The Distribution of Estrogen Receptor β Is Distinct to That of Estrogen Receptor α and the Androgen Receptor in Human Skin and the Pilosebaceous Unit

M. Julie Thornton, Anthony H. Taylor,* Kellie Mulligan,* Farook Al-Azzawi,* Calum C. Lyon,† John O'Driscoll,† and Andrew G. Messenger‡

Department of Biomedical Sciences, University of Bradford, Bradford, U.K.; *Department of Obstetrics and Gynaecology, Leicester Royal Infirmary, Leicester, U.K.; †Department of Dermatology, Hope Hospital, Salford, U.K.; ‡Department of Dermatology, Royal Hallamshire Hospital, Sheffield, U.K.

Both estrogens and androgens play important parts in skin and hair physiology, although studies of estrogen action in human skin have been rather limited. Recently, a second estrogen receptor (β) has been identified in many nonclassical target tissues, including androgen-dependent tissues. Therefore, we have revisited the role of estrogens in human skin and hair by comparing the pattern of expression by immunohistochemistry for both estrogen receptors (α and β) and the androgen receptor. Immunolocalization of androgen receptors was only seen in hair follicle dermal papilla cells and the basal cells of the sebaceous gland. Little specific staining of estrogen receptor α was seen anywhere except the sebaceous gland. In contrast estrogen receptor β was highly expressed in epidermis, blood vessels, and dermal fibroblasts, whereas in the hair follicle it was localized to nuclei of the outer root sheath, epithelial matrix, and dermal papilla cells. Serial sections also showed strong nuclear expression of estrogen receptor β in the cells of the bulge, whereas neither estrogen receptor α or androgen receptor was expressed. In the sebaceous gland, estrogen receptor β was expressed in both basal and partially differentiated sebocytes in a similar pattern to estrogen receptor α. There was no obvious difference in the expression of either estrogen receptor in male or female nonbalding scalp skin. The results of this immunohistochemical study propose that estrogen receptor β and not estrogen receptor α is the main mediator of estrogen action in human skin and the hair follicle. Further studies with androgen-dependent skin are required to determine whether estrogen receptor β has a regulatory role on androgen receptor expression in the hair follicle in parallel with its role in other androgen-dependent tissues. ***Key words: androgen receptor/estrogen receptor/hair follicle/human skin. JID Symposium Proceedings 8:100–103, 2003***

ESTROGEN RECEPTORS (ER): ERα AND ERβ

Since the isolation and cloning of the ER (Greene *et al*, 1986), it was thought that only a single nuclear ER existed until a second gene coding for an ER was cloned from rat prostate (Kuiper *et al*, 1996) and human testis (Mosselman *et al*, 1996) cDNA libraries. Owing to a significant homology with the DNA binding domain of the classical ER, this second receptor was termed ERβ, whereas the classical ER is now referred to as ERα. The two ER are not generated from alternate transcription sites of the same gene, as is the progesterone receptor (Taylor, 2001), but are distinct proteins encoded by separate genes located on different chromosomes. The ERα and ERβ proteins have ≈ 60% conservation of the residues in the ligand binding domain, yet each bind 17β-estradiol with nearly equal affinity and exhibit a very similar binding profile for a large number of natural and synthetic ligands (Kuiper *et al*, 1997); however, there are major differences between ERβ and ERα with respect to their tissue distribution (Fisher *et al*, 1998; Taylor and Al-Azzawi, 2000), the phenotype of the corresponding knockout mice and their transcriptional activities (Dechering *et al*, 2000). Additionally, studies have shown that ERβ and ERα can heterodimerize *in vitro*, indicating the possibility of cooperative, synergistic, or inhibitory actions between the two receptors (Cowley *et al*, 1997; Pettersson *et al*, 1997). Therefore, the discovery of ERβ has introduced a new level of complexity to the mechanism of estrogen signaling and physiology.

