Mechanism of Androgen Action in Cultured Dermal Papilla Cells Derived from Human Hair Follicles with Varying Responses to Androgens In Vivo

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Androgens are major regulators of human hair growth, but their effects vary: many follicles are stimulated by androgens, e.g., beard; some remain unaffected, e.g., eyelashes; whereas scalp follicles undergo regression and balding in genetically disposed individuals. Because the dermal papilla controls many aspects of the hair follicle, androgens may act via the dermal papilla, affecting the other follicular components indirectly. In this hypothesis androgens would alter dermal papilla cell production of regulatory substances, e.g., growth factors and/or extracellular matrix components. To test this theory the mechanism of androgen action has been compared in primary lines of dermal papilla cells cultured from androgen-dependent follicles and relatively androgen-independent non-balding scalp. Androgen receptor levels were assayed by saturation analysis (9–10 points; 0.05–10 nmol/l) using the synthetic androgen [3H]-mibolerone and specificity was confirmed by competition studies. Androgen metabolism was investigated both intracellularly and in the media after a 2-h incubation with 5 nM [3H]-testosterone. Carrier and [14C] steroids were added to the extracts before separation by thin-layer chromatography; steroid identity was confirmed by recrystallization.

Dermal papilla cells from androgen-dependent follicles contained higher levels of specific, high-affinity, low-capacity androgen receptors than non-balding scalp cells. Testosterone metabolism also varied with beard, pubic and scalp cells containing testosterone and androstenedione intracellularly, but only beard cells producing 5α-dihydrotestosterone, in line with the scanty beard growth found in 5α-reductase deficiency.

Elsewhere we have shown that cultured dermal papilla cells produce extracellular matrix components and mitogenic factors. These results all concur with our original hypothesis and suggest that further studies of such cells may elucidate the paradoxical effects of androgens on human hair follicles. J Invest Dermatol 98:86S–91S, 1992

Androgens have paradoxically different effects on human hair follicles depending on their body site. They stimulate hair growth in many areas, such as the beard and pubis, have little effect on non-hair follicles such as the eyelashes, but can cause regression and balding on the scalp in genetically disposed individuals (reviewed in [1–3]). Because much of adult human hair growth is involved in sexual and social communication (reviewed in [3]), androgen-potentiated hair conditions such as hirsutism and androgenetic alopecia (male pattern baldness) cause widespread distress [4,5]; however, these are currently poorly controlled because our knowledge of how the complex cell biologic system of the hair follicle functions and the mechanisms by which androgens regulate this are very limited.

THE PARADOXICAL EFFECTS OF ANDROGEN ON HUMAN HAIR GROWTH

The American anatomist, James Hamilton, carried out a range of studies that contributed significantly to our understanding of how androgens affect human hair follicles. He showed that although hair growth in many areas increased rapidly in both Caucasians and Japanese during adolescence, paralleling the rise in plasma androgens [6], it frequently continued to increase with age, with terminal hairs in some areas, such as the male chest and external auditory canal, often appearing in middle-age and even later [7]. He also demonstrated that beard growth was reduced by castration [6] and male pattern balding inhibited [8]. The essential role of androgens in adult human hair growth is demonstrated by the complete testicular feminization syndrome. These XY individuals have normal circulating androgens but lack functional androgen receptors and, therefore, exhibit a generally female phenotype; however, they do not produce any terminal hair at puberty (not even the female pattern of pubic and axillary hair growth), possess plentiful head hair, and exhibit no recession on the scalp [9–11].

Hamilton’s results show that the responses of hair follicles to androgens are complex because there is familial [8] and racial [6] variation as well as the differences related to body site [6]. This indicates that although circulating androgens are essential, their effects are strongly influenced within the target follicle both by inherited genes and the specific gene expression within the follicle’s cells, determined by the follicle’s location; the latter is presumably imposed during embryonic differentiation. The importance of this end-organ response is shown by the stimulation of only pubic and axillary follicles by female levels of androgens, the retention of donor site characteristics in transplanted hair follicles [12], and a case of unilateral hirsutism [13].

