In Vitro Main Pathways of Steroid Action in Cultured Hair Follicle Cells: Vascular Approach

Souâd Lachgar,*† Marie Charveron,* Jérôme Sarraute,† Michel Mourard,† Yvon Gall* and Jean-Louis Bonafe†

*Laboratoire de Biologie Cellulaire Cutanée, Institut de Recherche Pierre Fabre, Faculté de Médecine Rangueil, Toulouse, France; †Service de Dermatologie, C.H.U. Rangueil, Toulouse, France

The known role of steroids on the hair follicle leads us to investigate their effects on hair follicle cell angiogenic responses *in vitro*. We verified, using the immunohistochemical technique, whether human occipital scalp follicle cells express steroid receptors *in vitro*. We showed that androgen and estrogen receptors were expressed by dermal papilla cells (DPC) and keratinocytes from the outer root sheath *in vitro*. With regard to steroidal enzymes (type I and II 5α-reductases and Cytochrome-p-450-aromatase), the type I 5α-reductase gene is much more expressed in DPC than in dermal fibroblasts; however, the type II 5α-reductase gene is transcribed more in dermal fibroblasts than in DPC. The transcription of the two 5α-reductase isoform genes in cultured DPC is regulated by a 5α-reductase inhibitor. We also demonstrated that DPC, dermal fibroblasts, and outer root sheath keratinocytes expressed cytochrome-p-450-aromatase. Using ELISA and reverse transcriptase-polymerase chain reaction, we investigated the role played by some steroids (estrogens, androgens, anti-androgens) in the modulation of vascular endothelial growth factor (VEGF) expression by DPC. The association of different treatments of DPC (5α-reductase inhibitor and androgen receptor antagonist) shows a great stimulation of VEGF and aromatase expression. Strong stimulation of VEGF protein and gene expression is observed in the presence of 17β-estradiol. Also, the concentration-dependent inhibition of VEGF expression by DPC using the cytochrome-p-450-aromatase inhibitor, confirms the involvement of this estrogenic pathway in the regulation of VEGF expression *in vitro*. *Key words: VEGF/dermal papilla cells/5α-reductase/aromatase/17β-estradiol/finasteride. Journal of Investigative Dermatology Symposium Proceedings 4:290–295, 1999*

The hair dermal papilla, with its own blood supply, typical steroid receptors, and steroid-converting enzyme metabolism, appears to be a target for many circulating factors such as androgens, estrogens, and growth factors.

Androgens seem to act on hair follicles via dermal papilla cells, presumably by affecting the transcription of regulating factors by these cells. Indeed, many studies (Itami *et al*, 1991; Randall, 1994) have shown that dermal papilla cells (DPC) from hair scalp present high 5α-reductase (5αR) activities. These activities were higher than those in dermal fibroblasts. Moreover, only DPC from an androgen-dependent area (e.g., the scalp) have a great number of specific and saturable androgen receptors (Randall *et al*, 1991).

It is now widely accepted that hair growth regulation processes are related to vascular and hormonal components. In human androgenetic alopecia, elevated androgen levels seem to be responsible for follicular regression (Hamilton, 1942), and anti-androgen treatment is able to prevent it (Rittmaster *et al*, 1987). Estrogens also influence hair growth; e.g., topical estradiol is successfully used as a treatment of telogen effluvium in humans. It presumably acts by prolonging the duration of anagen (Orfanos and Vogel, 1980).

It is also known that the anagen growth stage of the hair is characterized by a high degree of vascularization (Parakkal, 1990).

We were interested in studying both hormonal and vascular components to determine the relationship between steroids and hair cell angiogenic responses. As a marker of angiogenesis we chose vascular endothelial growth factor (VEGF) (Folkman and Klagsbrun, 1987; Neufeld *et al*, 1994; Plate *et al*, 1994; Folkman, 1995). This was used for several reasons. It is regarded as the most important positive regulator of angiogenesis (Leung *et al*, 1989; Ferrara *et al*, 1992), vasodilation (Folkman and Shing, 1992), and vascular permeability (Keck *et al*, 1989) and it is mitogenic for vascular endothelial cells (Gospodarowicz *et al*, 1989; Plouet *et al*, 1989).

In the hair follicle, this factor is highly expressed by DPC both *in vivo* (Lachgar *et al*, 1996b) and *in vitro* (Lachgar *et al*, 1998). VEGF is autocrine regulated in the same cells *in vitro* (Lachgar *et al*, 1996a). A hair cycle-dependent change in the expression of VEGF protein and mRNA is observed with a strong expression of this factor by DPC at the anagen stage (Lachgar *et al*, 1996b), and VEGF has the property of not being expressed in hair follicles from androgenic alopecia (Goldman *et al*, 1995).