Manuscript accepted for publication February 1, 2003

Reprint requests to: Dr M.J. Thornton, Department of Biomedical Sciences, University of Bradford, Bradford, West Yorkshire, BD7 1DP, U.K. Email: M.J.Thornton@bradford.ac.uk

Abbreviations: ER, estrogen receptor; AR, androgen receptor.

ESTROGENS AND SKIN

The importance of estrogens in the maintenance of human skin is highlighted by the significant changes that occur in the skin of postmenopausal women, which may be reversed with hormone replacement. Estrogens not only improve collagen content and quality, they also increase skin thickness and enhance vascularization (reviewed Brincat, 2000). Although binding studies have demonstrated the presence of estrogen-binding sites in human skin (Punnonen *et al*, 1980), immunolocalization of ER has been difficult to correlate with those estrogen-binding sites. This has inevitably limited the number of studies on estrogen action in human skin. Estrogens also play a part in the regulation of the hair cycle in the rat, where estrogens slow down the moult cycle (Ebling, 1976), whereas in both sexes of mice, topical estradiol maintains

the hair follicle in telogen, blocking its transition into anagen, with a resultant inhibition of hair growth (Chanda *et al*, 2000). The effects of pregnancy hormones on hair growth are also well recognized, with postpartum alopecia a striking phenomenon seen in women. During pregnancy there is an increase in the number of hairs in anagen, which leads to an increased number entering telogen postpartum resulting in an increase in hair shedding (Lynfield, 1960).

Estrogens are also powerful suppressors of androgen-stimulated sebaceous activity in doses markedly lower than those required for anti-androgens (Ebling, 1974), the mechanism of action of estradiol in the sebaceous gland was concluded to be distinct from that of an anti-androgen (Ebling, 1974) suggesting that estrogens act via a non-androgen receptor (non-AR)-dependent pathway.

INTERACTION OF ESTROGENS AND ANDROGENS

The presence of ERβ in the prostate and testis, classical androgen-target tissues, and experiments with ER knockout mice have clearly indicated that ERβ plays an important part in prostate and testis development (Gustafsson, 2000; Makinen *et al*, 2001), which is supported by the fact that prostate cancer often responds favorably to estrogen treatment due to interaction with androgen-dependent signaling pathways. Studies with ERα, ERβ and AR knockout mice (termed ERKO, BERKO, and ARKO, respectively) have shown that the interaction of ERα, ERβ, and AR is of the utmost importance in the regulation of some tissues (Krege *et al*, 1998). In addition there are numerous reports that estrogen and androgen metabolites can interact with both receptor subtypes. For example, androst-5-ene-3β, 17β-diol a metabolite of dehydroepiandrosterone can interact presumably with both ERα and ERβ in MCF7 human breast cancer cells (Adams *et al*, 1981), whereas 5α-androstane-3β, 17β-diol, a metabolite of 5α-dihydrotestosterone interacts with ERβ in the prostate (Debé *et al*, 1979). Taken together, this indicates that the role of estrogens and androgens in the control of skin physiology and pathophysiology are probably interdependent (see **Fig 1**).

Although it is well established that androgens have an important role in the regulation of human hair growth (reviewed Randall, 2000), the exact interaction of estrogens and androgens is at present unclear. The complexity of androgen action on human hair growth is highlighted by the contradictory effects of androgens illustrated in individuals with androgenic alopecia. Such a biologic paradox implies that there are other regulatory factors at work. The role of ERβ in regulating the androgen-dependent prostate suggests that this may have a parallel role in other androgen-dependent tissues, including the human hair follicle.

EXPERIMENTAL DESIGN

As the interactions between estrogens and androgens in human skin are unclear it is essential to show that human skin has the necessary machinery to mediate both estrogenic and androgenic signals prior to investigations into the interactions of these steroid hormones. To determine whether the presence of both ER is important in human skin, or whether one ER predominates, we have compared the distribution of ERα and ERβ in human scalp skin. We have examined skin from similar sites of both sexes to establish whether there is a difference in the expression of ER between males and females. As there is strong evidence from studies using androgen-dependent tissues such as the prostate that ERβ can regulate AR production, the relative expression of ERβ and AR may also be of significance in the hair follicle, particularly those that are androgen dependent. Therefore, we have also compared the distribution of AR in the same skin samples.

