Hormones and Hair Growth: Variations in Androgen Receptor Content of Dermal Papilla Cells Cultured from Human and Red Deer (*Cervus Elaphus*) Hair Follicles

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Many hair follicles produce different types of hair in response to environmental changes or the mammals' age, that are translated to the follicle by hormones. Androgens cause many changes, such as transforming vellus follicles producing insignificant hairs on the face to terminal beard ones at puberty or the reverse on the scalp. In male red deer, the breeding season rise in androgens causes the annual production of a mane on the neck that is lost during the spring.

Because the dermal papilla situated at the base of the hair follicle is important in determining the type of hair produced, androgens may act via the dermal papilla. Therefore, primary cell lines of dermal papilla cells from human and red deer follicles with different responses to androgens have been established. Specific saturable androgen receptors were present in all human papilla cells examined, with higher levels in cells from androgen-dependent follicles, e.g., beard than in control, non-balding scalp cells. In preliminary investigations of red deer, androgen receptors were only present in cells derived from mane follicles and were undetectable in flank or spring neck follicles.

These similar results from both species support the hypothesis that androgens are acting on hair follicles via the dermal papilla. They also suggest that dermal papilla cells are potentially useful models for investigating the mechanism of androgen action because cultured cells appear to retain differences that relate to the androgen responsiveness of their parent follicle. The red deer seems particularly interesting in view of the much shorter hair-growth cycle than human scalp or beard follicles. *J Invest Dermatol* 101:114S–120S, 1993

Hair follicles often produce different types of hairs at various stages in a mammal's life; the hairs formed may change quite significantly in color, thickness, and length, as can be readily seen by comparing the facial hair of a man to those he had as a child. This transforming ability of the hair follicle allows many mammals to adjust to seasonal changes in the weather by increasing or decreasing the thermal insulating properties of their coat or, like some arctic mammals, altering the color to retain camouflage when the countryside colors change (reviewed in [1]). It also permits changes in hair types during the lifecycle of a mammal, which allow rapid distinction between young animals and adults and between the adult sexes. This can be readily seen in lions, where the young are speckled for camouflage and the adult males have a very obvious mane, but also occurs in human beings. Children have relatively little terminal hair (i.e., pigmented, relatively long and thick) except for that with protective roles on the scalp, eyebrows, and eyelashes, whereas both sexes develop axillary and pubic hair during adolescence. Sexually mature men are also readily identified by the characteristic hair of the beard, chest, and suprapubic regions. This major role of human hair growth in social and sexual communication explains why hair disorders such as hirsutism, androgenetic alopecia, and alopecia areata, though not in themselves life-threatening, often create psychologic problems (reviewed in [2], [3,4]).

To enable these changes to occur, hair follicles pass through regular cycles of hair development and growth (anagen) followed by periods of resting (telogen) ([5,6] (see Fig 1). The hair produced may be very similar to the previous one, as seen in many hair follicle cycles on the eyelids and the young human scalp, it may differ slightly or even be quite markedly altered, such as those in the neck region of the adult male red deer (*Cervus elaphus*), which produces a mane every year only during the breeding season [7]. The precise mechanisms of how alterations occur in the type of hair produced by a hair follicle are unknown. Further studies of hair follicle biology are needed to elucidate this problem so that better treatments can be developed to regulate the hair follicle disorders and increase hair production by domesticated animals such as sheep. Our approach is to study the hair follicular responses to hormones in both human and red deer follicles. In particular, the role of androgens in the control of hair growth is being investigated (reviewed in [2,8–10]), as androgens dramatically modulate the type of hair produced by hair follicles in both species.

**REGULATION OF HAIR-GROWTH CYCLES**

Seasonal coat changes occur twice a year in many mammals to accommodate alterations in the environment [11] (reviewed in [1]).

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The hair growth cycles in these species are synchronized and, generally, waves of new hair growth and molting of the previous season's hair type pass over the body (e.g., [12]); however, in some species, such as the guinea pig, molting is more diffuse and does not follow such a sequential pattern [13]. These molts are linked to climatic variation, particularly fluctuations in the length of daylight, but also of temperature. In addition, the type of new hair produced is affected by the availability of the food supply, presumably because of the large metabolic requirements involved in making new hairs [11]. Because these parameters are also important for the timing of seasonal reproduction, there is a correlation between the molting and reproductive cycles in many mammals, e.g., red deer [7].

