5α-Reductase Activity in Cultured Human Dermal Papilla Cells from Beard Compared with Reticular Dermal Fibroblasts

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The activity of 5α -reductase was assessed in cultured human beard dermal papilla cells and reticular dermal fibroblasts to elucidate the mechanism of androgen action in promoting the growth of beards in men. The monolayer was incubated with 50 nM of [1,2-^{3}H]-testosterone. Steroids were extracted from the medium and analyzed by thin layer chromatography. The major metabolite in the beard dermal papilla cells was dihydrotestosterone (DHT), the most potent androgen in the androgen target tissue. By contrast, the amount of DHT formed was similar to that of androstenedione in reticular dermal fibroblasts. The 5α-reductase activity in beard dermal papilla cells was three to five times as high as that in the reticular dermal fibroblasts from the same skin sample. The apparent Michaelis constant of 5α-reductase in the beard dermal papilla cells was 1.0×10^{-6} M, which was virtually equivalent to that of genital skin fibroblasts, typical androgen target cells. It was 4.0×10^{-5} M in reticular dermal fibroblasts. By contrast, the activities of 5α-reductase in dermal papilla cells from occipital scalp hair follicles were similar to those of reticular dermal fibroblasts of the same skin samples. These results strongly suggest that the beard dermal papilla cell is an androgen target cell, and that DHT plays a role in the growth of beards in men. *J Invest Dermatol 94: 150–152, 1990*

The growth of some sorts of hair is influenced by androgens and the response of hairs to the hormones is variable, depending upon where they grow. For instance, the growth of a beard is dependent upon the adult male level of circulating androgens, and the growth of pubic hair and axillary hair is dependent upon the female level. Like other androgen target tissues, human plucked hair follicles (which contain predominantly follicular epithelial cells) can metabolize testosterone to dihydrotestosterone (DHT) [1–3]. DHT, however, is not necessarily a major metabolite of testosterone even in the beard hair follicle [1]. Besides, the activity of 5α -reductase does not appear to correlate with androgen-mediated hair growth [2,3]. Dermal papilla cells, which are a mesenchymal component of the hair bulb, are considered to play a fundamental role in the induction of epithelial differentiation [4,5]. Recently, human dermal papilla cells were isolated and serially cultured in vitro [6]. These cells are morphologically and functionally differentiated from the reticular dermal fibroblast [7,8].

In this in vitro investigation, we studied metabolism of testosterone in dermal papilla cells from the human beard, and compared the activities of 5α-reductase of these cells with those of reticular dermal fibroblasts. As the controls of beard follicles, we also measured the activities of this enzyme in dermal papilla cells of occipital scalp hair and in fibroblasts obtained from the adjacent skin.

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The following trivial names are used:
dihydrotestosterone: 17β -hydroxy-5α -androstan-3-one
testosterone: 17β -hydroxy-4-androsten-3-one
androstanediol: 5α -androstane-3α, 17β -diol
androstenedione: 4-androstene-3, 17-dione
androstanedione: 5α -androstane-3, 17-dione

MATERIALS AND METHODS

Isolation and Culture of Dermal Papilla Cells The dermal papilla cells from human beard and occipital scalp hair were isolated from five skin samples, each obtained at plastic surgery and cultured according to the methods of Messenger [6]. The donors were all male and their ages ranged from 16 to 63. Reticular dermal fibroblasts from the same skin specimen were also cultured. Dulbecco's modified Eagle's medium (DMEM) buffered with sodium bicarbonate and supplemented with penicillin (50 U/ml) and 10% fetal calf serum (FCS) was used as in routine experiments. Cells were subcultured after trypsin dissociation (usually 3–4 weeks after the beginning of primary cell culture and at confluency thereafter) in a usual fashion. All experiments were performed after the fourth to sixth subculture and at confluency.

Androgen Metabolism When the cells became confluent, the medium was removed. The monolayer formed was washed with DMEM, and incubated with 2 ml of DMEM containing 50 nM [1,2-^{3}H]-testosterone for varying periods at 37°C in 5% CO_2. After incubation, the steroids were extracted from the medium and analyzed by thin layer chromatography. The cells were washed and lysed with 0.5 ml of 0.5 N NaOH, and the protein content was determined by the method of Lowry et al [9].

