DOI 10.1111/j.1365-2133.2005.06847.x

Gender differences and effects of sex steroids and dehydroepiandrosterone on androgen and oestrogen α receptors in mouse sebaceous glands

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Accepted for publication
13 May 2005

Key words:
androgens, dehydroepiandrosterone, intracrinology, oestrogens, sebaceous glands

Conflicts of interest:
None declared.

Summary

Background It is generally believed that the sebaceous gland is an intracrine organ which synthesizes its own active hormones to meet its local needs.
Objectives To understand further the mechanisms of sex steroid action in mouse sebaceous glands.
Methods We have used immunocytochemistry to examine the expression of oestrogen receptor α (ERα) and androgen receptor (AR) in mouse sebaceous glands.
Results In intact males AR is exclusively localized in the nuclei of basal and mature sebocytes, while in females it is present at a lower level in both the nuclei and the cytoplasm. Three weeks following gonadectomy (GDX), a marked decrease of AR labelling is observed in male sebocytes, while no change occurs in female sebocytes. Treatment of GDX animals with dihydrotestosterone (DHT) or dehydroepiandrosterone (DHEA) increases AR expression, while 17β-estradiol (E_2) decreases the stimulatory effect of DHT and DHEA. ERα is detected only in basal sebocytes of intact females but not in males. Following treatment with E_2, ERα expression becomes visible in GDX males while DHT and DHEA inhibit the effect of E_2.
Conclusions The present data show gender differences and demonstrate that DHT, E_2 and DHEA exert specific effects on the expression of AR and ERα in mouse sebocytes.

The main dermatological interest in the sebaceous glands relates to acne vulgaris and seborrhoea. It is well known that increased sebum production is an important element in the pathogenesis of acne vulgaris.[1,2] Endocrine mechanisms are known to play a major role in the proliferation and lipogenic activity of sebocytes.[3,4]

Previous studies have shown that the human sebaceous glands are capable of synthesizing cholesterol *de novo* while all the enzymes and cofactors required to use cholesterol and dehydroepiandrosterone (DHEA) as substrates for steroidogenesis are present in sebocytes.[5–7] Androgens are a major stimulus of sebaceous gland development and sebum secretion,[8] while oestrogens have been found to give direct suppression of human sebum secretion *in vivo*[9] and *in vitro*.[10] DHEA, on the other hand, exerts an exclusive androgenic stimulatory action on rat sebaceous glands.[11] It is thus generally believed that the sebaceous gland is an intracrine organ which synthesizes its own active hormones to meet its local needs.[5,6]

The objective of the present study is to understand further the role of androgens and oestrogens and to evaluate their differential effects in sebaceous glands of both male and female mice.

Materials and methods

Animals and treatments

In total, 63 male and 63 female C57BL6 mice (13–15 weeks old) weighing 24–32 g (males) and 20–28 g (females) were randomly distributed into nine groups of seven animals as follows: (i) intact control; (ii) intact + flutamide (Flu; 1·25 mg per mouse); (iii) gonadectomized (GDX) control; (iv) GDX + Flu; (v) GDX + 17β-estradiol (E_2; 0·025 mg per mouse); (vi) GDX + dihydrotestosterone (DHT; 0·1 mg per mouse); (vii) GDX + DHT + E_2; (viii) GDX + DHEA (6·25 mg per mouse); (ix) GDX + DHEA + E_2. On the first day of the study bilateral GDX was performed in the animals of the

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appropriate groups, while intact animals were sham-operated. Starting from the second day after surgery and for 3 weeks, DHT, E_2, DHEA and Flu were administered, alone or in combination. A subcutaneous injection of DHT (0·1 mg per mouse) was administered daily to the animals of the appropriate groups. Animals of the intact and GDX control group received by oral gavage the vehicle alone during the same period. The oral doses of E_2 and DHEA were selected based upon previous studies.[12–14] Three weeks after GDX, and 6 h after last drug administration, all animals were killed. The experiment was conducted in a facility approved by the Canadian Council on Animal Care and The Association for Assessment and Accreditation of Laboratory Animal Care in accordance with their Guide for Care and Use of Experimental Animals.

