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ORIGINAL PAPER

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Localization of steroid hormone receptors in the apocrine sweat glands of the human axilla

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Abstract The apocrine axillary glands, regarded as pheromone-producing scent glands, do not begin to function until puberty. Accordingly, sex hormones should have an impact on their activity, and the present study was designed to investigate the localization of androgen receptor (AR) and estrogen receptors (ERα and ERβ) in those glands. Strong nuclear immunoreactivity for AR and ERβ was found in the secretory epithelium. In AR especially, staining intensity was correlated with the height of the epithelium with more intense immunoreactivity in tall segments. Since the lower epithelium has been considered inactive or resting, our results suggest a correlation between steroid-receptor expression and secretory activity. Androgens are known to upregulate the cholesterol biosynthesis, and cholesterol may be used as precursor for pheromones. Accordingly, the results of this study establish a possible link between steroid hormone action and induction of pheromone production in the apocrine axillary glands.

Keywords Axilla · Apocrine glands · Androgen receptor · Estrogen receptor · Pheromones

Introduction

The apocrine glands of the human axilla are a major source of odorous signals and pheromones, and the role of body odour and pheromones in human communication has gained interest in recent decades (Weller 1998). Chemically, the human pheromones known up to now are volatile steroid molecules, such as the 16-androstenes 5α-androstenol or 5α-androstenone (Cowley and Brooksbank 1991; Sobel et al. 1999; Grosser et al. 2000). These substances act as pheromones in different ways: they activate specific regions within the brain (Sobel et al. 1999), affect social behaviour (Cowley and Brooksbank 1991; Cutler et al. 1998; Grosser et al. 2000), regulate ovulation (Stern and McClintock 1998) and modulate physiological parameters such as the serum levels of testosterone, luteinizing hormone and follicle stimulating hormone as well as respiration and cardiac frequency in a gender-specific way (Monti-Bloch et al. 1998; Shinohara et al. 2000). These functions, together with the well-known fact that the apocrine axillary glands are correlated in structure and function with approaching sexual maturity and do not begin their secretory activity until puberty (Hoepke 1927; Groscurth 2002), have implied that the apocrine axillary glands are stimulated by sex hormones. Accordingly, they should contain receptors for those hormones. Indeed, biochemical ligand binding assays in apocrine axillary glands of osmidrosis patients (Kurata et al. 1990) have suggested the occurrence of androgen receptor (AR). However, morphological studies have so far not considered the localization of AR or of estrogen receptors (ERα and ERβ) in the apocrine axillary glands. This study was designed to investigate the occurrence and the intracellular localization of AR and ERs in the apocrine glands of the human axilla by RT-PCR and immunohistochemistry.

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Materials and methods

Tissue

Samples were obtained from autopsies from the Anatomisches Institut and from the Institut für Rechtsmedizin, Universität Basel.

Antibodies

Epitope-specific polyclonal antibodies raised in rabbits were used. The antibody against AR (catalog E2721) and antibody against ERα (catalog M1641) were obtained from AMS Biotechnology (Bioggio, Switzerland). The ERβ antibody (catalog SC-8974) was obtained from Santa Cruz Biotechnology (Santa Cruz, USA).

Immunohistochemistry

The material was fixed in formaldehyde-containing fixative (10% formaldehyde in PBS overnight), dehydrated in a graded series of ethanol and embedded in paraffin (Paraplast Plus; Monoject Scientific, Athy, Ireland) at 57°C. Samples from one male and four female individuals were analysed. Paraffin sections (3 μm) were mounted on Histobond slides (Marienfeld Laboratoy Glasware, Lauda-Königshofen, Germany) and allowed to dry overnight at 37°C. After dewaxing with xylol and rehydration through graded stages of alcohol to water, the sections were subjected to antigen retrieval in 0.01 M citrate buffer, pH 6.0, for 20 min in a microwave oven (900 W), followed by cooling at room temperature for 20 min. Endogenous peroxidase activity was quenched using 3% hydrogen peroxide for 5 min at RT. Sections were blocked for 30 min with 10% goat serum in PBS and incubated overnight with the primary antibodies. The antibodies for AR and ERα were supplied as a ready-to-use dilution and were not diluted further. The antibody for ERβ was diluted 100 times.

The bound antibodies were visualized by a biotinylated secondary antibody followed by incubation with an avidin-peroxidase conjugate (Sigma ExtrAvidin peroxidase staining kit; Fluka, Buchs, Switzerland) and aminoethylcarbazol as substrate. The sections were counterstained with haematoxylin.