To further understand the role of estrogens, 5α-reductase inhibitors, and androgen receptors antagonists in hair follicle cell angiogenic responses, we have firstly verified in our experimental conditions the pattern of distribution of estrogen receptors *in vivo* and *in vitro* and the gene transcription of the two 5α-reductase

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Reprint requests to: Dr. S. Lachgar, Laboratoire de Biologie Cellulaire Cutanée, Institut de Recherche Pierre Fabre, Faculté de Médecine Rangueil, Toulouse, France. Email: souad.lachgar@pierre-fabre.com

Abbreviations: DF, dermal fibroblast; DPC, dermal papilla cell; VEGF, vascular endothelial growth factor.

isoforms (5αR1, 5αR2) and cytochrome-p-450-aromatase. As an *in vitro* model, we used cultured human hair DPC from the occipital region of scalp and compared them with other follicular cells: dermal fibroblasts and keratinocytes from the outer root sheath. Then, we investigated the relationship between these steroids (estrogens, androgens, 5α-reductase inhibitor, and androgen receptors antagonist) and the expression VEGF. In parallel, steroid action was evaluated on aromatase expression. This was done using ELISA and reverse transcriptase-polymerase chain reaction (RT-PCR) techniques for determining VEGF and aromatase expression in cultured human hair dermal papilla cells.

MATERIALS AND METHODS

Immunohistochemistry We used a mouse monoclonal antibody specifically directed against human androgen receptors or human estrogen receptors.

Frozen human scalp hair follicle cut in 5 μm sections or cultured hair follicle cells were fixed in acetone for 5 min, then incubated with monoclonal antiandrogen or antiestrogen receptor antibodies in phosphate-buffered saline buffer for 45 min. After rinsing the slides, antimouse IgG biotinalyted antibody was added for 10 min. Then streptavidin-fluorescein complex was incubated for 1 h. The slides were rinsed and mounted with Mowiol mounting medium.

Immunoenzymatic assay Human hair DPC from the occipital region of the scalp (P2) were plated on a 6 well plate at a density of 1×10^6 cells per well, and incubated for 24 h in serum-free medium containing different agents. VEGF protein production was measured in the cell supernatants and extracts using the ELISA method. All assays were done in triplicate for each agent.

RT-PCR analysis DPC were plated on a 25 cm^2 flask at a density of 1×10^6 cells per flask and incubated in serum-free medium.

Testosterone (0.1, 1, 10, and 100 nM), dihydrotestosterone (0.1, 1, 10, and 100 nM), Finasteride (6.5; 13 and 26 nM), cyproterone acetate (100 nM), 17β-estradiol (0.02; 0.36 and 1.8 μM), aromatase inhibitor (4-androsten-4-ol-3,17-dione) (2.5; 5 and 10 μg per ml), and progesterone (100 nM) were tested on DPC cultures.

Total RNA (1 μg) from cultured DPC or dermal fibroblasts (DF) or keratinocytes, was reverse transcribed, at 48°C for 60 min, into cDNA using Access RT-PCR System (Promega, France) in a 50 μl volume.

Polymerase reactions were performed using oligonucleotides complementary to the 5′ and 3′ ends of the coding sequence of 5αR1 (CTGCTCTCTTGGGTGCACTGC and CACCGCCTTGGCTTGTCACAT), 5αR2 (TGCACTGGAAATGGAGTCC and AAGCCACCTTGTGTGGAATCC), Cytochrome p-450 aromatase sequence (TTGTTGTTAAATATGATGCC and ATACCAGGTCCTGGCTACTG), and VEGF (CTGCTCTCTTGGGTGCACTGC and CACCGCCTTGGCTTGTCACAT).

Amplification was performed for 40 cycles of 94°C for 1 min, 50°C for 1.5 min, 72°C for 2 min (5αR1); 40 cycles of 94°C for 5 min, 50°C for 1.5 min, 68°C for 2 min (5αR2); 35 cycles of 93°C for 1 min, 42°C for 1 min, 72°C for 1 min (aromatase); and 30 cycles of 94°C for 40 s, 57°C for 1 min, 72°C for 1.5 min (VEGF) in a DNA thermal cycler heat block (Perkin Elmer, France, Gene Amp PCR system 2400).

Because of the high sequence homology between 5αR1 mRNA and its pseudogene, we used the *Mval* site restriction, present only in the mRNA sequence, to separate 5αR1 from its pseudogene.

5αR1 polymerase chain reactions (300 bp) were digested for 2 h at 37°C by the *Mval* restriction enzyme, which specifically cleaves the 5αR1 cDNA gene into two fragments (200 and 100 bp). The polymerase chain reaction and digestion products for 5αR1 were separated by 2% agarose gel electrophoresis.

RESULTS

Steroid receptor expression Firstly, by using an immunohistochemical technique, we compared the presence of estrogen receptors in skin from the occipital region of the scalp, and from human breast. Estrogen receptors were seen to be present in high numbers in scalp skin (**Fig 1**).

At the anagen stage, strong labeling of estrogen receptors was observed in dermal papilla and keratinocytes from inner and outer sheaths (**Fig 2**).

