

Activity of type 1 5 α -reductase is greater in the follicular infrafundibulum compared with the epidermis

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Summary

The enzyme 5 α -reductase converts testosterone (T) to dihydrotestosterone (DHT). Although this enzyme has been localized to various regions of the pilosebaceous unit, its activity has not been studied in the follicular portion of either vellus or sebaceous follicles. The goal of our study was to determine the relative activities of 5 α -reductase within various regions of these follicles with particular emphasis on the infrafundibulum. A finding of increased 5 α -reductase activity in upper follicles compared to epidermis might support the hypothesis that increased follicular production of DHT is involved in the hyperkeratinization observed in this region of the follicle in acne vulgaris. 5 α -reductase activity was determined at pH 5 (optimal for the type 2 isozyme) and pH 7 (optimal for the type 1 isozyme) in isolated infrafundibular segments from sebaceous and vellus follicles, homogenized epidermis from various anatomical areas and in microdissected segments of the pilosebaceous unit from breast skin of normal subjects. Enzyme activity was also determined at pH 7 in cultured infrafundibular keratinocytes and in interfollicular epidermal keratinocytes. Homogenates of infrafundibular segments demonstrated significantly greater activity at pH 7 compared to pH 5 ($P < 0.001$), confirming activity of the type 1 5 α -reductase in this region. Activity of 5 α -reductase was much lower in homogenized epidermis and did not demonstrate a clear pH preference. Keratinocytes cultured from the infrafundibulum demonstrated significantly greater 5 α -reductase activity compared to keratinocytes from interfollicular epidermis ($P = 0.04$). In the dissected segments of pilosebaceous units from breast skin, 5 α -reductase activity was greatest in the sebaceous gland followed by the sebaceous duct, infrafundibulum, whole skin and epidermis. These data indicate that 5 α -reductase activity varies within regions of the pilosebaceous unit and compared with interfollicular epidermal cells, infrafundibular keratinocytes have an increased capacity for producing androgens which may play a role in the follicular hyperkeratinization seen in acne.

Little is known regarding the exact mechanisms involved in the follicular hyperkeratinization observed in pilosebaceous follicles affected with acne vulgaris. It has been hypothesized that linoleic acid,¹ retinoids² and/or androgens^{3–5} may be involved. Androgens are known to be responsible for the conversion of terminal hair follicles to vellus follicles in the balding region of the male scalp. Conversely, androgens that are produced during sexual maturation are responsible for the conversion of vellus hairs to terminal hairs in genital skin, the axilla and the male beard and chest. Androgens are also necessary for sebaceous gland development. In

addition, it has been hypothesized that androgens may also play a role in the follicular hyperkeratinization of sebaceous follicles that is seen in acne vulgaris.^{3–5} Indirect evidence in support of this hypothesis includes the finding of androgen receptors in the outer root sheath of sebaceous follicles^{3,6} and the clinical observation that antiandrogens may reduce follicular casts in these regions.⁵

Dihydrotestosterone (DHT) is thought to be the principal hormone involved in acne and other androgen-related disorders such as male-pattern hair loss and hirsutism.⁷ DHT is produced by the action of the 5 α -reductase enzyme on testosterone (T). This conversion occurs principally in target tissues such as the prostate, sebaceous gland and hair follicle. Two

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isozymes of 5 α -reductase (type 1 and 2), which differ in their tissue distribution and biochemical properties, have been identified.⁸⁻¹⁵ Takayasu *et al.* were the first to note differences in the biochemical properties of 5 α -reductase in sebaceous glands compared to genital skin prior to identification of its isozymes.¹¹ The type 2 5 α -reductase isozyme is active in the prostate⁸ and cultured dermal papilla from the beard region,¹² and demonstrates optimal activity at pH 5.5.⁸⁻¹⁰ Within the skin, it has been immunolocalized to the cytoplasm of epidermal keratinocytes of the spinous layer, dermal fibroblasts, adipocytes and the non-keratinizing portion of the inner root sheath and cuticle.¹³ In contrast, the type 1 isozyme is active in whole scalp skin, chest skin and isolated sebaceous glands from the scalp, face, abdomen and extremities and exhibits optimal activity at pH 7.¹¹⁻¹⁷ This isozyme has been immunolocalized to the nucleus of basal keratinocytes, dermal fibroblasts, adipocytes, eccrine duct cells, dermal papilla and outer root sheath of hair follicles and the basal layer of sebaceous glands.^{13,16} The purpose of this study was to measure the biochemical activities of these isozymes in isolated infrainfundibular segments (from the dermoepidermal junction to the sebaceous duct) of sebaceous and vellus follicles, homogenized epidermis, segments from different regions of the pilosebaceous unit of breast skin and keratinocytes cultured from the infrainfundibulum and epidermis from various anatomical areas. Evidence for increased androgen production in the follicular infrainfundibulum compared to epidermis, may provide a rationale for further studies aimed at testing the hypothesis that these hormones might play a role in the follicular hyperkeratinization observed in acne.