Extraction and Isolation of Steroid Metabolites After incubation, a four-fold volume of chloroform-methanol (2/1, V/V) [10] containing 10 μg each of carrier steroids was added to the medium. The extracted steroids were analyzed by thin layer chromatography with the solvent system (methanol:chloroform, 99:1) as described by Gomez and Hsia [11]. The purity of each steroid was determined by the recrystallization method. The activity of 5α-reductase was expressed by the sum of DHT, androstanediol, and androstanedione formed.

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Chemicals [1,2-^{3}H]-Testosterone (55.2 Ci/mmol) was obtained from New England Nuclear Corporation (Boston, MA), and unlabeled steroids were purchased from Sigma Chemical Company (St. Louis, MO). FCS was supplied by Hazleton (Lenexa, KS), DMEM by Nissui Seiyaku Co., Ltd. (Tokyo, Japan), and Kieselgel 60 F_{254} by Merck (West Germany). All other chemicals were of reagent grade.

RESULTS

When the monolayer was incubated with [1,2-^{3}H]-testosterone, more than 95% of the metabolites were recovered in the medium as opposed to the cells. Therefore, the amount of metabolites formed was measured only in the medium in subsequent experiments. When beard dermal papilla cells were incubated with 50 nM [1,2-^{3}H]-testosterone, DHT formation increased linearly for about 3 h (Fig 1*A*). A much smaller amount of DHT was formed by reticular dermal fibroblasts than by dermal papilla cells, although such linear increase was sustained much longer (7 h) (Fig 1*B*). The levels of androstenedione were leveling off at 7 h in both types of cells. DHT was dominant over other metabolites in beard dermal papilla cells. By contrast, the amount of DHT formed by reticular dermal fibroblasts was nearly equivalent to that of androstenedione. Figure 2 shows the relation between the concentration of substrate testosterone and the rate of formation of 5α -reduced metabolites in both types of cells cultured from the same beard skin sample. The reaction almost culminated at 2 μM testosterone in dermal papilla cells. In reticular dermal fibroblasts it was not saturable under the present experimental conditions. The apparent Michaelis constant as estimated from Lineweaver-Burk plots was about 1.0×10^{-6} M for beard dermal papilla cells and 4.0×10^{-5} M for reticular dermal fibroblasts.

The findings on the 5α-reductase activity of beard dermal papilla cells compared with reticular dermal fibroblasts in five skin samples are summarized in Table I. The concentration of [1,2-^{3}H]-testosterone employed for this assay was 50 nM, which approximates the plasma level of testosterone in the adult male. The activities determined ranged from 3.04 to 5.55 pmoles/mg protein/h in beard dermal papilla cells. The age of the donors did not seem to have a bearing upon the enzyme activity. The relevant values for reticular dermal fibroblasts were 1.00 to 1.36 pmoles/mg protein/h. In each individual sample, beard dermal papilla cells showed three to five times as high an activity as that of reticular dermal fibroblasts.

Table II showed the 5α-reductase activities of dermal papilla cells and reticular dermal fibroblasts from the beard of five cases shown in Table I and from the occipital scalp hair of five other cases. The enzyme activities are significantly higher in beard dermal papilla cells than in other types of cells. There was no significant difference in the activity between the dermal papilla cells from the occipital scalp hair and reticular dermal fibroblasts.