Androgens are, therefore, the triggers that cause changes in follicular activity, but they are only able to promote the genetic programming existing within individual follicles. In many areas of the body androgens gradually transform vellus follicles, producing short, fine, non-pigmented hairs into larger terminal follicles forming longer.
thicker, pigmented, and often medullated hairs. On the scalp the
reverse gradual transformation of terminal to vellus follicles occurs
in genetically predisposed individuals, producing recession and
balding. These effects require not only the initial exposure to
androgens but also its continual presence to maintain the follicle’s
response; men castrated after the age of 20 maintain reduced levels
of beard growth [6] and scalp recession [8] rather than returning to
the pre-pubertal state. The gradual response of hair follicles could
suggest that androgens were only able to affect the follicles during
the pre-anagen or early anagen stages of the hair cycle; however,
the recent report by Randall and Ebling [14] of pronounced seasonal
variation in beard and thigh hair growth paralleling the apparent
circannual fluctuation in androgens implies that follicles can re-

c"on to androgens in mid-anagen, at least by growth, though
presumably not by changes in follicular type.

THE MODE OF ACTION OF ANDROGENS IN THE
HUMAN HAIR FOLLICLE

The gradual transformation of hair follicle type in response to
androgens must involve changes in the activity of the follicular epithel-
ial cells and melanocytes and, presumably, also of the dermal pap-
pilla cells because the dermal papilla maintains a constant size ratio
with the follicle and the hair produced [15,16]. This has recently
been discussed in more detail by Randall [1]. In theory, androgens
could act directly on each of these components of the follicle and/or
influence the other cell types indirectly via the dermal papilla.

The mesenchyme-derived dermal papilla found at the base of the
hair follicle has been shown to play a major regulatory role in the
hair follicle by an elegant series of experiments by the British group
led by Oliver and Jahoda, working mainly with the large rat vibrissa
follicle (reviewed in [17,18]); their research has revealed that the
dermal papilla induces new follicular development and strongly
influences the type of follicle and fiber produced, though the spec-
ific biochemical messengers are unknown. The dermal papilla
contains relatively few specialized fibroblast cells surrounded by
the extracellular matrix containing mucopolysaccharides [19], base-
ment membrane components, and interstitial collagen [20,21], all
of which is separated from the epithelial part of the follicle by a
trilaminar basement membrane. These cells may regulate the hair
follicle by secreting growth factors and/or the extracellular matrix
components in view of the constant size ratio between dermal pap-
illa and hair follicle [15,16] and the observations that basement
membrane proteoglycans influence morphogenesis and differentiation
in other tissues [22,23]. Because the dermal papilla has its own
blood supply, it seems a logical target site for any circulating factors
to act.

During the adult hair growth cycle the early stages of anagen
appear to partially repeat stages of the embryogenesis of the hair
follicle (reviewed in [3]): the dermal papilla and epithelial cells
grow down from the base of the telogen follicle to form another
hair bulb and new hair that enters the base of the telogen follicle
alongside the existing hair. In this way, the hair growth cycle rece-
pitates embryogenesis, probably to a greater extent than any other
adult tissue; androgens presumably influence the development of
individual hair follicles during successive growth cycles.

Androgens also determine the development of many mammalian
tissues during embryogenesis, as demonstrated by the female pheno-
type in testicular feminization [9–11]. They are essential for the
formation of the embryonic prostate during which androgen recep-
tors must be present in the mesenchyme-derived stroma, but are
unnecessary in the epithelial cells [24], i.e., during prostate develop-
ment androgens are acting on the epithelial cells indirectly via the
mesenchymal-derived stromal cells.

Taking all this into account, it seems very probable that andro-
gen act on the hair follicle through the dermal papilla, affecting
other follicular components indirectly by altering the production of
regulatory substances such as growth factors and/or extracellular
matrix components, by the cells of the dermal papilla (see Fig 1).

