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METABOLISM OF ANDROGENS BY ISOLATED HUMAN HAIR FOLLICLES

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SUMMARY

The *in vitro* metabolism of [4-^{14}C]dehydroepiandrosterone, [7-^{3}H]testosterone and [1,2-^{3}H]4-androstene-3,17-dione was investigated in isolated male human hair follicles taken from the scalp, beard and pubic areas. The freshly plucked follicles were incubated for 5 h in Krebs–Ringer phosphate solution containing glucose and co-factors. Metabolites were isolated and identified by paper and thin layer chromatography, followed by crystallization to constant specific activity or isotope ratio.

Testosterone was converted to androstenedione, 5α-dihydrotestosterone, 5α-androstanedione and androsterone. Formation of 5α-dihydrotestosterone was most intensive in the beard hair follicles. Androstenedione was mainly metabolized to 5α-androstanedione with limited transformation to testosterone. Dehydroepiandrosterone (DHA) was converted principally to 5-androstene-3β,17β-diol, 7α-hydroxy-DHA, androstenedione and 5α-androstanedione. Sulphoconjugation of DHA also occurred to a small extent. However, no free DHA formation was observed when scalp or beard hair follicles were incubated with tritiated DHA-S. The overall utilization of DHA by the hair follicles was far more intensive than that by human skin. The metabolism of DHA was altered in hair follicles taken from balding individuals.

DHA strongly inhibited the activity of glucose-6-phosphate-dehydrogenase isolated from the hair follicles. The presence of two dihydrotestosterone-binding components was also demonstrated in the hair follicle cytosol.

IT IS WELL known that the growth and distribution of scalp and body hair is influenced by androgens. Male pattern baldness is an inevitable consequence of continued androgenic stimuli in men with a genetic predisposition to baldness. Increased androgen production in women causes the loss of scalp hair in the pattern of male baldness and produces an increase of facial and body hair. This phenomenon accompanies adrenal hyperplasia and virilizing adrenal and ovarian tumors. However, in spite of our extensive empirical knowledge, the biochemical mechanism of action of androgens in the hair follicle remains to be clarified.

As a result of recent investigations it has become established that steroid hormone metabolites are the mediators of androgenic action. For example 5α-dihydrotestosterone (5α-DHT) is probably the active form of testosterone in several androgen sensitive tissues, such as the ventral prostate, seminal vesicles, preputial glands and epipidymis.

The transformation of testosterone into 5α-DHT has been demonstrated to occur in human skin and hair follicles. Northcutt and colleagues[1] found intensive DHT formation by pubic hair follicles *in vitro*. More recently, Sansone-Bazzano and coworkers[2] examined the testosterone metabolic activity of human scalp hair follicles. The principal metabolite was androstenedione while DHT could be identified only in some of the samples.

Dehydroepiandrosterone is another important circulating androgen with a possible influence on the function of the hair follicles. This steroid has been shown to be extensively metabolized by human skin.

In the present study we examined the *in vitro* metabolism of the known major androgens, such as testosterone, androstenedione and dehydroepiandrosterone by human scalp, beard and pubic hair follicles. Hair follicles were obtained by plucking from male individuals and incubated (20 follicles/flask) immediately with the radioactive steroid substrates ([7-^{3}H]-testosterone, 1 μCi, 40 pmol/flask; [4-^{14}C]-DHA, 0·3 μCi, 5220 pmol/flask; [1.2-^{3}H]androstenedione, 1 μCi, 20·8 pmol/flask) for 5 hours, at 37°C in 1·5 ml Krebs-Ringer phosphate solution, containing 200 mg% (w/v) glucose, NAD, NADH, NADP and NADPH (50 μg/flask). After extraction and addition of radioactive internal standards, the metabolites were isolated and purified by paper and thin layer chromatography and identified by serial crystallization to constant isotope ratio.

Figure 1 shows the basic steps of the metabolism of androgens in the human hair follicle. From testosterone, four principal metabolites were formed; androstenedione, 5α-DHT, 5α-androstanedione and androsterone. The conversion of androstenedione into testosterone and 5α-DHT was very limited. Dehydroepiandrosterone was converted to the following principal metabolites: 5-androstene-3β, 17β-diol, 7α-hydroxy-dehydroepiandrosterone, androstenedione and 5α-androstanedione. In addition, a small fraction of DHA was sulphoconjugated. However, no free DHA formation was observed when scalp or beard hair follicles were incubated with tritiated DHA-sulphate.