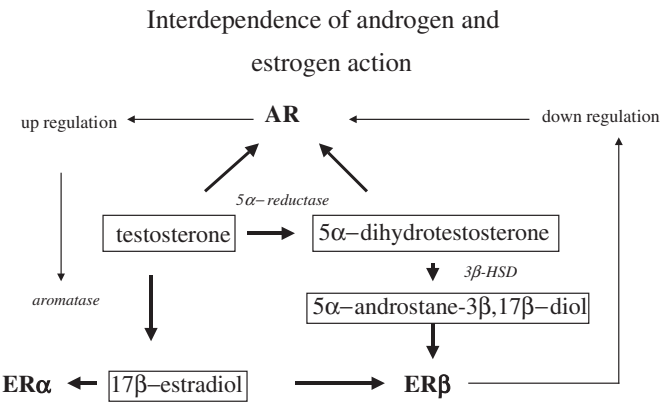

Figure 1. Hypothetical model for the interdependence of estrogens and androgens in human skin. Aromatase activity can be upregulated by either testosterone or 5α-dihydrotestosterone binding to AR, thereby increasing the availability of 17β-estradiol. 5α-dihydrotestosterone can be 3β reduced by 3β-hydroxsteroid dehydrogenase (3β-HSD) to produce 5α-androstane-3β,17β-diol. Both 17β-estradiol and 5α-androstane-3β,17β-diol can bind to ERβ, which can downregulate AR expression. Blocking the production of 5α-dihydrotestosterone may inadvertently upregulate AR by (i) downregulating aromatase via AR inactivation, and (ii) inactivation of ERβ by limiting the availability of 5α-androstane-3β,17β-diol.

MATERIALS AND METHODS

Normal human skin samples from nonbalding areas of scalp were obtained from patients at the time of surgery. Small pieces of skin were fixed in formol saline for a fixed period of 24 h before processing into paraffin wax. The nonbalding scalp of a total of 12 patients (six male and six female) were examined for the distribution of steroid receptors. Serial sections (4 μm) were mounted on to silane-coated slides and allowed to dry at 37°C, before incubating with either monoclonal mouse anti-bovine ERα (1 : 50) polyclonal rabbit anti-rat ERβ antibody (1 : 50) (Upstate Biotechnology, Lake Placid, NY), or a monoclonal antibody for the AR (1 : 25) (Novocastra, New Castle-upon-Tyne, U.K.), as described previously (Taylor and Al-Azzawi, 2000) and according to the manufacturers' instructions. Briefly, sections were dewaxed, rehydrated, and endogenous peroxidase, biotin, and nonspecific sites blocked with standard procedures before incubation with primary antibody at 4°C for 18 h. The antibody–antigen complex was amplified using horseradish peroxidase-linked avidin–biotin complexes (Vector Laboratories, Peterborough U.K.) after biotinylated secondary anti-rabbit and anti-mouse IgG incubation. Visualization was achieved with Cu^{2+} enhanced diaminobenzidine and light hematoxylin counterstaining, before mounting in XAM mounting medium (BDH, Poole, Dorset, U.K.). Specificity of immunostaining was confirmed using molar equivalents of rabbit IgG (ERβ) or mouse IgG (ERα, AR). Additionally, several control sections were produced by omission of the primary antibody. Positive control tissue included human ovary and human breast carcinoma for expression of ERα, human prostate for ERβ, and human testis for AR antigens.