Changes in the environment are interpreted for the hair follicle by the endocrine system. In many species this has been shown to involve melatonin from the pineal plus the hormones of the hypothalamic-pituitary system, which generally act indirectly at the level of the hair follicle via the gonadal, thyroid, and, possibly, corticosteroid hormones. Melanocyte-stimulating hormone (MSH) and prolactin are pituitary hormones that have been ascribed direct actions on hair follicles (reviewed in [1,2]). Despite exposure to the same circulating hormones, the reactions of follicles depend on the body site, varying from no effect on the eyelashes, to stimulation in many areas [28], and regression and balding on the scalp in genetically disposed individuals [29]. The gradual responses of these follicles to androgens, taking many years to have full effects [30], parallel the rat experiments with hormones eventually overriding the internal follicular cycle [26,27], but the situation in human beings is more complex; not only do human follicles vary in their reactions to androgens but they retain their endogenous responses when transplanted, this being the basis of corrective transplants for androgenetic alopecia [31]. Presumably, this characteristic response of each hair follicle to androgens is determined by alterations in gene expression in the cells of individual hair follicles during embryogenesis imposed by as-yet unknown factors. This makes the hair follicle a potentially interesting tissue to scientists from a range of disciplines.

In the red deer the neck follicles exhibit an intrinsic ability to react to male sex hormones, in contrast to other body follicles. However, the result differs markedly from the situation in humans because a quite dramatic enlargement occurs in one cycle and the follicle then produces a much smaller hair in the next cycle with the fall in testosterone during the non-breeding season.

HOW DO ANDROGENS REGULATE HAIR FOLLICLES?

Androgens are very obvious regulators of hair growth, as reviewed in [1,2,8-10,14,15]. In humans they stimulate the gradual transformation of small vellus follicles, making non-pigmented, fine, short hairs, in many areas to larger terminal follicles producing pigmented, thicker, longer hairs, as illustrated in Figure 2. This appears to occur more rapidly in other species, such as in the manes of the deer and lion, but these follicles are already producing terminal hairs as part of their normal coat. In the untransplanted producing a terminal follicle, an androgen-potentiated gradual regression of terminal hair follicles to vellus ones may occur on the human scalp [8] and has also been described on the scalp of both sexes of the stump-tailed macaque [32]. This appears to involve the opposite responses (see Fig 2). Because these paradoxical reactions are clearly end-organ responses to the same hormones, the key to understanding them and, hopefully, therefore further elucidating the control of the hair growth cycle, must lie within the hair follicles themselves.

To cause these modifications, androgens must alter, either directly or indirectly, the activity of many components of the hair follicle, in particular the melanocytes, and epithelial and dermal papilla cells. Randall's hypothesis for some years now [2,8-10]
combine with the receptors, which then undergo a change in configuration, revealing sites that can bind the specific hormone-response elements in the chromatin. Binding of the hormone-receptor complexes to these sites stimulates alterations in RNA production, processing, and translation, leading ultimately to changes in protein synthesis and modification that depend on the original specific gene expression in that cell type (reviewed in [39]). In other words, androgen-receptor complexes might stimulate the production of protein X in beard hair follicle cells although inhibiting it in scalp cells. More details of the mechanism of androgen action in hair follicles have recently been given elsewhere [2, 8–10].

The relevance of the general model for androgen action to androgen-dependent hair growth can readily be seen in people with testicular feminization. These genetic men are phenotypically female despite relatively normal androgen levels because they lack functional androgen receptors. They do not produce any androgen-dependent hair growth, not even the female axillary and pubic patterns, and do not go bald [40], providing very good evidence for the role of androgens in human hair growth. Recently, androgen receptors have been localized only in the dermal papillae of human hair follicles using a specific monoclonal antibody to the androgen receptor [41]. This gives strong support to our hypothesis that androgens act via the dermal papilla.

**INVESTIGATIONS INTO ANDROGEN ACTION IN HUMAN AND RED DEER FOLLICLE DERMAL PAPILLA CELLS**

**Experimental Design** Based on this hypothesis, we are trying to understand how androgens affect hair follicles by investigating the mechanism of androgen action in cultured dermal papilla cells from human and red deer hair follicles. The main thesis behind our approach is that comparisons of the mode of androgen action in cells from follicles that are androgen-dependent, such as human beard or red deer mane, with those from control follicles, such as non-balding scalp, deer flank or non-breeding-season deer neck follicles, should reveal important information about the androgenic control of hair follicles.