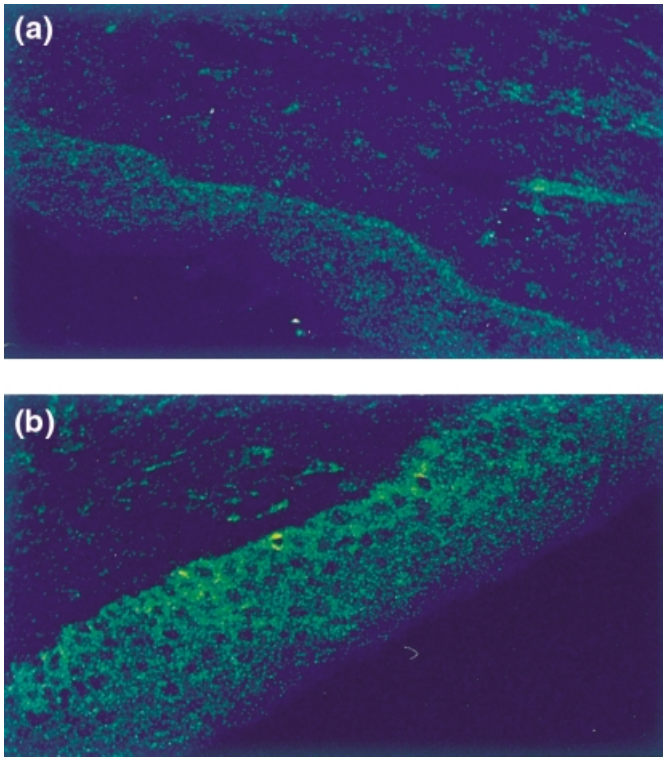

Figure 1. Immunolocalization of estrogen receptors in interfollicular occipital scalp skin and in normal breast skin. (*a*) Normal breast skin; (*b*) interfollicular occipital scalp skin. Note the strong immunostaining of epidermis in scalp skin (magnification, ×825).

We showed strong expression of estrogen and androgen receptors by follicular cells *in vitro* (**Fig 3**). The expression by outer root shealth keratinocytes was greater than that by dermal papilla fibroblasts.

Steroid enzyme expression According to our RT-PCR analysis of steroid-converting enzyme genes, both DPC and DF from occipital scalp hair expressed type I (**Fig 4**) and II (**Fig 5**) 5α-reductase cDNA. The type I 5α-reductase gene was expressed much more in DPC than in DF; however, the type II gene was transcribed more in DF than in DPC.

A semiquantitative study clearly shows a variation of the two isoforms (**Fig 6**). The comparison of the expression of 5αR isoenzymes related to β_2-microglobulin in each cell type showed that 5αR1 gene transcription in DPC was 1.5-fold greater than in DF; however, the 5αR2 gene was 1.6-fold more transcribed in DF than in DPC (**Fig 6**). The ratio (5αR1/5αR2) was 1.8 for DF and 3.8 for DPC.

The transcription of the type I 5α-reductase isoform gene is affected by a 5α-reductase inhibitor: finasteride. DPC treated with different concentrations of Finasteride showed a high concentration-dependent inhibition of 5αR1 (**Fig 7**).

As regards the results of RT-PCR studies on aromatase gene expression in some cultured hair follicle cells, we demonstrated that hair follicle fibroblasts and keratinocytes expressed cytochrome-p-450-aromatase. There was an increase in aromatase gene expression after treatment with progesterone (**Fig 8**).

Steroids and VEGF protein production The presence of these steroidal enzymes and receptors led us to choose dermal papilla fibroblasts as a model to evaluate and compare the effect of different steroids on VEGF gene and protein expression. The effect of some steroids was also tested on the expression of the aromatase gene.

We demonstrated that neither testosterone nor dihydrotestosterone caused a significant stimulation of VEGF synthesis by cultured dermal papilla cells.

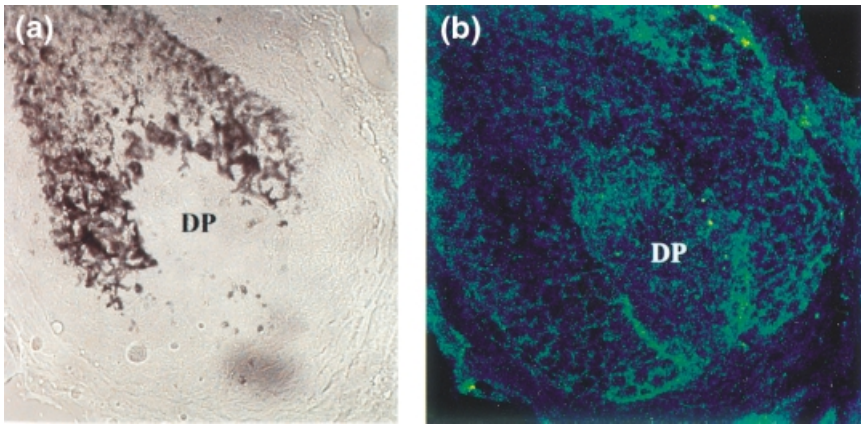

Figure 2. Immunolocalization of estrogen receptors in hair follicle from the anagen stage. Note the strong immunostaining of dermal papilla (DP) (magnification, ×523).

