[a]	n = 7	n = 3
	DIOLS	AADION	DIOLS	AADION
^{3}H-DHT	7.79±1.63	1.75±0.87	5.28±1.6	5.03±3.06
	n = 7	n = 7[a]	n = 7	n = 5

[a] Values obtained from different patients to those of the hairy skin.

Table II. *Metabolism of DHT in hairy and alopecic skin biopsies.*
^{3}H-DHT was incubated at 4 nM and 3-α-, and 3,β-androstanediols are formed. Data are expressed as the mean percent values of each androstanediol obtained from only those patients in which both diols are formed.

	αDIOL	n	βDIOL	n
Alopecic	17±5.96	6	83±5.96	6
Hairy	9.5±5.75	2	90±7.77	2

Dihydrotestosterone metabolism

Kinetic studies.— In the alopecic areas DHT was mainly converted into androstanediols. AADION being also obtained in some cases. β-DIOL was the main diol observed and the only one found in most cases. When both diols were formed, β-DIOL amounted to between 80 and 90 % (table II). The enzyme activity was represented in calculations as the sum of the two diols formed. A complete kinetic study was performed in 8 subjects. The activity of the 3-α,β-OH was linear for up to 60 min of incubation, which was the time used for the experiments. The reaction was also linear up to 0.4 mg of protein/tube. Using a range of DHT concentrations between 10 and 100 × 10^{-6} M, the V_{max} was 15 ± 4.5 nmol/mg prot/h and $K_m = 24 \pm 9 \times 10^{-6}$ M (fig. 2).

Comparative regional studies.— For the same reasons as for the testosterone metabolism, only one concentration of ^{3}H-DHT was used (4×10^{-9} M). In seven patients the 3-α,β-OH activity was determined in both alopecic and hairy areas. The results were 1.3 ± 0.2 pmol/mg prot/h in the alopecic scalp and 1.1 ± 0.5 pmol/mg prot/h in the hairy scalp. If these results are expressed per g of tissue, the values are 12.4 ± 2.6 pmol/g tissue/h in alopecic areas and 8.4 ± 2.7 pmol/g tissue/h in the hairy scalp. These differences were significant for $p < 0.05$.

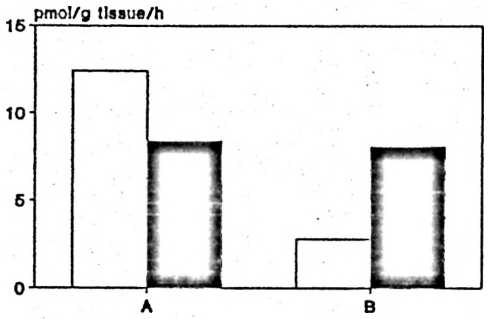

Fig. 4. *Regional differences in dihydrostestosterone metabolism.*
^{3}H-dihydrotestosterone (4 nM) was incubated with scalp skin homogenates during 60 min. 3-α,β-hydroxysteroid oxoreductase activity was evaluated by the formation of 3-α- and 3-β-, androstanediols (A). 17,β-hydroxysteroid oxoreductase was evaluated by the formation of androstanedione (B). Filled bars: hairy tissue; empty bars: alopecic tissue.

The activity of 17,β-HO was studied by the formation of AADION in some of the subjects. In the seven patients referred to above, AADION formation was observed in five cases in the hairy area (mean value = 8.1 ± 4.8 pmol/g tissue/h) and in only one case also in the alopecic scalp. In the latter case, the amount of AADION formed was 5.2 and 15.3 pmol/g tissue/h for the alopecic and hairy areas, respectively. In seven different patients the formation of AADION was also observed in alopecic areas, with a mean value of 2.8 ± 1.4 pmol/g tissue/h. Comparing all the values of the AADION formed, the greater values observed in the hairy areas were significant for $p < 0.05$. Figure 4 depicts these results and table I shows the amount of DHT transformed at the end of the incubation time.

Discussion

Chromatographic methods are commonly used to study androgen metabolism. Thin-layer chromatography is the easiest procedure for separating, identifying and recovering the formed metabolites. Nevertheless, the detection method to localize and quantify these metabolites is the critical point of any such procedure. In our case, the availability of the radiochromatograph simplifies all the classical problems mentioned and the steroids were detected with a precision of more than 0.25 mm. On the other hand, the common problem for the separation of both diols was resolved with the double run proposed by MORPHIN *et al.* (12). There is a great variety of results in the literature concerning androgen metabolism. The results are controversial, the percentage of metabolites formed varies greatly from one author to another, metabolism is clearly different among animal species and in man each type of tissue gives different results. For this heterogeneity experimental conditions are of capital importance, especially those concerning the initial substrate and the substrate concentrations. Many studies related to androgen metabolism reveal that inappropriate substrate concentrations are used to evaluate enzyme activity and that it is important to differentiate between the experimental conditions to obtain complete kinetic parameters (K_m and V_{max}) and those necessary only to facilitate the production of certain metabolites when the enzyme activity is low. Therefore, large concentrations of testosterone and DHT were used as precursors for the analysis of the kinetic parameters when large quantities of tissue (alopecic scalp), were available a percentage of conversion below 5 % being obtained. On the contrary, for the comparative regional studies between alopecic and hairy areas, because the availability of tissue was too small (occipital- donor area) and hence the total activity of the enzyme was very low, lower-undiluted concentrations of the radioactive precursor (4×10^{-9} M) were used to achieve a high percent of production of radioactive metab-

olites and to facilitate their detection. Moreover, these low concentrations of substrate agree better with the physiological amounts of the circulating hormones.