As a negative control, the primary antibodies were replaced by molar equivalents of rabbit non-immune serum. Positive control tissue included human prostate for expression of AR and ERβ and human mammary carcinoma for ERα.

RNA isolation and RT-PCR

For RNA isolation, apocrine glands were removed from axillary skin with a scalpel. Total RNA was isolated by means of the RNeasy mini kit (Qiagen, Basel, Switzerland). The RNA concentration was determined by measuring the absorbance at 260 nm, and the purity and integrity of the RNA was checked by running a formaldehyde gel (1% agarose).

RT-PCR (reverse transcriptase-polymerase chain reaction) was performed employing the superscript one-step RT-PCR kit (Invitrogen). As template, 200 ng total RNA were used; the RT was performed for 30 min at 50°C, followed by 40 thermal cycles of 95°C for 15 s, 58°C for 30 s and 72°C for 30 s. The first cycle was preceded by denaturation at 94°C for 2 min, and the last cycle was followed by extension at 72°C for 10 min. As a negative control, the RNA (equal amount) was added after the RT step. PCR products were analysed by electrophoresis on a 2% agarose gel stained with ethidium bromide to visualize DNA bands. Primers for AR were 5′-ATGTCCTGGAAGCCATTGAG-3′ and 5′-GCTGTACATCCGGGACTTGT-3′ yielding a 323-bp fragment; they represent the sense sequence 3142–3161 and the antisense sequence 3445–3464, respectively (GenBank NM000044). Primers for ERβ were 5′-GTTCGTGGTGATGGAGGACT-3′ and 5′-CACTTCTGTGCTGGTGCATT-3′ yielding a 701-bp fragment; they represent the sense sequence 217–236 and the antisense sequence 898–917, respectively (GenBank BC011899).

Primers for ERα were 5′-TCGCTGTCTGACCAGATGTG-3′ and 5′-CCTCGAGCATCTCCAAGAAC-3′ yielding a 502-bp fragment; they represent the sense sequence 387–406 and the antisense sequence 869–888, respectively (GenBank BC033701).

Results

Generally, the results of immunohistochemistry (Fig. 1) and of RT-PCR analysis (Fig. 2) corresponded to each other, demonstrating the expression of AR and ERβ in the apocrine glands. No sex-specific differences for both AR or ERs were detected between male and female samples.

Androgen receptor

Strong AR immunoreactivity was restricted to the nuclei of the apocrine secretory epithelium whereas the cytoplasm showed only a weak diffuse reaction (Fig. 1A, B). In contrast, the nuclei of myoepithelial cells and fibroblasts were devoid of immunoreactivity (Fig. 1B). The majority of the segments of the apocrine glands consists of cuboidal or columnar epithelial cells. In some segments, however, the epithelium is very flat. In comparison to the tall epithelial cells, the nuclei of the flat epithelium segments were significantly weaker stained or even unstained (Fig. 1C). Furthermore, AR immunoreactivity was found in the nuclei of sebaceous glands (Fig. 1D). The nuclei of the secretory epithelium of the eccrine sweat glands showed no immunoreactivity. A positive granular staining, however, was found in their cytoplasm (not shown) which most probably is an unspecific reaction of secretory vesicles.

Estrogen receptor β

The apocrine secretory epithelium exhibited strong positive ERβ immunostaining in nuclei and in the cytoplasm (Fig. 1E, F) while the nuclei of myoepithelial cells and fibroblasts were devoid of immunoreactivity (Fig. 1F). Sebaceous glands showed similar staining as with the AR antibody (results not shown).

Estrogen receptor α

Immunoreactivity was found in the nuclei of the peripheral cells of sebaceous glands (Fig. 1G, H) while the secretory epithelium of the apocrine glands lacked ERα immunostaining in general. Solitary stained nuclei were only sporadically found in single glandular profiles (*arrow* in Fig. 1H). This finding of an absent or a very low occurrence of ERα was confirmed by the RT-PCR analysis (Fig. 2), indicating that the amount of ERα mRNA was below the detection limit.

Discussion

Androgen receptor and ERs belong to the steroid hormone nuclear receptor superfamily and function as hormone-dependent transcriptional factors that regulate genes for cell proliferation and differentiation (Lamb et al. 2001; Thornton 2002; Lee and Chang 2003). The receptors are required for normal development and function in tissues which are affected by those hormones during development, as well as after puberty. Our data, demonstrating the localization of AR and ERβ in the apocrine axillary gland indicate that those glands are direct targets of steroid hormone signalling.