This hypothesis is supported by the autoradiographic localization of
3H-testosterone only in the dermal papillae of rat hair follicles [25].

MECHANISM OF ANDROGEN ACTION IN HUMAN
HAIR FOLLICLES

Steroids released by the endocrine glands circulate in the blood
either in solution or bound to specific plasma carrier proteins such as
sex hormone binding globulin, the predominant carrier of andro-
gen. Steroid hormones are now generally believed to diffuse across
the plasma membrane, through the cytoplasm, and into the nucleus
where they bind to the appropriate specific nuclear receptor. Hor-
mones binding stimulates the receptor to undergo a conformational
change, exposing sites that can then interact with the relevant hor-
mon hormone-response elements in the chromatin, causing the appropriate
changes in DNA transcription and protein synthesis for that particu-
lar hormone and cell type (reviewed in [26]).

In this manner testosterone can bind with the androgen receptor
in target cells, such as skeletal muscle and the embryonic Wolffian
duct, and stimulate androgenic responses (reviewed in [27]) (see Fig
2). However, the situation for androgens is more complex in that
testosterone may be metabolized intracellularly by the enzyme 5α-
reductase to 5α-dihydrotestosterone, which can also bind and activ-
ate the androgen receptor [27] (see Fig 2) and is often discussed,
incorrectly, as if it is the only active intracellular androgen.

The mechanism of androgen action in human hair follicles ap-
ppears to vary with the body site of the follicle. Androgen receptors
are required for any androgen-dependent responses, as shown by the
absence of body hair and scalp recession in the testicular feminiza-
tion syndrome [9–11]. However, hair distribution in men with
5α-reductase deficiency who have relatively normal levels of
plasma testosterone, but cannot convert testosterone to 5α-dihy-
drotestosterone intracellularly, points to specific roles for testoster-
one and 5α-dihydrotestosterone in different follicles. These individ-
uals produced little or no beard growth and do not go bald, but do
form terminal hair in the female pubic pattern and in the axilla
[11,28,29]. This suggests that 5α-dihydrotestosterone is the active
androgen in beard and balding scalp follicles, but is not required for
axillary and pubic growth.

The mechanism of androgen action in skin has been studied ex-
tensively both in whole skin and skin fibroblasts, and has recently
been reviewed by Randall [1]. The relevance of these studies to the

Figure 1. Diagram of a possible model for the mode of action of androgens
on the human hair follicle.

? = Soluble mitogenic substances and/or extracellular matrix components
MECHANISM OF ANDROGEN ACTION IN DERMAL PAPILLA CELLS DERIVED FROM FOLLICLES WITH VARYING RESPONSES TO ANDROGENS IN VIVO

We have investigated the mechanism of androgen action in human dermal papilla cells working on the hypothesis described above (Fig 1). Because androgens have different effects on hair follicles from various sites, we have established primary lines of dermal papilla cells from androgen-sensitive hair follicles, particularly the beard, and control, relatively androgen-independent follicles from non-balding areas of the scalp, and compared their mechanism of androgen action.

**Growth Studies** Initially, the effects of increasing concentrations of androgens on the growth of dermal papilla cells were investigated, because a positive result would indicate the presence of androgen receptors and androgen-responsive genes. However, neither beard nor scalp cells responded to testosterone with altered DNA synthesis as assessed by \(^3\)H-thymidine uptake [40]; to avoid the possibility of metabolism inactivating the testosterone, the experiments were repeated with the synthetic, non-metabolizable androgen, mibolerone, with similar results [40] (Fig 4). This inability to detect a growth response does not mean that the cells are incapable of responding to androgens in a more subtle manner, such as alterations in protein production, but does mean that further investigations into androgen receptor content are required.