Tissue preparation

After shaving the long hair, an area of dorsal skin (2 × 1 cm) was carefully excised, flattened and immediately immersed in a solution of 10% buffered formalin. After fixation, whole mount technique was performed as described.[15]

Immunohistochemistry

Paraffin sections (4 μm) were deparaffinized in toluene and rehydrated through ethanol. Endogenous peroxidase activity was eliminated by preincubation in 3% H_2O_2 in methanol for 30 min. A microwave retrieval technique using citrate buffer was applied[16] and nonspecific binding was blocked using 10% goat serum. The sections were then incubated for 90 min at room temperature with antibodies against androgen receptor (AR) (1 : 300, N-20; Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.) or oestrogen receptor α (ERα) (1 : 200, MC-20; Santa Cruz Biotechnology). Zymed SP kit (San Francisco, CA, U.S.A.) was used and, under microscope monitoring, diaminobenzidine was applied as the chromogen to visualize the biotin/streptavidin-peroxidase complexes. As a negative control, an excess of the respective neutralized blocking peptide was incubated for 2 h at room temperature with the primary antibody.

Sebaceous gland histomorphometry

Light microscope examination of the skin whole mount revealed that, in the dorsal skin, each pilosebaceous unit of the overhair contains a pair of sebaceous gland lobes. Images of sebaceous glands were captured using a DC-330 3 CCD colour camera and the total area of seven pairs of sebaceous glands was measured in each animal using Image-Pro Plus (Leica Microsystems, Willowdale, ON, Canada).

Statistical analysis

Data were expressed as mean ± SEM of seven animals per group. Statistical significance was determined according to the multiple range test of Duncan–Kramer.[17]

Results

Immunohistochemical localization of androgen receptor and oestrogen receptor α

Androgen receptor expression

In intact males, AR was found to be expressed exclusively in the nuclei of basal and mature sebocytes, while no reaction was detected in the degenerating cells situated close to the gland neck (Fig. 1a). Interestingly, in intact females, a low level of expression of AR was detected in both the nuclei and cytoplasm of basal and mature cells, while no reaction was seen in the degenerating cells (Fig. 1j). When intact males received Flu, no change was observed in AR expression (Fig. 1b). However, in intact females treated with Flu, AR localization disappeared from the cytoplasm and became exclusively and strongly localized in the nuclei, thus leading to a pattern similar to that seen in males (Fig. 1k).

Three weeks after castration, AR expression decreased dramatically in male sebocytes and a weak nuclear and cytoplasmic labelling comparable with that of intact females was observed (Fig. 1c). No significant change in AR expression was observed in GDX females (Fig. 1l). When GDX mice of both sexes received Flu, AR was strongly and exclusively expressed in the nuclei of basal and mature sebocytes (Fig. 1d,m). Similarly, when GDX animals of both sexes received DHT or DHEA, a strong level of AR expression became comparable with that observed in intact males (Fig. 1e,g,n,p). In E_2-treated male and female GDX mice, AR was expressed at a low level comparable with that of intact females; AR was thus weakly expressed in the nuclei and was found in the cytoplasm (Fig. 1f,o). In contrast, when GDX male or female mice received the combination of DHT and E_2 or DHEA and E_2, AR expression was found to be similar to that observed in animals that had received DHT or DHEA alone (Fig. 1h,i,q,r).

Oestrogen receptor α expression

In the sebaceous glands of all groups, ERα immunostaining revealed some cytoplasmic background in sebocytes which persisted after the use of the blocking peptide.

In intact males ERα was not detected in the sebaceous glands (Fig. 2a), while in intact females a strong labelling was detected exclusively in the nuclei of basal cells (Fig. 2j). In male mice, the absence of ERα labelling persisted after Flu treatment (Fig. 2b) and GDX (Fig. 2c) as well as following DHT treatment (Fig. 2e), while after DHEA administration some basal cell nuclei became slightly labelled (Fig. 2g).

When GDX males received E_2 alone, ERα became detectable in the nuclei of basal cells (Fig. 2f). Meanwhile, in GDX females that received E_2, ERα was strongly expressed in the nuclei of basal cells (Fig. 2o). In intact or GDX females, administration of the pure antiandrogen Flu did not affect the strong expression of ERα in basal sebocytes (Fig. 2k,m).