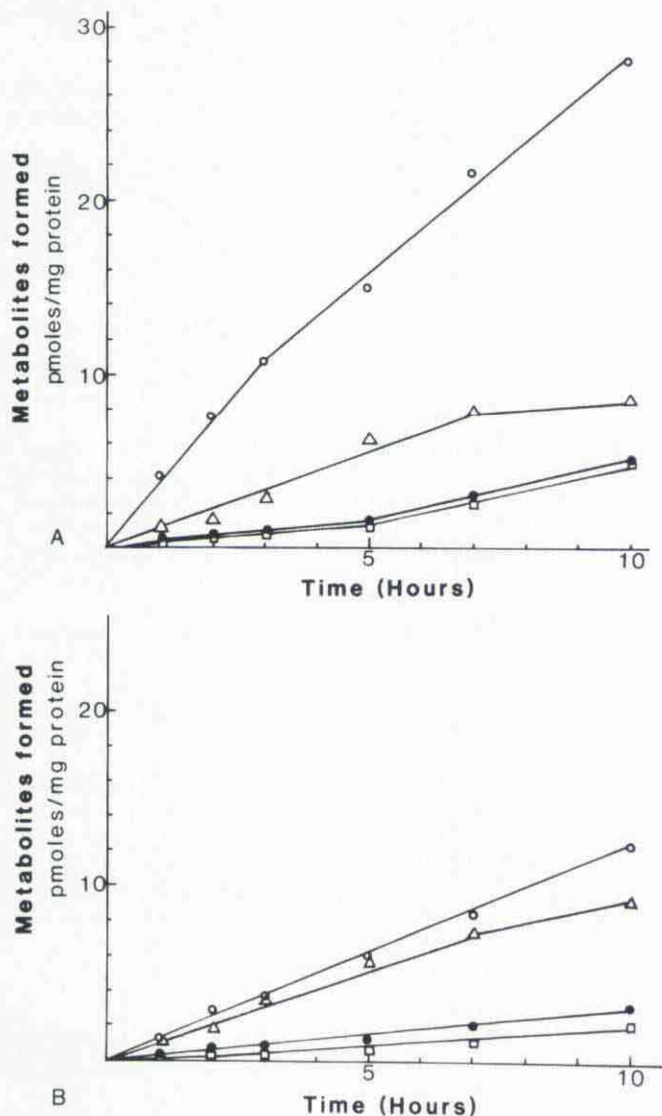

Figure 1. Time course of formation of testosterone metabolites by dermal papilla cells from beard *(A)* and reticular dermal fibroblasts *(B)*. Both types of cells were obtained from *case B* shown in Table I. When the cells became confluent, the medium was replaced by 0.2 ml of fresh medium containing 50 nM of [1,2-^{3}H]-testosterone. The medium was removed at the indicated time and metabolites were analyzed by thin layer chromatography. Each point represents a mean of two determinations. *Open circles,* dihydrotestosterone, *open triangles,* androstenedione; *open squares,* androstanedione; *solid circles,* androstanediol.

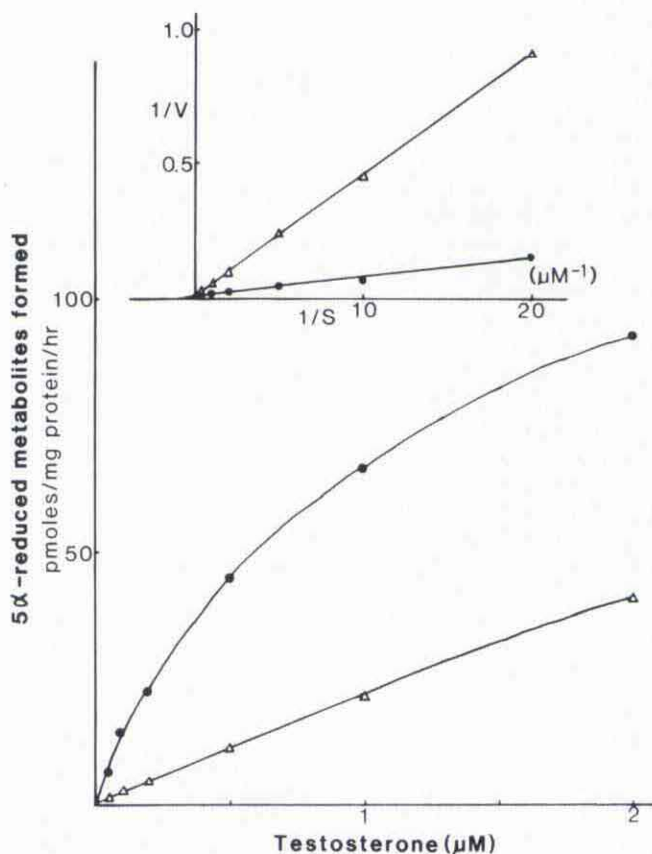

Figure 2. The effect of substrate concentration on 5α-reduction of testosterone in beard dermal papilla cells and reticular dermal fibroblasts from *case E*. The inset graph represents a Lineweaver-Burk plot. Each point represents a mean of two determinations. *Solid circles,* dermal papilla cells; *open triangles,* reticular dermal fibroblasts.