Table 1 demonstrates the quantitative aspects of testosterone metabolism by the beard, scalp and pubic hair follicles of two normal adult males. The principal metabolite was androstenedione, followed by 5α-androstanedione and DHT. The formation of this tissue active androgen was most intensive in beard follicles, but the quantities were much less than those reported for human prostatic tissue [3].

Table 2 presents the quantitative aspects of dehydroepiandrosterone metabol-

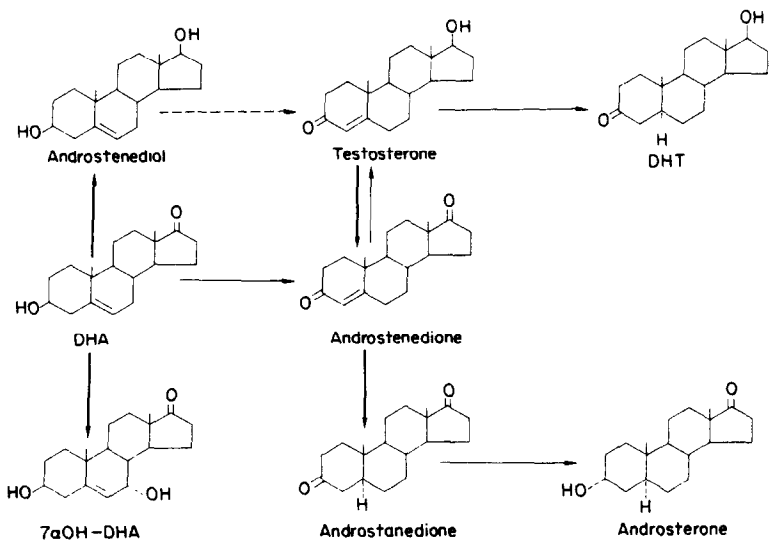

Fig. 1. Metabolism of androgens in the human hair follicle.

Table 1. Testosterone metabolic activity of human hair follicles

Product	Beard	Scalp	Pubic
DHT	4·2	2·7	1·6
	8·9	1·8	—
Androstenedione	76·3	120·0	25·0
	198·0	71·8	—
Androstanedione	8·3	16·0	—
	25·2	10·4	—
Androsterone	0·6	—	—
	0·5	—	—

Quantities of radiometabolites are expressed as pmol/100 mg tissue/h.

Table 2. DHA metabolic activity of human hair follicles

Product	Beard	Scalp	
		Frontal	Occipital
5-Androstenediol	2,500	3,200	3,650
	2,350	5,060	8,130
7-Hydroxy-DHA	1,650	1,320	954
	1,450	1,610	3,450
Androstenedione	196	148	162
	267	794	1,100
Androstanedione	—	42	20
	—	278	212

Quantities of radiometabolites are expressed as pmol/100 mg tissue/h.

ism by the beard and scalp hair follicles of the same individuals. There is a very intensive formation of 5-androstene-3β,17β-diol, followed by 7α-hydroxy-dehydroepiandrosterone and androstenedione. The overall rates of formation of 5-androstenediol and 7α-hydroxy-DHA were much higher than those by human skin. Hair follicles formed 100 times more androstenediol and 10 times more 7α-hydroxy-DHA from DHA than did human skin incubated under similar conditions. The transformation into 4-androstene-3,17-dione occurred at rates within those described for human skin [4].

By studying the metabolism of dehydroepiandrosterone in the hair follicles of non-balding and balding individuals, we have been able to recognize a type of baldness which seems to be characterized by a defect in the function of 17β-hydroxysteroid-oxidoreductase. The net result is a decreased formation of 5-androstene-3β,17β-diol, a principal metabolic product in the hair follicles of control individuals. The above observation led us to believe that this metabolic step has a special importance in the hair follicles. Consequently, we were searching for

possible sites of action, where these steroids could interfere with cellular metabolism in the hair follicles. One of the most likely sites is glucose-6-phosphate-dehydrogenase. The activity of this enzyme increases most dramatically during the growing phase of the human scalp hair follicle as shown by Adachi and coworkers[5]. This is a key enzyme of the pentose cycle, which supplies building blocks for the synthesis of nucleic acids and also an important source of NADPH. It is well established that the activity of this enzyme is inhibited by various steroids[6–8], among them dehydroepiandrosterone. Therefore, we examined the effect of this steroid on the activity of human hair follicle glucose-6-phosphate dehydrogenase. Enzyme activity was measured by spectrophotometry[8].

Figure 2 illustrates the effect of various androgens on the activity of glucose-6-phosphate dehydrogenase, extracted from human scalp hair follicles. The most effective inhibitors were dehydroepiandrosterone and epiandrosterone. 5-Androstene-3β,17β-diol and the other androgens examined had only marginal effects.