Materials and methods

Subjects

Normal skin was obtained from 82 patients (ages 18–81 years, mean 62 years) undergoing routine surgery performed in the Division of Dermatology and Division of Plastic and Reconstructive Surgeries at the Pennsylvania State University College of Medicine.

Microdissection of the pilosebaceous follicle

Microdissection was performed using a stereomicroscope under 15 \times magnification. Follicles from the ear, forehead, temple and nose were characterized as sebaceous follicles by virtue of the large size of their sebaceous glands. Hair follicles from the abdomen, arm

and breast were identified as vellus follicles by virtue of the diminutive size of their associated hairs and sebaceous glands. The lower follicle (beneath the sebaceous duct to the base of the follicle) was dissected from surrounding collagen. The sebaceous gland and attached duct were dissected from the follicle and the ducts were removed using a combination of blunt and sharp dissection with Dumont no. 5 forceps, a no. 11 scalpel blade and an 18-gauge needle. The remaining infrainfundibular portion of the follicle was detached just beneath the dermoepidermal junction. Isolated portions of the follicle were cleaned of attached collagen fibres. All segments were visually inspected for evidence of residual collagen or sebaceous gland remnants. The epidermis was separated from the dermis following incubation in collagenase using previously published methods.¹⁸ Infrainfundibular segments and epidermis were stored at -80°C in Hank's balanced salt solution (HBSS) until 5 α -reductase incubation assays were performed. Preliminary studies indicated that the enzyme is stable for at least 4 months under these conditions.

Keratinocyte culture

Isolated infrainfundibular segments were grown in 35 mm plastic plates (three segments per plate) over mitomycin-inactivated 3T3 fibroblast feeder layers in DMEM/Hams F-12 3:1 containing 10% fetal calf serum, cholera toxin 10^{-10} mol/l, hydrocortisone $0.4\ \mu\text{g/ml}$, insulin $5\ \mu\text{g/ml}$, epidermal growth factor $10\ \text{ng/ml}$ and antibiotics. Single cell suspensions of epidermal keratinocytes were prepared and plated in the same media according to the method of Boyce and Ham.¹⁸ These methods were chosen as they resulted in optimal plating efficiency. Media were changed three times weekly.

Incubation assay of 5 α -reductase activity

Incubation studies with [1,2-³H] T were performed on pooled isolated infrainfundibular segments obtained from sebaceous follicles from the forehead, ear, temple and vellus follicles from the abdomen, arm and breast in order to estimate 5 α -reductase activity. Homogenates of infrainfundibular follicular segments were prepared in HBSS containing 1 mmol/l phenylmethylsulphonyl fluoride (PMSF), 1 mmol/l dithiothreitol (DTT), 0.25 mol/l sucrose and 20% (v/v) glycerol in a Radnoti precision microgrinder with a 500 μl capacity. Protein content of the homogenates was determined using the

Pierce BCA protein assay. Each sample of infrainfundibular segments contained a minimum of 115 μg of protein (representing approximately 100 pooled follicles). Samples were incubated at pH 5 and 7 for 30 min C with 10 $\mu\text{mol/l}$ cold T, 500 $\mu\text{mol/l}$ NADPH, 10^6 d.p.m. [1,2- ^3H] T at 37°C, extracted and androgens separated using thin layer chromatography, according to previously described methods.¹⁵ Because of the increased availability of skin from reduction mammoplasty, we were able to isolate epidermis, infrainfundibular segments, sebaceous ducts, sebaceous glands, lower follicles and full-thickness skin from breast skin from seven females (ages 20–61 years) in order to compare 5 α -reductase activity within various regions of the pilosebaceous unit. Pooled samples of sebaceous duct, infrainfundibular segments and lower follicles from eight additional subjects (ages 21–53 years) were also studied.