RESULTS

ERβ was the predominant steroid receptor in both male and female human nonbalding scalp skin. In the hair follicle, ERβ was immunolocalized to the cell nuclei of the outer root sheath, epithelial matrix, and dermal papilla cells (see **Fig 2**). This was in contrast to ERα, which did not stain the hair follicle cells, and AR, which only stained dermal papilla cells. Serial sections of one follicle clearly showed ERβ strongly expressed in the bulge, whereas there was no staining for either ERα or AR. ERβ was also expressed in the nuclei and cell membranes of the partially differentiated sebocytes (see **Fig 3**); a similar pattern of nuclear and membrane staining was seen with ERα. In the epidermis, strong ERβ expression was seen in the keratinocytes of the stratum basale and stratum spinosum, whereas the cells of the stratum granulosum were less immunoreactive and the stratum

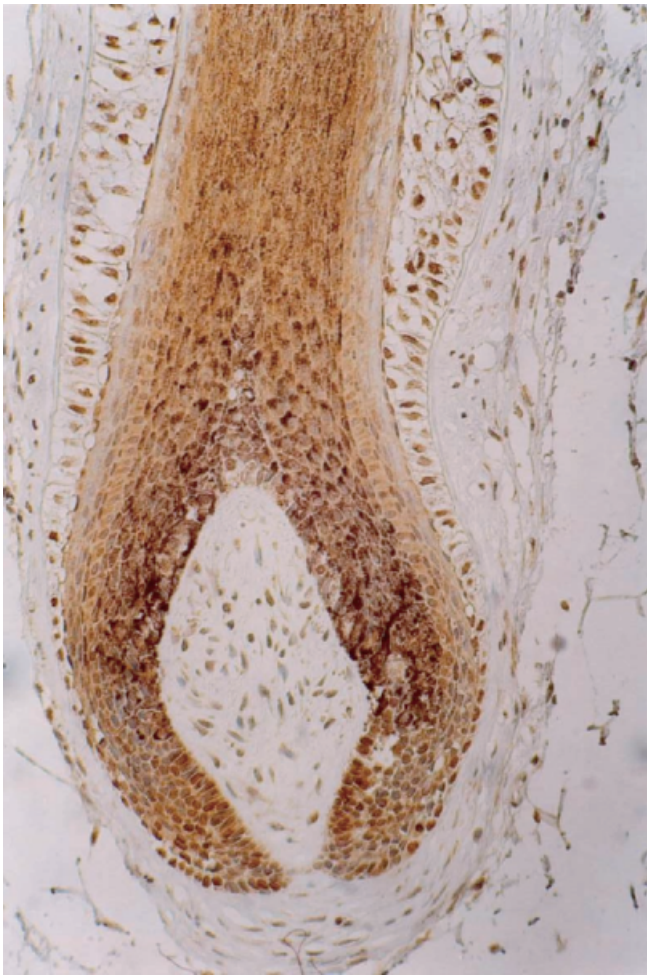

Figure 2. Immunohistochemical localization of ERβ to human occipital scalp hair follicles (original magnification × 250). Bound antibodies were visualized with diaminobenzidine and is seen as brown staining. Sections were counterstained with hematoxylin (blue nuclei). ERβ was widely expressed in the hair follicle bulb. Specific nuclear staining was seen in the mesenchymal dermal papilla and epithelial outer root sheath and hair matrix cells.