Figure 3 shows the Dixon plot of the inhibition of hair follicle glucose-6-phosphate dehydrogenase by dehydroepiandrosterone. The inhibitor constant ($K_i = 1{\cdot}20 \times 10^{-6}$M) is about 5 times lower than that found for the human red blood cell enzyme[8].

These results clearly demonstrate that dehydroepiandrosterone has a strong inhibitory effect on the activity of this important enzyme in the hair follicles. In this framework the conversion of dehydroepiandrosterone into 5-androstenediol represents an inactivation step when the enzyme inhibitor becomes deactivated. Consequently, a defect in the function of the deactivating enzyme, 17β-hydroxysteroid oxidoreductase, results in higher intracellular concentrations of dehydroepiandrosterone and inhibition of glucose-6-phosphate dehydrogenase. This in turn would slow down the growth of hair and the hair cycle. In this manner, the

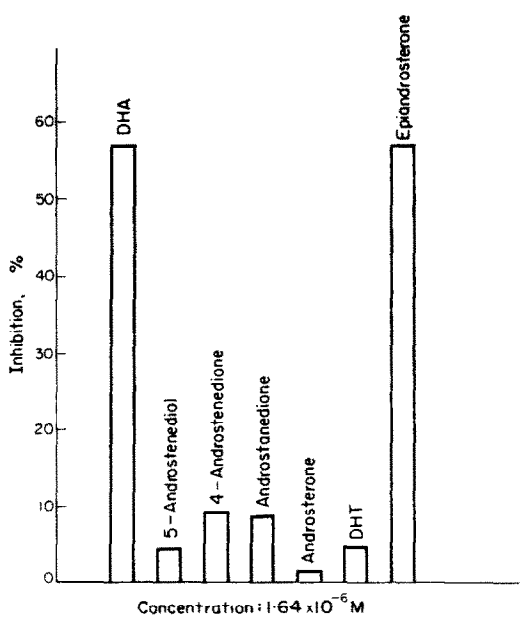

Fig. 2. Inhibition of human hair follicle G-6-P-DH by various androgens.

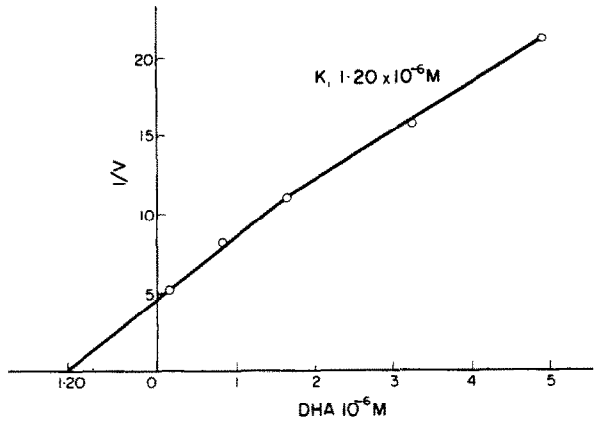

Fig. 3. V = enzyme velocity, K_i = inhibitor constant, DHA = dehydroepiandrosterone (inhibitor concentration).