When primary keratinocyte cultures were at least 30% confluent, the fibroblast feeder layers were removed using 0.02% EDTA pH 7.4. Plates were washed three times in phosphate-buffered saline. Serum-free keratinocyte media (MCDB-153, Sigma Corp., U.S.A.) was reconstituted with water and bicarbonate and pH was adjusted to 7. No growth factors, steroids, bovine pituitary extract or other additives were included. This media was supplemented with 10^6 d.p.m. of [1,2- ^3H] T and cold testosterone was added to a final concentration of 10 $\mu\text{mol/l}$. Plates were incubated for 60 min at 37°C then media was removed, extracted and androgens separated as above. Residual cells were washed with phosphate-buffered saline and were removed from the plate using trypsin/EDTA. Cell counts were performed using a Coulter counter.

Enzyme activity was calculated using the net per cent conversion of [1,2- ^3H] T to DHT, the molar concentration of T, and the incubation volume. Data are expressed as pmol product/min per mg protein for homogenized epidermis and infrainfundibular segments and as nmol product/ 10^6 cells per h for cultured keratinocytes. A paired *t*-test was used to compare 5 α -reductase activity at pH 5 and 7 in isolated infrainfundibular segments and enzyme activity at pH 7 in cultured follicular and epidermal keratinocytes.

Results

5 α -reductase activity in homogenates of infrainfundibular segments and epidermis

More than 700 infrainfundibular segments were isolated from sebaceous or vellus follicles and cleaned

of attached collagen fibres (Fig. 1). Pooled infrainfundibular segments from seven anatomical areas and pooled epidermal homogenates from five anatomical areas were studied (Tables 1 and 2). Radiolabelled T was converted only to DHT and negligible amounts of androstenedione (data not shown). Production of 5 α -androstenedione and 5 α -androstane diols was not detected. 5 α -reductase activity was observed in all infrainfundibular samples studied. Maximal values were obtained at pH 7, confirming that the type 1 isozyme is active in this region. Activity at pH 7 was significantly greater than activity at pH 5 ($P < 0.0001$). Highest activity levels were observed in sebaceous follicles from the retroauricular region and temple and in vellus follicles from the abdomen. Epidermal homogenates in general had negligible 5 α -reductase activity when assayed at the same cellular protein concentrations as the infrainfundibular segments. Due to the extremely low activity, the data do not demonstrate a clear pH optima. Infundibular homogenates from the ear, forehead and temple had significantly greater 5 α -reductase activity at pH 7 compared to samples of homogenized epidermis from these same regions ($P = 0.005$, 0.02 and 0.004, respectively, paired *t*-test, $\alpha = 0.05$).

5 α -reductase activity in cultured infrainfundibular and epidermal keratinocytes

Growth of keratinocytes from infrainfundibular explants and single cell suspensions of epidermal

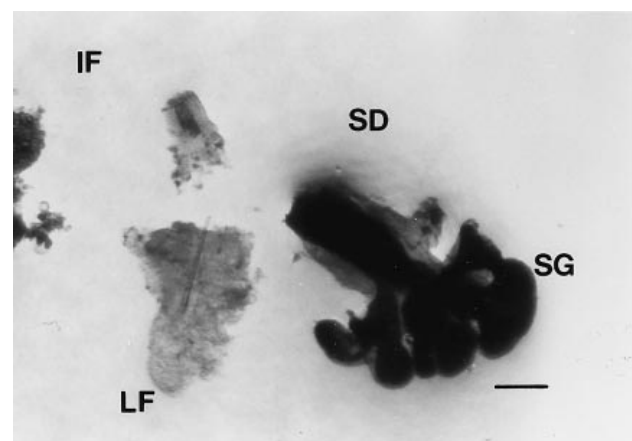


Figure 1. Microdissection of infrainfundibular follicular segments from a sebaceous follicle from the posterior auricular region of 76-year-old man. The sebaceous gland (SG) and attached sebaceous duct (SD) are seen and the infrainfundibulum (IF) and lower follicle (LF) are seen (bar, 660 μ).

Table 1. 5 α -reductase activity in infrainfundibular follicular segments

Site	Donors* Sex: ages (years)	5 α -reductase activity (pmol/ min per mg protein) [†]	
		pH 5	pH 7 \ddagger
Ear	M: 67, 70, 73, 76, 77, 78, 79, 81	0.27/0.39	5.68/5.61
Forehead	M: 67, 72, 74, 76, 77, 81 F: 56	0.03/0.09	2.40/2.29
Temple	M: 53, 71, 75, 82, 83	0.22/0.22	3.91/3.86
Nose	M: 37, 61, 76, 89 F: 58, 81, 81	0/0	1.66/1.53
Abdomen	F: 18, 39	0/0	4.10/3.69
Arm	M: 83, 63 F: 77	0/0	1.32/1.17
Breast	F: 29, 29, 31, 34 F: 28, 30, 34, 36	0/0 0/0	0.04/0.02 0.37/0.39
Mean \pm SD activity		0.08 \pm 0.12	2.37 \pm 1.9

*90–150 follicles were pooled for each sample.