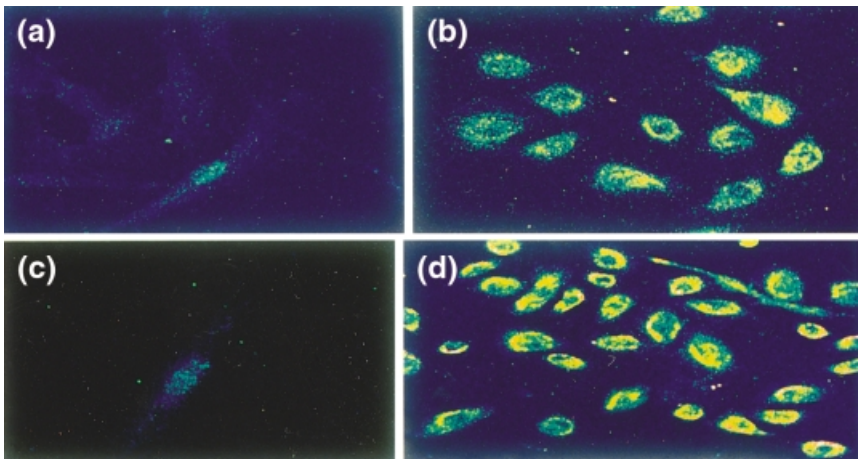

Figure 3. Immuunolocalization of estrogen and androgen receptors in cultured hair follicle cells from the occipital region of the scalp. (*a*, *b*) Androgen recptors; (*c*, *d*) estrogen receptors; (*a*, *c*) dermal papilla fibroblasts; (*b*, *d*) outer root shealth keratinocytes. Strong labeling is observed specifically in the cell nucleus (magnification, ×523).

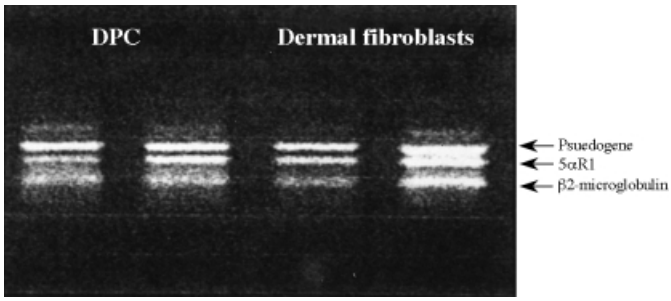

Figure 4. Comparison of 5αR1 gene expression by RT-PCR in DPC and DF.

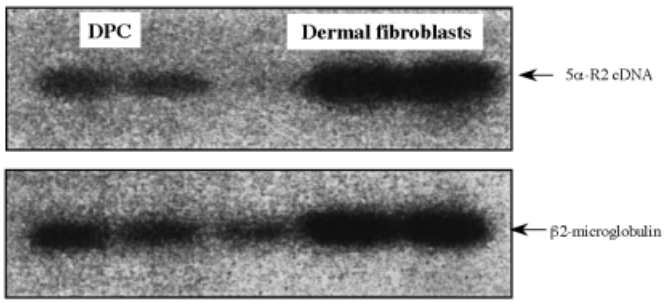

Figure 5. Comparison of 5αR2 gene expression by RT-PCR in DPC and DF.

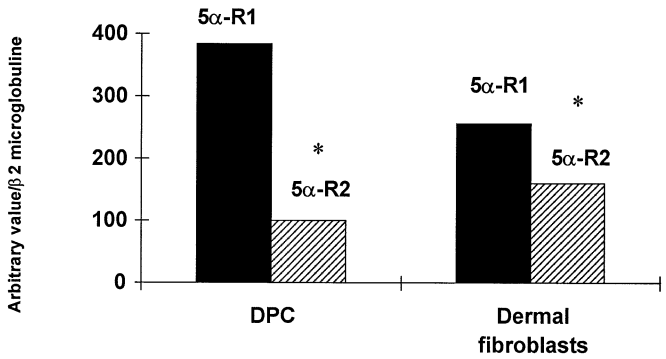

Figure 6. Semi-quantification of 5αR1 and 5αR2 transcripts in each cell type: DPC and DF. To quantify the enzymes, we measured the average pixel density per band. In this histogram, the vertical axis is an arbitrary value related to β2-microglobulin. The asterisks indicate a statistically significant difference ($p < 0.05$) using Dunnett test.

Finasteride, however, induces a significant stimulation of VEGF with a maximal effect (+30%) obtained when DPC are incubated with 13 nM Finasteride (**Fig 9**).

We also chose to evaluate, by ELISA, 17β-estradiol and aromatase inhibitor effects on the production of VEGF. 17β-estradiol induces a significant concentration-dependent stimulation of VEGF production in the supernatants of DPC (**Fig 10**).

A significant concentration-dependent inhibition of the synthesis of VEGF, however, is observed in the presence of aromatase inhibitor (**Fig 10**).

Steroids and VEGF gene expression Using RT-PCR we determined whether VEGF gene expression by DPC was affected by some steroids.

Stimulation of VEGF gene expression was observed in the presence of progesterone or finasteride; however, no effect was observed with the androgen receptor antagonist, cyproterone

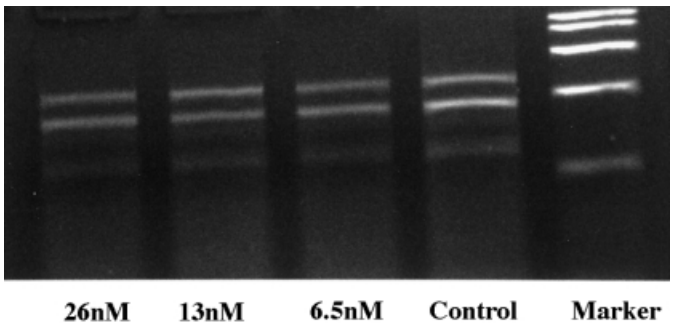

Figure 7. Effect of the finasteride on 5αR1 gene expression by RT-PCR in cultured DPC.