**Androgen Receptors** The presence of androgen receptors was investigated using \(^3\)H-mibolerone (Amersham International plc...
UK) in a 9–10-point saturation analysis (0.05–10 nm). Cells were incubated in 100-mm petri dishes [2,41] with and without the addition of 100× excess of unlabeled 5α-dihydrotestosterone to saturate non-specific binding sites after 24 h in serum-free media to remove endogenous hormones; 1000× excess triamcinolone acetonide was added to all incubations to prevent any possible binding to progesterone receptors. The Shinogi 115 androgen-responsive mouse mammary carcinoma line was assayed as a positive control; the results agreed with previous reports [42]. Competition studies with a range of unlabeled steroids showed that the binding was to specific androgen receptors [2,41].

Specific high-affinity, low-capacity androgen receptors with similar characteristics to the classical target tissues were detected in all dermal papilla cells and dermal fibroblasts studied. The levels of androgen receptors, calculated by Bmax from Scatchard plots, in relation to the number, protein, or DNA content of the cells, were significantly higher in dermal papilla cells from androgen-dependent sites, e.g., beard, moustache (0.033 fmol/10⁶ cells; 17 fmol/mg protein; 0.32 fmol/μg DNA) than in non-balding scalp (0.01 fmol/10⁶ cells; 6.0 fmol/mg protein; 0.052 fmol/μg DNA) (p < 0.05; p < 0.02; p < 0.05); levels in cells from female pubic and male scrotum (n = 2) were even higher (0.063 fmol/10⁶ cells; 30.5 fmol/mg protein) (Fig 5). Similar results were obtained with skin fibroblasts cultured from adjacent dermis.

Although the function of androgen receptors in fibroblasts is unclear, higher levels of androgen receptors have been reported in genital than non-genital fibroblasts [43]. The presence of androgen receptors in non-balding scalp follicles might not have been expected unless it is a general characteristic of fibroblast type cells, but, in fact, in some individuals almost all scalp follicles can be lost due to androgenetic alopecia, so the precise definition of scalp follicles as non-responsive is difficult.

**Figure 4.** The presence of increasing concentrations of either testosterone (a) or the non-metabolizable androgen, mibolerone (b), had no significant effect on the rates of ³H-thymidine incorporation in either beard [(a) five cell lines, (b) four] or non-balding scalp [five cell lines] hair follicle dermal papilla cells. Mean ± SEM. Reprinted from [40] with permission of the Journal of Investigative Dermatology.

**Figure 5.** Androgen receptor levels (Bmax) measured by saturation analysis in hair follicle dermal papilla cells from non-balding scalp (four lines), beard (six lines), and genital skin (two lines). Mean ± SEM.

**Metabolism of Androgens** The metabolism of testosterone in cultured dermal papilla cells from beard, pubis, and non-balding scalp follicles was also investigated to determine whether the pattern reflected that anticipated from hair growth in 5α-reductase deficiency. Confluent dermal papilla cells were incubated with 5 nM ³H-testosterone for 2 h after prior incubation in serum-free medium for 24 h. The steroids present both inside the cells and in the media were analyzed separately for each cell type [2,44]. Unlabeled carrier steroids and ³H-carrier steroids were added to each extract prior to separation by thin-layer chromatography; steroid identity was confirmed by re-crystallization.

The cells all took up testosterone intracellularly and metabolized it to androstenedione, but only beard cells contained 5α-dihydrotestosterone; 5α-dihydrotestosterone was also found in the media from beard cells (n = 5) and not in that from pubic (n = 2) or scalp cells (n = 6) [44]. These results agree with a previous report of higher levels of 5α-reductase activity in the media of beard than occipital scalp cells [45]. The ability to form 5α-dihydrotestosterone intracellularly by beard, but not pubic and non-balding scalp, cells closely parallels the distribution of hair growth in 5α-reductase deficiency and supports the importance of the dermal papilla in androgen action in human hair follicles.