Dermal papilla cells seem a particularly useful model system for studying hair follicles not only because of the important functions of the dermal papilla and its putative role in androgen action, as discussed above, but also because they are relatively easy to isolate as a distinct population and culture and because rat and human cells have been shown to retain the ability to induce hair growth after culture (reviewed in [36]).

**Characteristics of Cultured Dermal Papilla Cells** Dermal papilla cells are a specialized type of fibroblast with distinct properties in culture [42, 43]. Confluent fibroblasts normally show an elongated spindle shape and grow in characteristic arrays on plastic dishes, whereas dermal papilla cells from both species maintain a more spread-out morphology and aggregate into little groups, frequently lying partially on top of each other and leaving areas of the dish surface uncovered [9]. Human dermal papilla cells grow much more slowly than dermal fibroblasts and the cultures are normally much more short-lived [43]. Our preliminary studies on red deer dermal papilla cells have shown that they differ from the human pattern and grow faster than deer dermal fibroblasts [1]; this could be related to the shorter anagen of red deer follicles or the much younger age of the red deer at adulthood.

**The Growth of Dermal Papilla Cells in the Presence of Androgens** Because the measurement of cell growth is much easier than the assay of androgen receptors, we first investigated the effects of androgens on the growth of beard and non-balding scalp dermal papilla cells. An alteration in growth rates would have indicated the

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activation of both androgen receptors and a range of androgen-responsive genes. Unfortunately, neither cell type showed significant changes in growth rates as assessed by [3H]-thymidine uptake over a range of concentrations of either testosterone or the non-metabolized androgen mibolerone [33]. As the lack of growth in response to androgens does not mean that the cells are unable to respond to androgens in a more delicate manner than the gross response of alterations in cell growth, androgen receptor content was measured in dermal papilla cells from both species.

MATERIALS AND METHODS

Culture of Dermal Papilla Cells In both species dermal papilla cells were derived from full-thickness skin samples by microdissecting the dermal papilla from the lower part of the follicle. The dermal papillae of the androgen-activated hair follicles were larger in both species than those from the control follicles [8] and unpublished observations). Dermal papillae explants were transferred to small, sterile, culture-treated, plastic Petri dishes and cultured in medium E199 supplemented with 20% fetal bovine serum as previously described [42]. All experiments described in this paper have been carried out on individual primary cell lines at passages between 3 and 6, inclusive.

Measurement of Androgen Receptor Content The binding of the synthetic non-metabolizable androgen [3H]-mibolerone (7α, 17α-dimethyl-19-nortestosterone, specific activity 2.74-3.33 TBq/mmol) (Amersham International plc) was measured in dermal papilla cells from both species cultured until almost confluent in 100-mm Petri dishes in normal medium; they were then grown in medium without serum for 24 h prior to the assay to reduce intra-cellular androgens. A saturation analysis was carried out over the range 0.05-10 nM [3H]-mibolerone involving nine or ten points in each of the 12 primary cell lines investigated; non-specific binding was assessed in parallel incubations in the presence of 100 X excess 5α-dihydrotestosterone to saturate any non-specific binding sites and 1000 X excess triamcinolone acetonide was included in all incubations to counter any possible binding of [3H]-mibolerone to progestosterone receptors. After incubation for 2 h at 37°C, the medium was removed and retained for counting to estimate the amount of unbound radioactivity, i.e., "free" radioactivity for Scatchard analysis. The cells were then washed 4 X with cold phosphate-buffered saline to remove any radioactivity loosely associated with the cell surfaces and removed by scraping into cold phosphate-buffered saline before separation by centrifugation. The cell pellets were resuspended in chloroform-methanol (1:1, v:v) to extract the radioactivity and, after centrifuging out the cellular residue, the radioactivity in each supernatant was measured on an LKB scintillation spectrometer with a counting efficiency of 50%. Scatchard plots were constructed for each cell line to enable the calculation of the affinity (Kd) of the receptors for [3H]-mibolerone and the concentration of androgen receptors (i.e., Bmax) [34]. The androgen-responsive mouse mammary carcinoma cell line, Shinogi 115, was assayed as a positive control [34].

The ability of various steroids to inhibit the binding of 1 nM [3H]-mibolerone to the dermal papilla cells was assessed by incubating the cells in duplicate or triplicate with a range of unlabeled steroids at 100 nM using the same methodology.