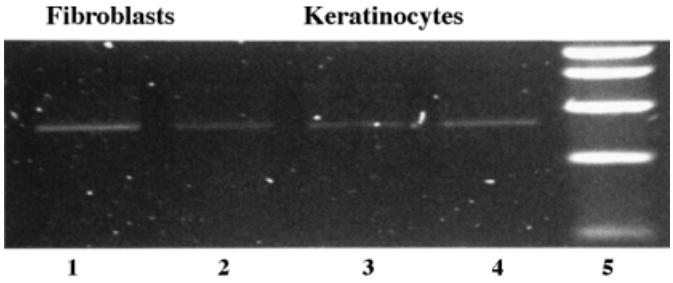

Figure 8. RT-PCR detection of cytochrome-p-450-aromatase gene in cultured hair follicle fibroblasts and outer root sheath keratinocytes. Reverse transcription was performed as described in the *Materials and Methods*.

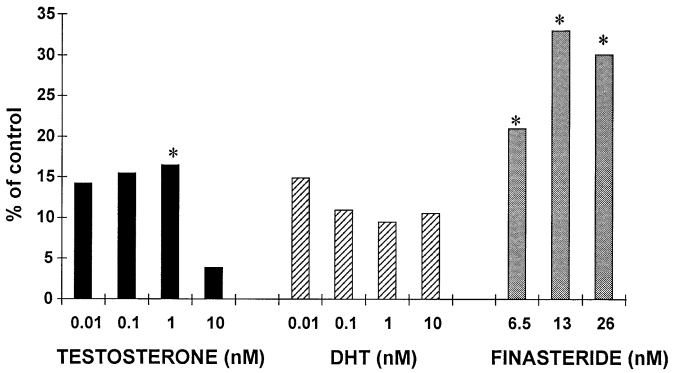

Figure 9. VEGF production by cultured DPC following 24 h treatment with testosterone, dihydrotestosterone, or Finasteride. VEGF protein was measured in the cell supernatants. The asterisks indicate a statistically significant difference ($p < 0.05$) compared with the control using Dunnett test.

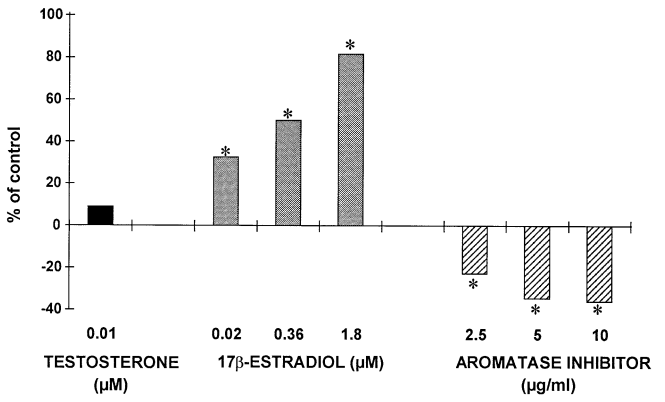

Figure 10. Concentration–response curve of VEGF expression in DPC. The DPC were cultured in the presence of testosterone, 17β-estradiol, or aromatase inhibitor for 24 h, then VEGF protein was measured in the cell supernatants. The asterisks indicate a statistically significant difference ($p < 0.05$) compared with the control using Dunnett test.

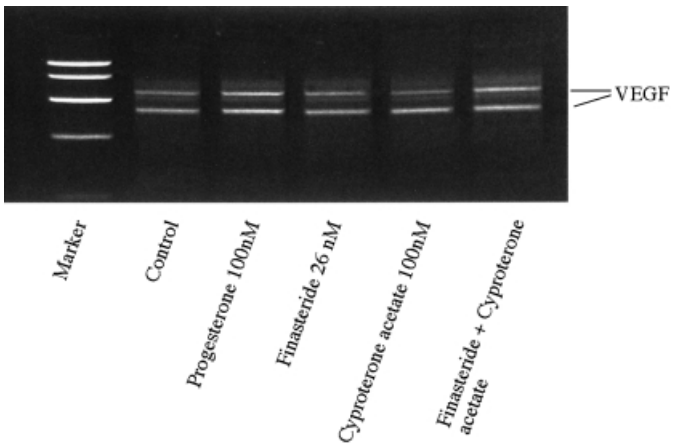

Figure 11. RT-PCR detection of VEGF gene in cultured hair follicle DPC. Reverse transcription was performed as described in the *Materials and Methods*.

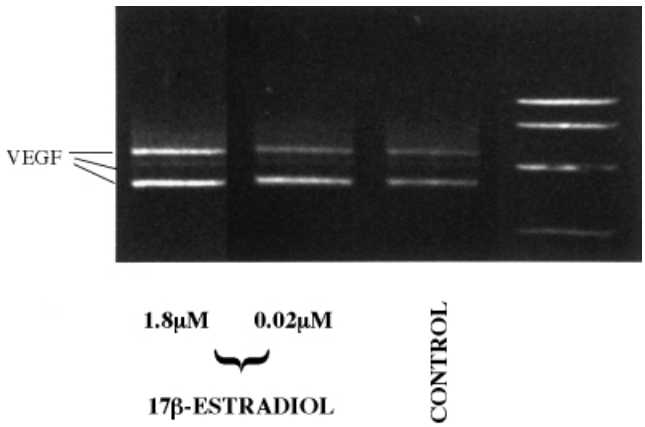

Figure 12. RT-PCR detection of VEGF gene in hair follicle DPC. Reverse transcription was performed as described in the *Materials and Methods*.