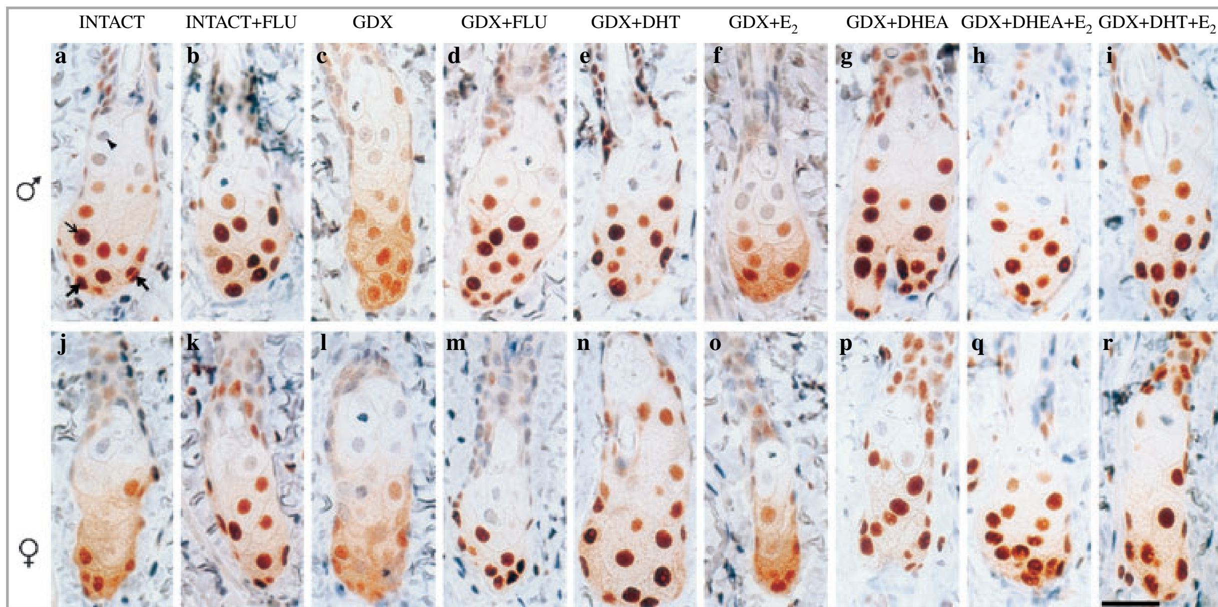

Fig 1. Comparison of androgen receptor (AR) labelling in male and female sebaceous glands of mouse dorsal skin of intact animals, intact animals that received 3 weeks of treatment with flutamide (FLU), control gonadectomized (GDX) animals, and GDX animals after 3 weeks of treatment with FLU, dihydrotestosterone (DHT), 17β-estradiol (E_2), dehydroepiandrosterone (DHEA), DHEA + E_2 and DHT + E_2 (×500). In most of the paraffin sections, AR is detected exclusively in the nuclei as seen in a, b, d, e, g, h, i, k, m, n, p, q and r. However, nuclear as well as cytoplasmic AR labelling is seen in c, f, j, l and o. In general, basal cells (thick arrows) and mature cells of the lower part of the gland (thin arrow) are labelled, while mature cells of the upper gland gradually lose their labelling and the degenerating cells (arrowhead) are not labelled. Bar = 20 μm.