RESULTS

Human Studies The androgen receptor assays demonstrated the presence of specific, high-affinity, low-capacity androgen receptors in cultured dermal papilla cells from both androgen-dependent and non-balding scalp hair follicles with properties similar to those of classical androgen target tissues. Binding was inhibited by around 50% by the androgens testosterone, 5α-dihydrotestosterone, and mibolerone, in both beard and scalp cells. The antiandrogen cyproterone acetate and estradiol also caused reductions but progesterone, triamcinolone acetonide (with progestational and corticosteroidal binding), and cortisol had little effect (Fig 3). The same pattern of specificity was seen in dermal papilla cells devised from beard or non-balding scalp follicles. These results follow the normal pattern for competition studies of androgen receptors and demonstrate the presence of specific androgen receptors.

Cells from beard, moustache, pubis, and scrotum (Kd = 0.22 nmol/l; n = 8) and also Shinogi 115 cells had a similar affinity for [3H]-mibolerone, but that of non-balding scalp cells was, rather surprisingly, even greater (Kd 0.081 nmol/l; n = 4) [34]. Androgen receptor content was calculated in relation to cell number, protein, and DNA content, but gave similar values by all three methods [9,34]. The receptor concentration in androgen-dependent cells was significantly higher (p < 0.05) than that of non-balding scalp cells (Fig 4); in six different cell lines of beard and moustache cells the Bmax was 0.033 fmol/10⁶ cells, 17 fmol/mg protein, and 0.32 fmol/μg DNA, whereas that of four scalp cell lines was only 0.01 fmol/10⁶, 6.0 fmol/mg protein, and 0.052 fmol/μg DNA. Cells from female pubis and male scrotum contained even higher levels (0.063 fmol/10⁶ cells, 30.5 fmol/mg protein, and 0.32 fmol/μg DNA). The binding affinity [34] and receptor content (Fig 4) were related to passage number to see if there were any significant trends such as a fall off in receptor content with increasing passaging, as has been detected in the inductive capacity of rat dermal papilla cells [44]. No significant changes were detected in relation to the passage number with either binding affinity or receptor content, but the actual range investigated was small, ranging from P3 to P6; earlier passage cells are difficult to obtain in the numbers necessary for a full androgen receptor assay.

Red Deer Investigations Primary cell lines have been established from large autumn breeding season neck, i.e., mane follicles, from the smaller spring neck follicles, and from the flank in both seasons. In our preliminary investigations saturable androgen receptors have been detected in cells derived from male mane follicles (n = 2), but not in cells derived from the corresponding non-breeding-season neck follicles (Fig 5). Little or no specific binding was seen in cells obtained from flank hair follicles in the breeding season (Fig 5). These are very interesting observations, which merit further study.

DISCUSSION

Cultured Dermal Papilla Cells from Human and Deer Hair Follicles: A Model System for Studying Androgen Action in Hair Follicles The findings of significant levels of androgen receptors in cultured dermal papilla cells are in accordance with our
culture suggest that the presence of androgen receptors, in these
direct correlation to their androgen responsiveness at the time of
in androgen receptor content of cells from deer neck follicles in
advantages should outweigh the quite major drawbacks of obtain-
ing the initial full-depth skin material in both species and the slow-
growing, short-lived nature of the cells produced.

This makes both human and red deer dermal papilla cells appear to
contain more androgen receptors than those from non-balding areas of scalp.

Nevertheless, we have already detected significant differences in
many aspects of the mechanisms of androgen action in human der-
mal papilla cells from beard, pubis, and scalp follicles, as reported
elsewhere [8,9]. This includes a greater ability of beard cells to form
5α-dihydrotestosterone [45], believed to be the active intracellular
androge in some hair follicles, a longer mitogenic response to me-
dium conditioned by other dermal papilla cells, and the production
only by beard cells of an as-yet unknown factor that stimulates the
growth only of beard dermal papilla cells [46]. All of these differ-
ences support the hypothesis that androgens are acting through the
dermal papilla in hair follicles, though of course they do not exclude
actions on other follicular cell types. The differences in both human
and red deer between dermal papilla cells from anogen-dependent
and anogen-independent follicles suggest that alterations in gene
expression, presumably determined by their location on the body
and related to their anogen responsiveness, are retained in culture.
This makes both human and red deer dermal papilla cells appear to
be good model systems for further studies of androgen action. These
advantages should outweigh the quite major drawbacks of obtain-
ing the initial full-depth skin material in both species and the slow-
growing, short-lived nature of the cells produced.