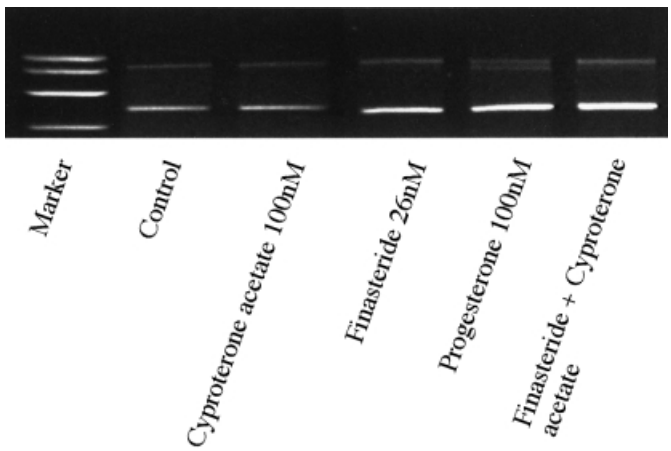

Figure 13. RT-PCR detection of cytochrome-p-450-aromatase gene in cultured hair follicle DPC. Reverse transcription was performed as described in the *Materials and Methods*.

acetate. An increase of stimulation was obtained by associating Finasteride and cyproterone acetate (**Fig 11**).

17β-estradiol also stimulated VEGF gene expression, strong stimulation was observed in the presence of 1.8 μM 17β-estradiol (**Fig 12**).

Steroids and aromatase Concerning aromatase gene expression (**Fig 13**), the results obtained were similar to those with VEGF gene expression. Finasteride and progesterone stimulated aromatase expression; however, cyproterone acetate had no effect but the association of Finasteride and cyproterone acetate greatly stimulated aromatase expression.

DISCUSSION

Many factors influence the expression of androgen and estrogen receptors (age, sex, site of body, etc). In reviewing data obtained on the localization and the content of androgen receptors, various studies have described in detail their precise location in the different structures of the hair follicle, but the labeling intensity and the localization of these receptors varied with the methodology used: the type of antibodies (monoclonal or polyclonal) and the scalp regions where the hair follicles were (occipital, frontal).

Liang *et al* (1993), using monoclonal antibodies, demonstrated a strong *in vivo* expression of androgen receptors in the outer root sheath, in the hair bulb epithelium, and in dermal papilla.

Other work (Choudhry *et al*, 1992) showed that androgen receptor staining was restricted only to dermal papilla. No staining was observed in outer root sheath keratinocytes.

The results obtained by Itami *et al* (1994; 1995) are comparable with ours. They demonstrated by using polyclonal anti-androgen receptor antibodies a low expression of androgen receptors in DPC from occipital scalp hair follicles.

As regards estrogen receptors, their involvement in hair growth has been studied less extensively in normal human scalp skin. Therefore we investigated their localization *in vivo* and *in vitro*. The only work reported in this domain (Wallace *et al*, 1998) has compared the localization of estrogen receptors in androgenetic alopecia *versus* alopecia areata. No comparison was made with normal human scalp skin; however, other recent work (Oh and Smart, 1996) reports on estrogen receptor localization, but in an animal model. They demonstrate the presence of a weaker staining of the derma papilla of early anagen follicles and no detectable staining in dermal papilla of mid to late anagen. Perhaps the highly synchronous nature of the mouse hair follicle cycle may control the expression of the estrogen receptors in dermal papilla. Thus the reason for carrying out immunostaining of estrogen receptors is that we have not enough precise data relating specifically to normal human occipital scalp skin.

Our finding concerning their localization in human hair follicle *in vivo* showed a high expression of these receptors in human hair dermal papilla at the anagen stage, and contradicted the results described below obtained on mouse hair follicle by Oh and Smart (1996). Perhaps this difference is due to the highly synchronous nature of the mouse hair follicle cycle, which may control the expression of the estrogen receptors in dermal papilla. In cultured cells, the staining for estrogen receptors was particularly prominent in outer root shealth keratinocytes.

Our results concerning type I 5α-reductase gene expression *in vitro* are consistent with a previous publication (Nakanishi *et al*, 1996), in which occipital DPC express type I 5α-reductase. In contrast to our findings, however, these authors report an absence of type II 5α-reductase on occipital DPC. This difference may be due either to the advanced passage of DPC used in their experiments or to the nature of the oligonucleotide sequence, which differed from ours.

The novelty of our work is that we have quantitatively compared the expression of two isoenzymes of the 5α-reductase gene in DPC and DF and demonstrated a high expression of type I 5α-reductase in DPC *versus* DF.

These results confirmed the presence of the effector steroids (receptors and enzymes) targetting to cultured hair DPC and may be a good *in vitro* model to study the intracrine system regulation of steroids in this unit.

In addition to the strong 5αR1 expression in DPC, we demonstrated that one of the 5α-reductase inhibitors, Finasteride, inhibits the transcription of types I and II 5α-reductase gene by cultured DPC. A significant concentration-dependent inhibition by finasteride of 5α-reductase activity was also obtained on the same cultured cells (results not shown). Such inhibition of 5α-reductase activity was recently shown on microdissected dermal papilla (Hoffman and Happle, 1999).