Recent studies of human skin suggest that ERβ is the predominant ER in epidermis, hair follicles and sebocytes

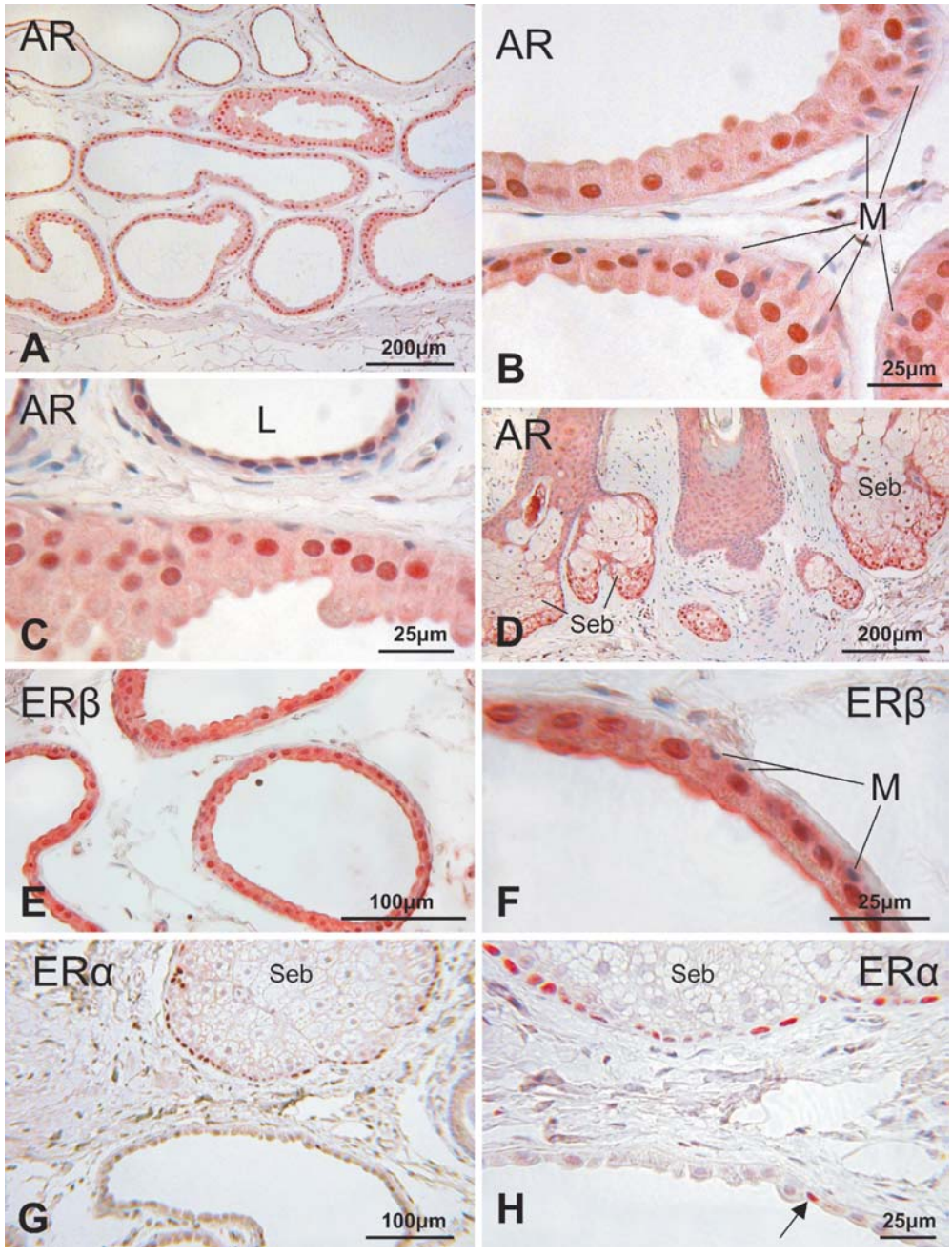

Fig. 1A–H Light micrographs from sections of axillary skin immunostained with antibodies to androgen receptor (*AR*), estrogen receptor α (*ER*α) and estrogen receptor β (*ER*β). Counterstaining with haematoxylin. **A** Apocrine glands, overview, immunostaining for AR. **B** Detail from **A**. Note the specific (*red*) immunostaining of the nuclei in the secretory epithelium of the apocrine glands. Nuclei of myoepithelial cells (*M*) are devoid of immunoreactivity. **C** Detail from **A** showing a segment with low epithelium (*L*) with weak or absent nuclear immunoreactivity. **D** Sebaceous glands (*Seb*) and basic hair follicles incubated with an antibody to AR. Note the specific nuclear immunostaining of the sebaceous gland cells. **E** Incubation with an antibody to ERβ showing immunostaining of cytoplasm and nuclei of the secretory epithelium of the apocrine glands. **F** Detail from **E**, nuclei of myoepithelial cells (*M*) are devoid of immunoreactivity. **G** Incubation with an antibody to ERα, overview with sebaceous gland (*Seb*) and apocrine gland. **H** Detail from **G** showing positive staining of peripheral cells of sebaceous glands and one single positive nucleus in the apocrine gland (*arrow*)

(Thornton 2002, 2003; Pelletier and Ren 2004). Our findings demonstrate that ERβ is the dominant, if not the only ER also in the apocrine axillary glands.

Androgen receptor has been localized by immunostaining in sebocytes (Choudry et al. 1992; Kimura et al. 1993; Bayer-Garner et al. 1999), sweat glands (Ruizfeld de Winter et al. 1991; Kimura et al. 1993), dermal papillae (Choudry et al. 1992; Kimura et al. 1993; Ando et al. 1999; Thornton et al. 2003) and in the apocrine glands of female genital skin (Choudry et al. 1992). The apocrine glands of the axilla had not been included in any of those studies. Earlier investigations using biochemical ligand binding assays (Kurata et al. 1990) localized AR in the cytosolic fraction of axillary apocrine glands of osmidrosis patients. However, they were unable to detect AR in the nuclear fraction which led to discussing the possibility that AR might have relocalized from nuclei to cytosol during tissue preparation. Our results confirm the nuclear localization of AR in the apocrine glands of the axilla.