In our opinion the most relevant results of this work are those concerning the regional differences between alopecic and non alopecic samples. In the alopecic areas there is a greater activity of the 5-α-R and the 3-β-HO systems, with a higher production of βDIOL. On the contrary, hairy tissue exhibits a predominant activity of the 17,β-HO system.

Using testosterone as the precursor, DHT was mainly obtained as the sole metabolite in both alopecic and hairy areas. AEDION was obtained only in some patients in the alopecic area, whereas it was practically constant in the hairy occipital areas. These results agree with those obtained by other authors in human skin. In other working models, androstanediols were also observed (13, 16). In isolated hair follicles and in fetal skin, AEDION was the principal metabolite observed (15, 17).

When DHT was used as the precursor, βDIOL was the main metabolite observed and the αDIOL was absent or present but to a lesser extent. These differences appear in the alopecic and hairy areas. Similarly with the results obtained with testosterone, AADION production was generally observed in the hairy areas but it was rarely observed in the alopecic skin. Other authors have also observed the production of βDIOL as the major metabolite coming from DHT (16, 18) using skin samples from different body regions. Data about kinetic experiments similar to those carried out by us could not be found, but several studies concerning the activity of the 5-α-R and the 3-α,β-HO systems in other working models give similar results for the K_m values (3, 10, 19, 20).

Histological controls of our biopsies demonstrated that in alopecic areas sebaceous glands occupies the greater part of the tissue, accounting for 80 %, whereas in hairy skin these glands were of normal size, accounting for about 15 % of the pieces. Our findings would be in accordance with those described by DIJSTRA *et al.* (9), the oxidative pathway being highly developed in the sebaceous glands whereas the 17,β-HO system is probably more concentrated in hair follicles.

All these data could explain the lack of a specific DHT receptor (6) and the unusual binding pattern for the βDIOL of the cytosolic fraction coming from the alopecic-seborrhoeic tissue (7) described previously. This picture raises the question about the implications of the 3-α,β-HO system in the physiopathology of the sebaceous gland and reinforces our opinion and that of other authors, about the increasing evidence for the activity of βDIOL in the sebaceous gland and for the existence of a specific binding protein for this metabolite. If the sebaceous hypertrophy is secondary or primary to this metabolic pattern remains to be elucidated. In any case these results open new possibilities for the understanding of the ethiopathogenics of the MPB and other forms of seborrhoea, but also suggest that the selective inhibition of the 3-β-HO system can be helpful in treating such disturbances.

Resumen

Se estudian las diferencias regionales en el metabolismo androgénico en zonas alopécicas y no alopécicas de pacientes afectos de alopecia seborreica masculina. La actividad 5-α-reductasa (5-α-R) se valora a través de la dihidrotestosterona (DHT) formada a partir de ^{3}H-testosterona cuya actividad es superior en las zonas alopécicas (3,4 pmol/g tejido/h) que en las pobladas (1,5 pmol/g tejido/h). La actividad 3-α,β-hidroxiesteroide oxorreductasa se valora con ^{3}H-DHT como precursor y cuantificando la producción de 3-α- y 3-β-androstanodioles (αDIOL y βDIOL). La actividad global del enzima es superior en la zona alopécica (12,4 pmol/g tejido/h) que en la poblada (8,4 pmol/g tejido/h) y, de los dos dioles, el βDIOL es el más frecuente y abun-

dante. La actividad 17,β-hidroxiesteroide oxorreductasa muestra que la androstenodiona partiendo de la testosterona es superior en las zonas pobladas (12 pmol/g tejido/h) que en las alopécicas (6 pmol/g tejido/h); que la androstanodiona a partir de la DHT también es superior en las zonas pobladas (8,1 frente a 2,8 pmol/g tejido/h). La predominancia de formación de βDIOL en las zonas alopécicas con una gran hipertrofia sebácea refuerza la hipótesis del posible papel de dicho metabolito en los mecanismos de regulación de la secreción sebácea.

Palabras clave: Alopecia seborreica masculina, Metabolismo androgénico, 5-α-reductasa, 3-α,β-hidroxiesteroide oxorreductasa, 17,β-hidroxiesteroide oxorreductasa.

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