[†]Duplicates of each sample were assayed.

\ddagger Paired *t*-test at 95% confidence interval for pH 5 vs. pH 7 < 0.0001.

keratinocytes was observed between 3 and 10 days. 5 α -reductase assay was performed when cells reached approximately 30% confluence (usually within 3 weeks). Control experiments (data not shown) demonstrated no significant differences in 5 α -reductase activity based on method of keratinocyte preparation. A total of eight infrainfundibular and epidermal samples from

various anatomical areas were studied. At pH 7, 5 α -reductase activity was greater in keratinocytes cultured from the infrainfundibulum compared to those obtained from the epidermis in all samples tested (Fig. 2). As a group, activity in follicular infrainfundibular keratinocytes was significantly higher than that observed in epidermal keratinocytes ($P = 0.04$).

Table 2. 5 α -reductase activity in homogenized epidermis

Site	Donors* Sex: ages (years)	5 α -reductase activity (pmol/ min per mg protein) [†]	
		pH 5	pH 7
Ear	M: 62, 69	0/0.15	0/0.02
Forehead	M: 61, 65, 74, 75 F: 40 M: 39, 52, 69, 76, 82, 83	0/0.05 0.65 0/0	0/0.007 0.15 0/0.14
Temple	M: 57, 61, 70 M: 71, 71, 78, 80, 80	0/0 0/0	0/0 0/0
Nose	M: 62, 69, 73	0.9/0.13	0/0.67
Cheek	M: 52, 59 F: 86	0/0 0/0	0/0 0.2/0.8
Mean \pm SD activity		0.11 \pm 0.25	0.12 \pm 0.24

*Epidermal samples were pooled to achieve a minimum of 60 μ g of protein.

[†]Duplicates of each sample were assayed.

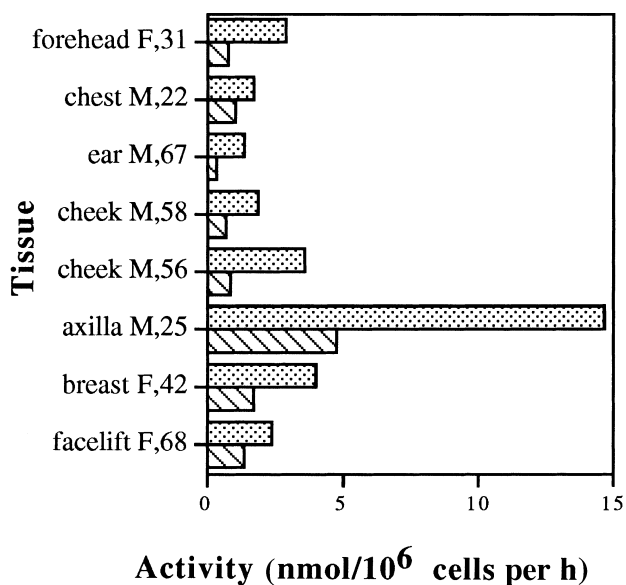


Figure 2. Type 1 5 α -reductase activity in cultured follicular vs. epidermal keratinocytes. Source of the tissue, subject's sex and age (years) are depicted. \square , 5 α -reductase activity in follicular keratinocytes; and \square , 5 α -reductase activity in epidermal keratinocytes.

Profile of 5 α -reductase activity in dissected segments of pilosebaceous follicles of breast skin

5 α -reductase activity in homogenates of sebaceous gland, sebaceous duct, infrainfundibular segments, epidermis and whole skin from pooled samples of female breast skin revealed that there is a gradient of 5 α -reductase activity within the pilosebaceous unit. Activity is greatest in the sebaceous gland, and then progressively declines from the sebaceous duct to the epidermis (Table 3). Activity in full-thickness skin is greater than epidermis, most likely as a result of the contribution from the sebaceous glands present in the whole skin specimens. In general, activity is greatest at pH 7 in the sebaceous gland, sebaceous duct, and infrainfundibulum, suggesting the presence of the type 1 isozyme in these areas. 5 α -reductase activity in the isolated epidermis is too low to assess preferential activity at either pH 5 or 7. In the breast samples tested, the lower follicle (from the level of the sebaceous duct to dermal papillae) exhibited slightly higher activity at pH 5 compared to pH 7 (Table 3), suggesting possible activity of the type 2 isozyme of 5 α -reductase in these areas.