The secretory epithelial cells of the apocrine glands vary in size from a high columnar type to a flat type. The height of the epithelium has been correlated with the secretory state of the cells, with the flat epithelium cells considered as resting or inactive (Hoepke 1927; Hurley and Shelley 1960). Accordingly, our results that show a significantly weaker AR immunoreactivity in the nuclei

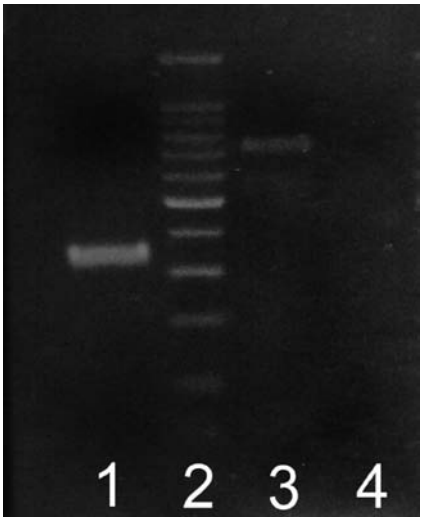

Fig. 2 Results from RT-PCR with primers for AR yielding a 323-bp fragment (*lane 1*) and for ERβ yielding a 701-bp fragment (*lane 3*). No PCR product was detected for ERα (*lane 4*, expected size: 502 bp). On each lane, 0.5 μl PCR product were applied. *Lane 2* 100-bp ladder

of the flat epithelial cells suggest a correlation between the activity of the cells and the expression of AR.

In sebaceous glands, androgen simulates the secretory activity while estrogen elicits a reduction in size and secretion of sebaceous glands in both men and women (Pochi and Strauss 1974). The occurrence of both AR and ERβ in the apocrine axillary glands, together with the earlier observation that levels of 5α-androstenone in axillary secretions are significantly higher in men than in women (Gower et al. 1985), suggest that the hormonal regulation of the axillary apocrine glands may be at least partially similar to that of the sebaceous glands. This hypothesis gains further support by the fact that androgens are known to upregulate multiple enzymes of the cholesterol synthesis (Swinnen et al. 1997; Heemers et al. 2003). Cholesterol biosynthesis presumably plays an important functional role within the apocrine axillary glands (Rothardt and Beier 2001) as cholesterol could serve as precursor for the biosynthesis of those volatile steroid molecules which act as pheromones (Cowley and Brooksbank 1991; Sobel et al. 1999; Grosser et al. 2000). Consequently, the induction of cholesterol biosynthesis by androgens may have an impact on the synthesis of pheromones. Thus, the results of the present study establish a possible direct link between androgen action and the induction of pheromone production in the apocrine glands of the human axilla.

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