Despite the current preliminary nature of the results, the changes
in androgen receptor content of cells from deer neck follicles in
direct correlation to their anogen responsiveness at the time of
culture suggest that the presence of androgen receptors, in these
cells at least, requires circulating levels of androgens. In other
words, androgens appear to be inducing the dermal papilla cells to
alter their gene expression and induce androgen receptors so that
they can respond to androgens. Because androgen receptors are
present only in cells from follicles producing large hairs as a result of
androgen stimulation, it seems probable that the alteration of an-
drogen receptor gene expression has taken place either prior to, or
during, early anagen; very interestingly, these alterations are main-
tained during anagen and retained when these cells are cultured. If
androge have to cause alterations in gene expression before or

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**Figure 4.** Differences in the concentration of androgen receptors in cult-
tured human dermal papilla cells derived from scalp, beard, and genital skin
hair follicles. Androgen receptor content was measured by assaying the
binding capacity of confluent 10-mm dishes of primary cell lines over a
range of concentration of [3H]-mibolerone (0.05-10 nM; 9 or 10 points)
after 2-h incubation at 37°C. Non-specific binding was assessed by parallel
incubations in the presence of 100 X excess 5α-dihydrotestosterone and
1000 X excess of triamcinolone acetonide was added to all dishes to prevent
binding to any progesterone receptors. The cells had been incubated for 24 h
previously in serum-free medium to reduce endogenous androgens. The
specific binding (i.e., the difference between total and non-specific binding)
is that due to binding to the low-capacity, high-affinity receptors; it was
equivalent to about 175 cpm at each point between 1 and 10 nM [3H]-mibo-
lerone. P, passage number. Reprinted by permission of Blackwell Publica-
tions Ltd, from Randall VA, Thornton MJ, Messenger AG: Cultured dermal
papilla cells from androgen-dependent human hair follicles (e.g. beard) con-
tain more androgen receptors than those from non-balding areas of scalp.
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**Figure 5.** Variations in androgen receptor content in cultured dermal pa-
pilla cells derived from mane and flank follicles of a red deer stag at different
stages of the year. Androgen receptor measurements were carried out as
described in the legend of Fig 4. Cells were derived from mane (a) and flank
(b) follicles in the autumnal breeding season and from the neck region of the
same animal in the spring when no mane was present (c). Solid circles, total
binding of [3H]-mibolerone: open circles, non-specific binding: dotted line,
specific binding.
very early in the hair cycle, this could account for the gradual changes seen in human hair follicles in response to androgens (Fig 2) because anagen in many areas, including the scalp and face, is very long. Nevertheless, if the seasonal fluctuations in beard and thigh growth [24] are related to androgens, as seems likely, this means that at least growth rate is able to be altered during the cycle. On the other hand, the biannual fluctuation in anagen in the thigh appeared independent of whatever was controlling the growth rate. The length of anagen has been suggested to play a major role in the response of hair follicles to androgen [47] and it is possible that this may be controlled in a different way from the rate of growth. The complex actions of hormones, in general, and androgens, in particular, on hair follicles are still very little understood.

Further studies on the models described here should hopefully yield important information about how androgens affect hair growth. Beard or mane, scalp, or spring neck/flank follicles offer the possibility of comparing androgen-potentiated and relatively independent tissues. The red deer is particularly promising, as it offers androgen-dependent hair follicles with much shorter hair-growth cycles. Our human studies have focussed on androgen-potentiated follicles rather than balding follicles on the basis that positive changes are probably easier to detect than inhibitory ones; however, it is probable that information from such studies should be relevant to androgen inhibition of hair growth and should lead to better control of hair disorders in general, whether androgen-related or not. In addition, the relatively accessible hair follicle has strong parallels to other androgen targets, such as the prostate (discussed in [2,8,10]); further studies may well provide greater understanding of androgen action in human tissues, which would be valuable in understanding hormone-related cancer.

This paper is dedicated to the late Professor F. John G. Ebling, without whose classic research and personal training of one of us (VAR) this work may well not have been carried out.

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ANDROGEN RECEPTORS IN HUMAN AND DEER HAIR CELLS