Consequently these data indicate the specificity of these cells as a model to compare 5αR inhibitor effects, and the efficiency of 5αR inhibitors on the metabolism and genome, in alopecia treatment, can be estimated with this model.

From our previous data on VEGF, we demonstrated that its expression varies with the hair cycle. It is strongly expressed in DPC from the anagen stage (Lachgar *et al*, 1996a, b). Most of the work undertaken until now has concentrated on the effects of testosterone metabolism via the 5α-reductase pathway. So, to develop another concept, we were particularly interested to study the effect of 5α-reductase inhibitors on VEGF regulation in cultured DPC. Did 5α-reductase inhibitors act directly on VEGF expression or did they act via the aromatase pathway? The inhibition of 5α-reductase leads to an accumulation of testosterone. This excess of testosterone may activate other steroid pathways like the aromatase pathway.

To validate our *in vitro* experimental model concerning VEGF and aromatase expression, we chose commercially available molecules: an inhibitor of 5α-reductase (finasteride), a blocker of androgen receptors (cyproterone acetate), an inhibitor of aromatase (4-androsten-4-ol-3,17-dione), and some androgens (testosterone, DHT) or estrogens (17β-estradiol). We used progesterone as a positive control of our experiments (this is an estrogen-like molecule). Several works have shown that the treatment of other types of cells with progesterone induces an increase of aromatase expression by preventing the loss of testosterone *via* 5a-reductase way (Sawaya and Price, 1997)

Treatment with Finasteride stimulated both VEGF and aromatase expression in DPC. Cyproterone acetate alone had no effect on VEGF or aromatase expression; however, when combined with finasteride we observed an increased effect on both VEGF and aromatase expression. So we think that in order for cyproterone acetate to act, a modification of testosterone metabolism is necessary.

Our finding that finasteride stimulates VEGF and aromatase is very interesting and suggests two modes of action: finasteride may act like estrogen because it directly stimulates VEGF, or it acts via aromatase because it also stimulates aromatase expression.

The studies on the effects of 17β-estradiol and aromatase inhibitor on VEGF synthesis were undertaken to verify if the estrogenic way (metabolism of testosterone to 17β-estradiol by aromatase) was involved in the regulation of VEGF in hair DPC. The finding that 17β-estradiol or progesterone increased VEGF protein and gene expression by DPC is exciting and for the first time suggests the involvement of this estrogen pathway in the regulation of VEGF expression by hair DPC *in vitro*.

The same increase by 17β-estradiol or progesterone was observed in other cellular models, e.g., McLaren *et al* (1996) demonstrate that VEGF expression by human peritoneal fluid macrophages is greatly enhanced by treatment in culture with 17β-estradiol or progesterone.

Estrogen also regulates transcription of both aromatase and VEGF in the mammary gland (Nakamura *et al*, 1999).

These preliminary findings suggest that 17β-estradiol, Finasteride, and cyproterone acetate influence cultured DPC angiogenic responses by stimulating VEGF protein and gene expression. The modification of aromatase gene expression in DPC by these molecules supports its involvement in regulating local

(DPC) estrogen concentrations and controlling VEGF expression by these cells.

It is conceivable that the modulation of VEGF and aromatase expression may be necessary to maintain vascular supply to the hair bulb, albeit at the expense of metabolizing testosterone to dihydrotestosterone.

This hypothesis constitutes another indirect way in which some 5α-reductase inhibitors and estrogens affect hair cell angiogenic responses *in vitro*.

One question for the future for the prevention of hair loss is the possible correlations between these *in vitro* results and the *in vivo* situation to define the effectiveness of estrogens and antiandrogens (5α-reductase inhibitors, androgen receptor antagonists, etc.) on initiating or maintaining hair-bulb vascularization during the anagen stage through the expression of different angiogenic markers in hair follicle cells.

REFERENCES

Choudhry R, Hodgins MB, VanderKwast TH, Brinkmann AO, Boersma WJ: Localization of androgen receptors in human skin by immunohistochemistry: implication for the hormonal regulation of hair growth, sebaceous glands and sweat glands. *J Endocrinol* 133:467–475, 1992

Ferrara N, Houck K, Jakeman L, Leung DW: Molecular and biological properties of the vascular endothelial growth factor family of proteins. *Endocr Rev* 13:18–32, 1992

Folkman J: Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nat Med* 1:27–31, 1995

Folkman J, Klagsbrun M: Angiogenic factors. *Science* 235:442–447, 1987

Folkman J, Shing YJ: Angiogenesis. *J Biol Chem* 267:10931–10934, 1992

Goldman CK, Tsai JC, Soroceanu L, Gillespie Y: Loss of vascular endothelial growth factor in human alopecia hair follicles. *J Invest Dermatol* 104 (Suppl.):18–20, 1995

Gospodarowicz D, Abraham JA, Schilling J: Isolation and characterization of a vascular endothelial cell mitogen produced by pituitary-derived folliculo stellate cells. *Proc Natl Acad Sci USA* 86:311, 1989

Hamilton JB: Male hormone stimulation is prerequisite and an incitant in common baldness. *Am J Anatomy* 701:451–480, 1942