**CONCLUDING REMARKS**

Androgens have varying effects on human hair follicles depending on their body site and the individual's genetic tendencies. The mechanisms of androgen action in the hair follicle are not well under-
stood, but presumably involve differential gene expression within the cells of the hair follicles. However, androgens must alter the activity of the follicular epithelial cells, melanocytes, and dermal papilla cells to carry out the gradual transformations of vellus to terminal follicles and vice versa. By analogy with other tissues and bearing in mind the important regulatory role of the dermal papilla, it seems probable that androgens may act on the hair follicle via the dermal papilla.

Our studies on primary lines of human dermal papilla cells have shown that they contain the appropriate specific, saturable androgen receptors to enable them to respond to androgens, and that they are able to metabolize testosterone. Most interestingly, differences were apparent between androgen-sensitive dermal papilla cells and those from the relatively androgen-independent regions of non-balding scalp. Androgen-sensitive cells contained higher levels of androgen receptors than scalp cells and the patterns of androgen metabolism varied with beard, but not pubic and scalp, cells metabolizing testosterone to 5α-dihydrotestosterone intracellularly in line with terminal hair distribution in 5α-reductase deficiency. These results support the importance of the dermal papilla cells in androgen action in human hair follicles. They also show that dermal papilla cells retain an altered gene expression in culture dependent on their androgen response in vivo.

The varying requirement to metabolize androgens to 5α-dihydrotestosterone in hair follicles from different sites is intriguing. Presumably, because androgens are stimulating the transformation of vellus to terminal follicles in many areas although different intracellular hormones are involved, similar genes are being activated within the follicles to produce the appropriate proteins to initiate this response. It is generally believed that there is only one androgen receptor due to the widespread effects in many tissues of its deficiency in testicular feminization and the recent cloning studies (reviewed in [46]). Because both testosterone and 5α-dihydrotestosterone can bind to the androgen receptor, it appears that in some cells the testosterone-receptor complex is not able to regulate genes in the same manner as the 5α-dihydrotestosterone-receptor complex. The reason for this is unclear; it could possibly be a spatial effect of a different shaped complex not being able to bind to the appropriate hormone response element(s) in the chromatin or that appropriate regulatory transcription factors are also only able to bind to the hormone response element when the 5α-dihydrotestosterone-receptor complex is present. Further studies on cultured dermal papilla cells from sites with different requirements for 5α-dihydrotestosterone could clarify the mechanism of androgen action further. Indeed, such cells offer good potential models for androgen action in general because androgen-responsive and control cell lines can be obtained relatively non-invasively from normal people. Such studies could be particularly relevant to understanding the development of prostate-dependent cancer, as the hair follicle has parallels to these processes as discussed elsewhere [1–3].

Although this symposium is concerned with receptors and intracellular signaling, the hypothesis under investigation eventually requires extracellular paracrine signals to be produced as a result of the receptor stimulation. We have shown elsewhere that cultured dermal papilla cells produce extracellular matrix components [21], secrete, and respond to, mitogenic factors [2], and that beard cells produce an androgen-regulated mitogenic factor or factors [47]. All these results are consistent with the hypothesis that androgens influence the hair follicle via the dermal papilla cells (Fig 1) by altering their production of unknown regulatory substances, which may include growth factors and/or extracellular matrix components. Despite the difficulties of obtaining and isolating dermal papillae and the slow growth and short-lived nature of the dermal papilla cells, they appear to offer a good model system for studies of the mechanism of androgen action in human hair follicles. Further investigations may elucidate the intriguing biological paradox of how androgens can stimulate some hair follicles while simultaneously causing recession elsewhere in the same individual.

REFERENCES

22. Bernfeld M, Banerjee SD, Koda JE, Rapaeger AC: Remodelling of
41. Randall VA, Thornton MJ, Messenger AG: Cultured dermal papilla cells from androgen-dependent human follicles (eg beard) contain more androgen receptors than those from non-balding areas. J Endocrinol (in press)