Discussion

Follicular hyperkeratinization is a key aetiological factor in acne. The role of androgens in follicular keratinization

Table 3. 5 α -reductase activity in segments of the pilosebaceous unit of breast skin

Sample	Activity (pmol/min per mg protein)						
	pH	SG	SD	LF	IF	EPI	WS
A*	5	3.2	0.9	0.11	0.03	0.007	ND
	7	37	9	ND	0.17	0.06	0.46
B†	5	NT	0.42	0.45	ND	NT	NT
	7	NT	4.54	0.16	ND	NT	NT

* Sample A represents pooled segments obtained from seven females ages 20–61 years.

† Sample B represents pooled segments obtained from eight females ages 20–53 years.

SG, sebaceous glands; SD, sebaceous duct; LF, lower follicle; IF, infrainfundibulum; EPI, epidermis; WS, whole skin; ND, not detectable; NT, not tested.

has been hypothesized, but not extensively explored. Using immunohistochemistry and biochemical methods, enzymes involved in androgen production such as 3 β -hydroxysteroid dehydrogenase, 17 β -hydroxysteroid dehydrogenase and 5 α -reductase have been identified in epidermis, sebaceous glands and hair follicles.^{13,17,20–22} This study is the first to examine the biochemical activity of the type 1 5 α -reductase in the infrainfundibular region of sebaceous and vellus follicles, the region which is affected by hypercornification in acne vulgaris.

It is extremely time consuming and tedious to dissect follicular segments from skin and this has hampered experimentation in this field. None the less, we managed to isolate a sufficient quantity of follicular segments to assay for 5 α -reductase activity. Our data demonstrate that infrainfundibular segments of sebaceous and vellus follicles have the capacity to produce DHT from T via the type 1 isozyme of 5 α -reductase and that this enzyme activity is greater in the infrainfundibulum than in epidermis. The isolated epidermis had the lowest 5 α -reductase activity compared to each of the other regions within the pilosebaceous unit. Greater activity was noted at pH 7 compared with pH 5 in epidermal homogenates, but the overall activity was too low to make any definitive statements regarding identification of the isozyme(s) of 5 α -reductase in the epidermis.

5 α -reductase activity has been previously studied in cultured epidermal keratinocytes.²⁰ Levels of 0.1–5 nmol/10⁶ cells per h were reported in cultured interfollicular epidermis from female breast, male breast and male thigh skin using a variety of assay conditions.²⁰ We found remarkably similar levels of 5 α -reductase activity in primary cultures of interfollicular

epidermis in our samples (range 0.3 to 5 nmol/10⁶ cells per h). In our study, 5 α -reductase activity (at pH 7) was significantly higher in cultured infrainfundibular keratinocytes and ranged from 1.3 to 14.6 nmol/10⁶ cells per h.

Our studies demonstrate that 5 α -reductase activity varies within different regions of the pilosebaceous unit, with the greatest activity found in the sebaceous gland followed by the sebaceous duct, infrainfundibulum and epidermis. It is known that the sebaceous ductal cells possess morphological features common to both sebocytes and follicular keratinocytes. Accordingly, sebaceous ducts demonstrate levels of 5 α -reductase activity which are intermediate to levels found in the sebaceous gland and infrainfundibulum. Whether the gradient in this enzyme's activity within the pilosebaceous unit exists to metabolize further the testosterone found in sebum or whether it is a function of the intermediary nature of ductal and follicular cells is not known.

These data support the long-standing observation that the pilosebaceous unit represents the major site of sex steroid hormone metabolism within the skin and suggests that androgens may be involved in follicular keratinization. Our preliminary findings provide a rationale for further investigation of the role of androgens in follicular keratinization. For example, it would be of interest to determine if there is a difference in sebaceous follicles of normal vs. acne subjects. From a therapeutic standpoint, pharmacological agents, such as 5 α -reductase inhibitors, which target androgen production within the pilosebaceous unit, could greatly enhance our ability to treat such conditions as acne vulgaris, hirsutism and male-pattern hair loss.

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