Hoffmann R, Happle R: Finasteride is the main inhibitor of 5α-reductase activity in microdissected dermal papilla of human hair follicles. *Arch Dermatol Res* 291:100–103, 1999

Itami S, Kurata S, Sonoda T, Takayasu S: Characterization of 5α-reductase in cultured human dermal papilla cells from beard and occipital Scalp hair. *J Invest Dermatol* 96:57–60, 1991

Itami S, Sonoda T, Kurata S, Takayasu S: Mechanism of action of androgen in hair follicles. *J Dermatol Sci* 7 (Suppl.):S98–S103, 1994

Itami S, Kurata S, Sonoda T, Takayasu S: Interaction between dermal papilla cells and follicular epithelial cells in vitro: effect of androgen. *Br J Dermatol* 132:527–532, 1995

Keck PJ, Hauser SD, Krivi G, Sanzo K, Warren T, Feder J, Connolly DT: Vascular permeability factor, an endothelial cell mitogen related to PDGF. *Science* 246:1309–1312, 1989

Lachgar S, Moukadiri H, Jonca F, *et al*: VEGF is an autocrine growth factor for cultured hair dermal papilla cells. *J Invest Dermatol* 106:17–23, 1996a

Lachgar S, Charvéron M, Ceruti I, Gall Y, Bonafé JL: Expression of VEGF mRNA in different hair follicle stages by fluorescence *in situ* hybridization: analysis by confocal laser microscopy. In: *Hair Research for the Next Millenium*, eds Van Neste DJJ, Randall VA. Amsterdam, Elsevier, 1996b, pp. 407–411

Lachgar S, Charvéron M, Gall Y, Bonafé JL: Minoxidil upregulates the expression of vascular endothelial growth factor in human hair dermal papilla cells. *Br J Dermatol* 138:407–411, 1998

Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N: Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science* 246:1306–1309, 1989

Liang T, Hoyer S, Yu R, Soltani K, Lorincz AL, Hiipakka RA, Liao S: Immunocytochemical localization of androgen receptors in human skin using monoclonal antibodies against the androgen receptor. *J Invest Dermatol* 100:663–666, 1993

McLaren J, Prentice A, Charnock-Jones DS, Millican SA, Müller KH, Sharkey AM, Smith SK: Vascular Endothelila growth factor is produced by peritoneal macrophages in peritoneal fluid macrophages in endometriosis and is regulated by ovarian steroids. *J Clin Invest* 98:482–489, 1996

Nakamura J, Lu Q, Aberdeen G, Albrecht Brodie A: The effect of estrogen on aromatase and vascular endothelial growth factor messenger ribonucleic acid in the normal nonhuman primate mammary gland. *J Clin Endocrinol Metab* 84:1432–1437, 1999

Nakanishi J, Itami S, Adachi K, Nakayama Y, Takayasu S. Expression of androgen receptor, type I and type II 5α-reductase in human dermal papilla cells. In: *Hair Research for the Next Millenium*, eds Van Neste DJJ, Randall VA. 1996, pp. 333–337

Neufeld G, Tessler S, Gitay-Goren H, Cohen T, Levi BZ: Vascular endothelial growth factor and its receptors. *Prog Growth Fact Res* 5:89–97, 1994

Oh HS, Smart RC: An estrogen receptor pathway regulates the telogen anagen hair follicle transition and influences epidermalcell proliferation. *Proc Natl Acad Sci* 93:12525–12530, 1996

Orfanos CE, Vogel L: Lokaltherapie der alopecia androgenetica mit 17α-Ostradiol. *Dermatologica* 161:124–132, 1980

Parakkal PF. Catagen and telogen phases of the growth cycle. In: *Hair and Hair Diseases*, eds Orfanos CE, Happle R. Berlin: Springer-Verlag, 1990, pp. 99–116

Plate KH, Breier G, Risau W: Molecular mechanisms of developmental and tumor angiogenesis (review). *Brain Pathol* 4:207–218, 1994

Plouët J, Shilling J, Gospodarowicz D: Isolation and characterization of a newly identified endothelial cell mitogen produced by AtT-20 cells. *EMBO J* 8:3801–3806, 1989

Randall VA: Androgens and human hair growth. *Clin Endocrinol* 40:439–457, 1994

Randall VA, Thornton MJ, Hamada K, Redfern CPF, Nutbrown M, Ebling FJG, Messenger AG: Androgens and the hair follicle: cultured human dermal papilla cells as a model system. *Ann NY Acad Sci* 642:355–375, 1991

Rittmaster RS, Uno H, Povar ML, *et al*: The effects of N,N-diethyl-4-methyl-3-oxo-4-aza-5 alpha-androstane-17 beta-carboxamide, a 5α-reductase inhibitor and anti-androgen, on the development of baldness in the stumptail macaque. *J Clin Endocrinol Metab* 65:188–193, 1987

Sawaya ME, Price VH: Different level of 5α-reductase type I and II, aromatase, and androgen receptor in hair follicles of women and men with androgenetic alopecia. *J Invest Dermatol* 109:296–300, 1997

Wallace ML, Bruce R, Smoller MD: Estrogen and progesterone receptors in androgenetic alopecia versus alopecia areata. *Am J Dermatopathol* 20:160–163, 1998