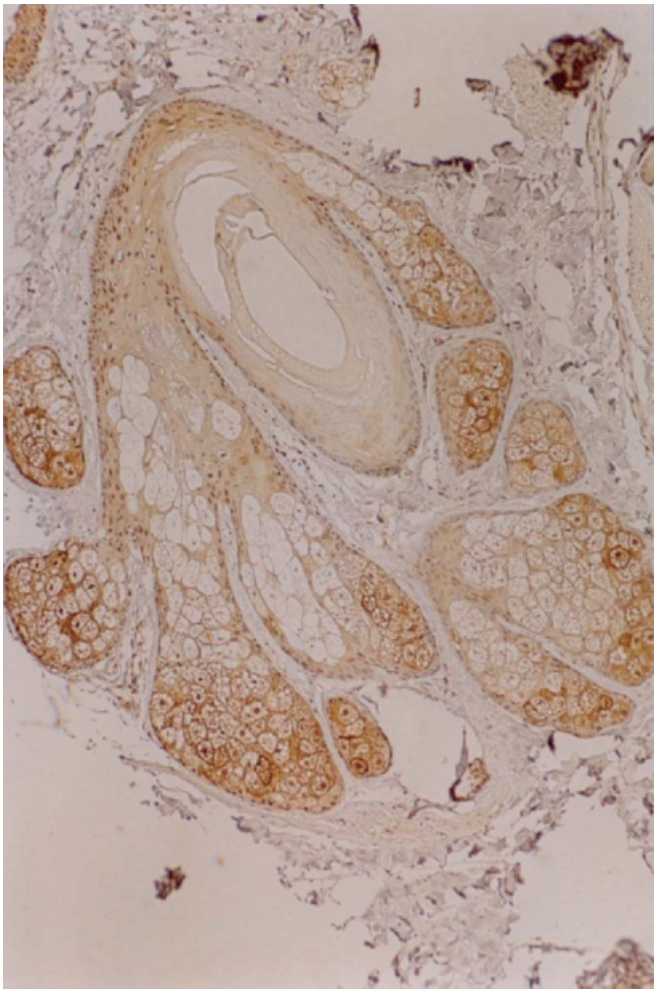

Figure 3. Immunohistochemical localization of ERβ to human occipital scalp sebaceous glands (original magnification × 128). Bound antibodies visualized with diaminobenzidine are seen as brown nuclear staining. Sections were counterstained with hematoxylin (blue nuclei). ERβ was widely expressed in the sebaceous gland, particularly in the partially differentiated sebocytes. Staining was not restricted to the nucleus, but was also present in the cytoplasm, particularly concentrated around the cell membrane.

corneum was devoid of immunoreactivity (see **Fig 4**). By contrast, there was no expression of ERα or AR in these structures. In addition to the staining seen in the epidermis, the underlying fibroblasts of the papillary dermis also showed strong nuclear expression of ERβ. There was no obvious difference in the expression patterns of either ER in male or female skin.

DISCUSSION

Studies of estrogen action in human skin and hair have been limited to some extent because immunohistochemical expression of ER has been difficult to demonstrate, even though radio-labeled estradiol was known to bind to structures within skin and hair. The results of the present immunohistochemical study demonstrate that ERβ and not ERα is the main mediator of estrogen action in human skin and the hair follicle. The inability to demonstrate ER staining in human skin by immunohistochemistry is hardly surprising as until recently only one ER was thought to exist, i.e., ERα; however, these data are contrasted by studies in the mouse where ERα and not ERβ was identified in hair follicles (Chanda *et al*, 2000). These apparent inconsistencies between ERα and ERβ expression in human and mouse need to be interpreted with care as the expression of ERβ protein also differs markedly in other human tissues when compared with the mouse or rat (Fisher *et al*, 1998; Taylor and Al-Azzawi, 2000). Interestingly, we have recently reported that the distribution of ERβ in red deer skin is also in complete contrast to that described in human skin (Thornton *et al*, 2000). Although ERβ was strongly expressed in the blood vessels of red deer skin in common with human skin, there was no expression of ERβ in epidermis, dermis, or the pilosebaceous unit (Thornton *et al*, 2000). These observations are noteworthy as it may signify that ERβ plays a unique part in human skin and that the predominant mediator of estrogen action in other mammals is the ERα.