Table I. 5α-Reductase Activities in Beard Dermal Papilla Cells and Reticular Dermal Fibroblasts from Five Cases[a]

Cells	Case A (59 years old)	Case B (17 years old)	Case C (22 years old)	Case D (43 years old)	Case E (40 years old)
Dermal papilla cells	3.04 ± 0.08	4.00 ± 0.05	3.45 ± 0.06	3.38 ± 0.02	5.55 ± 0.23
Reticular dermal fibroblasts	1.14 ± 0.03	1.11 ± 0.07	1.19 ± 0.01	1.36 ± 0.06	1.00 ± 0.08

[a] Each value is a mean ± S.E. of triplicate determinations, expressed as pmoles/mg protein/h.

DISCUSSION

The present observations clearly indicate that dermal papilla cells from beard resemble androgen target tissues in character: they show a high 5α-reductase activity comparable to that of cultured genital skin fibroblasts [12,13]. Dermal papilla cells metabolized testosterone more actively to DHT than to androstenedione. On the other hand, 17β-oxidation and 5α-reduction occurred at a similar rate in the reticular dermal fibroblasts obtained from the same skin samples (Fig 1). In other strains of cultured reticular dermal fibroblasts, 17β-oxidation was predominant over 5α-reduction (in *case E*, 17β-oxidase activity was 1.82 ± 0.05 pmoles/mg protein/h, and 5α-reductase activity was 1.0 ± 0.08 pmoles/mg protein/h). Such differences in testosterone metabolism were demonstrated between genital and nongenital skin [14]. Further studies using cell-free systems are required in order to clarify whether these results reflect the difference of enzyme activity itself or that of the level of cofactors in cells.

There are also some differences in the characteristics of 5α-reductase between beard dermal papilla cells and reticular dermal skin fibroblasts. As shown in Fig 2, the reaction reached a nearly maximum level at 2 μM testosterone in beard papilla cells, whereas it was not saturable at this substrate concentration in reticular dermal fibroblasts. The apparent Michaelis constant for beard dermal papilla cells was 1.0×10^{-6} M, which is comparable to that for genital skin or cultured genital skin fibroblasts [12,15]. The value was much larger for the reticular dermal fibroblasts. These differences might be related to the fact that dermal papilla cells are morphologically and functionally differentiated from the reticular dermal fibroblasts [7,8]. Further studies using purified enzyme sources are essential for clarifying whether or not these results can be ascribed to the presence of different forms of 5α-reductase in these two types of cells.

It is well known that the growth of human beard hair follicles is androgen dependent but that the occipital scalp hair growth is not. However, experiments using plucked hair follicles proved that DHT formation is a ubiquitous property of hairs [2,3]. Besides, beard hair follicles do not have a higher activity of 5α-reductase than hair follicles from any other site of the skin [1–3]. These results might be due to the fact that plucked hairs lack dermal papillae and have often lost part of the bulbar epithelium [16]. The results demonstrated in Table II clearly indicate that androgen-dependent dermal papilla cells have high 5α-reductase activities. These results strongly suggest that dermal papilla cells may play an important role in the initiation of androgen-dependent growth of a beard, and that they might mediate the action of androgen on the epithelium, as in the prostate [17] and mammary gland [18].

Table II. 5α-Reductase Activities in Dermal Papilla Cells and Reticular Dermal Fibroblasts from Beard and Occipital Scalp Hair Follicles[a]

Cells	Beard (n = 5)	Occipital scalp hair (n = 5)
Dermal papilla cells	3.88 ± 0.23	1.08 ± 0.05
Reticular dermal fibroblasts	1.15 ± 0.04	0.99 ± 0.05

[a] Each value is a mean ± S.E. of five cases, each of which is computed from triplicate determinations expressed as pmoles/mg protein/h.

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