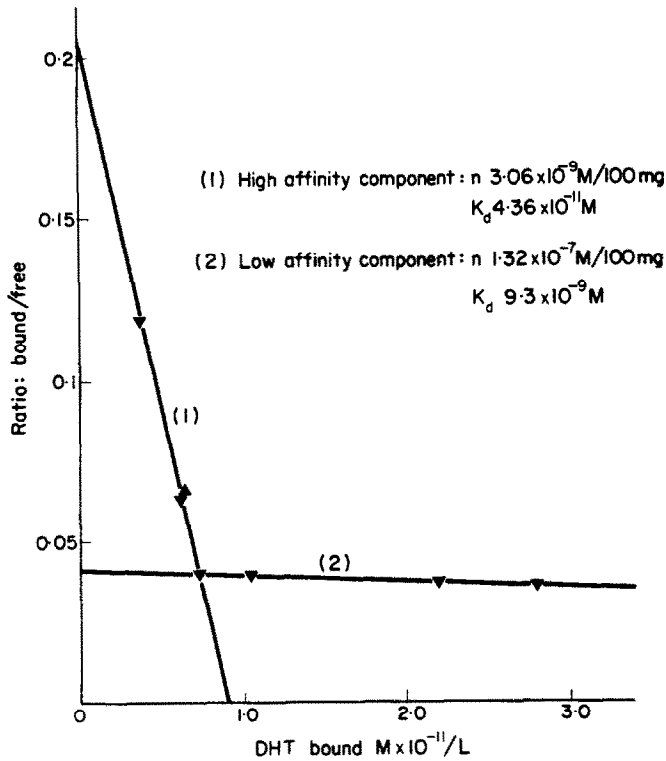

Fig. 4. Scatchard plot of dihydrotestosterone binding in hair follicle cytosol as measured by the florisil adsorption method. 100 hair follicles were plucked from a male individual, homogenized in 0·01 M Tris-HCl buffer, containing 0·002 M EDTA, pH 7·4, then centrifuged for 1 h at 105,000 *g*. 1 ml aliquots of the cytosol were added to tubes containing [1,2-^{3}H]dihydrotestosterone (20 pg; 8.000 d.p.m.) and variable amounts of non-radioactive dihydrotestosterone. The tubes were incubated by shaking at + 3°C for 2 h. After incubation 40 mg of washed florisil was added to each tube to absorb free steroid. Tubes were shaken for 2 min, left to stand for 10 min at + 3°C, then 0·5 ml aliquots of the supernatant were taken for measurement of bound radioactivity. The dissociation constant (K_d) and number of binding sites (n) were calculated from the intercepts with the y and x coordinates (n/K_d and n respectively).

intracellular levels of free dehydroepiandrosterone might regulate hair growth by controlling the activity of glucose-6-phosphate-dehydrogenase. Unfortunately, the concentration of free dehydroepiandrosterone in the hair follicle is not known at present. Physiological blood concentrations are between 0·5–2 μg/100 ml[9]. At these concentrations the inhibition of the enzyme is around 5–10 per cent. However, the actual concentration could be much higher, depending on the sulphatase activity of the surrounding skin.

Since cytoplasmic receptors have been implicated as mediators during the action of androgens in the target cells[10, 11], we attempted to demonstrate the presence of such macromolecules in human hair follicle cytosol. The binding of tritiated dihydrotestosterone was studied by absorption of the free steroid on florisil after equilibration at + 3°C. Two binding components were demonstrated by using the same amount of tritiated DHT and increasing amounts of cold DHT, as shown on the Scatchard plot (Fig. 4).

One of the components possessed a high affinity for dihydrotestosterone, with relatively small binding capacity, while the other component had a much larger quantity of binding sites but a lower affinity for the steroid.

These binding components of the human hair follicle cytosol could be part of a receptor mechanism enabling the intracellularly formed DHT to interact with nuclear components as demonstrated for rat prostate cytosol[12].

ACKNOWLEDGEMENTS

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DISCUSSION

Liao: I wonder if you have tried adenyl-cyclase. There was a report that DHT would inhibit adenyl cyclase in hair follicles.

Fazekas: Yes, I know the work by Adachi (*J. Soc. Cosmet. Chem.* **21** 1970 901).

Liao: Has anyone confirmed this?

Fazekas: I don't know about any follow-up work. I found this a little strange, because as far as I know, adenyl-cyclase is located in the cell membranes, and DHT is formed within the cell.

Liao: This may not be the problem since a large amount of 5α-dihydrotestosterone binding protein can be isolated from the microsomal fractions of prostate and adenyl-cyclase activity has been detected in the intracellular particulate fractions.

Fazekas: No, I used (^{14}C)-DHA in these experiments, and 15α tritiated DHA in the experiments with DHA.
activity. My results concerning the formation of dihydrotestosterone in the hair follicles indicate a tendency to higher dihydrotestosterone activity in balding individuals compared with non-balding individuals, but this study must be extended to a larger number of cases.
O'Malley: Your Scatchard plots were with hair from what source.
Fazekas: Human hair follicle cytosol.
O'Malley: From what area was the hair taken?
Fazekas: The hair was taken from the occipital scalp region.
O'Malley: Have you tried any other sources or sex hair?
Fazekas: No, that's the only source I've used.
Morfin: After you extracted your incubation medium, what kind of recovery did you have.
Fazekas: Over 90%.
Morfin: Have you any evidence for unidentified very polar metabolites?
Fazekas: Yes, I do have unidentified metabolites present, especially from dehydroepiandrosterone.
Morfin: And from testosterone?
Fazekas: From testosterone, I indicated the presence of the diols, 3α,3β-diols from testosterone, in a paper which is published (*Steroids* **18** (1971) 367).
Morfin: But if you had a 7-hydroxylated compound, that wouldn't be shown, would it, especially if you incubate it with a 7-tritiated steroid?
Fazekas: No, I used [^{14}C]-DHA in these experiments, and [15α-^{3}H]-DHA in the experiments with tritiated DHA.