Our observed AR expression in nonbalding scalp dermal papilla cells supports the observations of AR expression from previous studies (Choudry *et al*, 1992; Randall *et al*, 1992; Hibberts *et al*, 1998). Androgens, such as testosterone and 5α-dihydrotestosterone, are not required for scalp hair growth, as patients with AR defects have good hair growth; however, androgens, particularly 5α-dihydrotestosterone appear to be a requirement for androgenetic alopecia (Sawaya, 2000). This leads to the hypothesis that estrogens or androgenic metabolites might be more important molecules for hair growth control in the human scalp. Clues

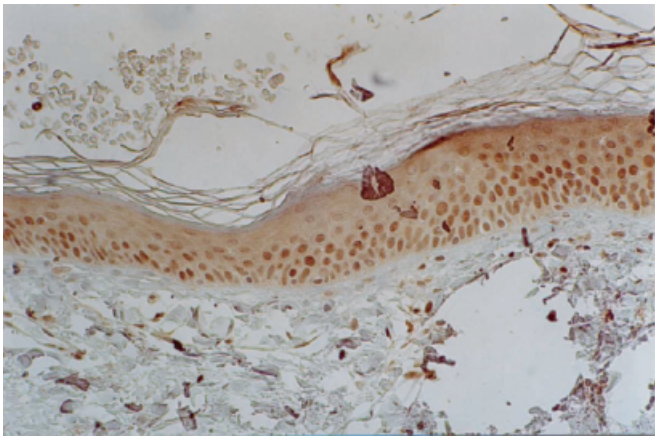

Figure 4. Immunohistochemical localization of ERβ to human occipital scalp skin (original magnification × 100). Immunoreactive ERβ visualized with diaminobenzidine appears as brown nuclear staining, whereas nuclei counterstained with hematoxylin appear blue. Strong ERβ staining was restricted to the keratinocytes of the stratum basale and stratum spinosum, whereas the cells of the stratum granulosum were less immunoreactive. The stratum corneum was devoid of immunoreactivity for ERβ. In addition to the staining seen in the epidermis, the underlying fibroblasts of the papillary dermis also showed strong nuclear staining for ER.

from other androgen-target tissues such as the prostate in the ERβ knockout mice, which shows a distinct hyperproliferation, suggests that ERβ is important in controlling prostate growth, probably by downregulating AR expression (Gustafsson, 2000). By extension, an important role for ERβ in the human hair follicle may be to regulate androgen-dependent hair growth by modulating AR expression or androgen signaling pathways. In this respect, it is noteworthy that finasteride, an inhibitor of 5α-reductase, has only been shown to be effective in men and not postmenopausal women to treat androgenetic alopecia (Price, 2000). Also other recent studies by Lachgar *et al* (1999) have shown that finasteride upregulates aromatase expression in cultured scalp dermal papilla cells, indicating that finasteride might have an estrogen promoting effect because it increases the amount of testosterone that is converted to 17β-estradiol (**Fig 1**). Furthermore, Sawaya (2000) has reported that there are differences in aromatase expression in scalp follicles taken from men and women and also between occipital and frontal follicles. That study showed higher levels of aromatase activity in occipital scalp follicles compared with frontal scalp follicles in both sexes; however, aromatase activity was 6-fold higher in frontal hair follicles of females compared with males. Interestingly, the expression of aromatase in human genital skin fibroblasts has been shown to be androgen dependent, with much lower levels of expression in cells from patients with androgen insensitivity syndromes (Stillman *et al*, 1990). In addition, androgens can significantly increase aromatase activity in cultured genital skin fibroblasts, which can be blocked by an anti-androgen (Stillman *et al*, 1991), demonstrating that the response is mediated via the AR.

In order to understand the effects of reproductive hormones on skin aging, skin cancer, hair growth, and loss, there is an overwhelming need to widen our understanding of the physiology and interaction of steroid hormones and their receptors in human skin. Clearly further studies of the role of ERβ in human skin are warranted, particularly in relation to the expression of AR and modulation of androgen action pathways in the pilosebaceous unit. Studies such as those suggested could contribute to an understanding of the paradoxical, site-dependent, effects of androgens on hair growth.

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