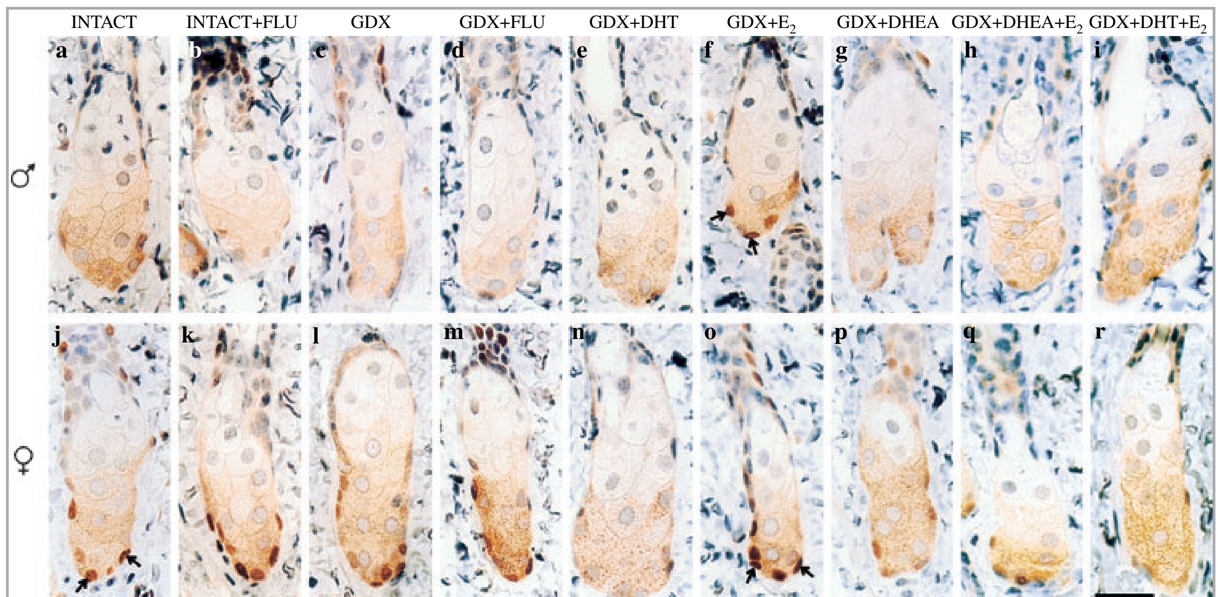

Fig 2. Comparison of oestrogen receptor α (ERα) labelling in male and female sebaceous glands of mouse dorsal skin of intact animals, intact animals that received 3 weeks of treatment with flutamide (FLU), control gonadectomized (GDX) animals, and GDX animals after 3 weeks of treatment with FLU, dihydrotestosterone (DHT), 17β-estradiol (E_2), dehydroepiandrosterone (DHEA), DHEA + E_2 and DHT + E_2 (×500). In male mice, all sebocytes show an absence of clear ERα labelling. However, when GDX males received E_2, ERα was detected in the basal cell nuclei (arrows) as seen in (f). In intact, GDX and GDX females that received E_2, labelled basal cell nuclei (arrows) could be detected (j, l, o, respectively). FLU treatment did not affect ERα labelling in intact (k) or GDX (m) animals while DHEA and DHT decreased E_2-induced ERα labelling (q, r). Nonspecific background labelling of ERα was present in the cytoplasm of all treated and control sebaceous cells. Bar = 20 μm.

The GDX females treated with DHT did not express ERα (Fig. 2n), while in the GDX females treated with DHEA a weak expression of ERα was detected in some basal cells (Fig. 2p). Interestingly, when GDX animals of both sexes were treated with the combination of DHT and E_2, the strong expression of ERα seen in the E_2-treated groups (Fig. 1f,o)

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Table 1 Comparison of the effect of gonadectomy (GDX) and of the indicated treatments on sebaceous gland size in male and female mice after 3 weeks of treatment

Treatment	Dorsal skin sebaceous gland surface area (μm^2) Male	Female
Intact	4287 ± 166**	2962 ± 88**
Intact + FLU	3754 ± 189**·+	3009 ± 173**
GDX	2936 ± 143	3534 ± 214
GDX + FLU	2913 ± 115	3118 ± 116*
GDX + E_2	2872 ± 137	2796 ± 138**
GDX + DHT	4604 ± 164**	5134 ± 185**
GDX + DHT + E_2	3874 ± 122**	3906 ± 22*
GDX + DHEA	3423 ± 114*	3186 ± 93
GDX + DHEA + E_2	2794 ± 124	2756 ± 60**

FLU, flutamide; E_2, 17β-estradiol; DHT, dihydrotestosterone; DHEA, dehydroepiandrosterone. Values are expressed as mean ± SEM ($n = 7$ animals) obtained from measurements of the total area of sebaceous glands. *$P < 0{\cdot}05$, **$P < 0{\cdot}01$ experimental vs. GDX control, +$P < 0{\cdot}05$ experimental vs. intact control (Duncan–Kramer multiple range test).

was markedly decreased (Fig. 2i,r). A similar inhibitory effect on ERα expression was observed when DHEA was added to E_2 (Fig. 2h,q).

Sebaceous gland size

In the intact male group, sebaceous glands were significantly larger than in females by 45% ($P < 0{\cdot}01$) (Table 1). When intact animals received the antiandrogen Flu, sebaceous gland size decreased by 14% ($P < 0{\cdot}05$) in males (Fig. 3) while no significant change was observed in females (Fig. 4).

Three weeks after GDX, a 46% atrophy was observed in males ($P < 0{\cdot}01$) while, in GDX females, sebaceous gland size increased by 19% when compared with the intact female group ($P < 0{\cdot}01$). When GDX animals received Flu, a significant 13% decrease in sebaceous gland size ($P < 0{\cdot}05$) was observed only in females when compared with the GDX female control group ($P < 0{\cdot}01$). E_2 treatment, in contrast, decreased the size of GDX female sebaceous glands by 26% ($P < 0{\cdot}01$), while in males the size of sebaceous glands did not change.

DHT treatment of GDX animals significantly increased the size of sebaceous glands by 57% ($P < 0{\cdot}01$) in males and by 45% ($P < 0{\cdot}01$) in females. When GDX males received DHT treatment in combination with E_2, a significant reduction of 19% ($P < 0{\cdot}01$) was observed when compared with DHT treatment alone. Similarly, in GDX females treated with both DHT and E_2, a 31% reduction ($P < 0{\cdot}01$) was observed in comparison with DHT treatment alone.

After DHEA treatment, sebaceous gland size of GDX animals increased significantly by 17% ($P < 0{\cdot}05$) in males, while no significant change was observed in females. In contrast, combined administration of DHEA and E_2 to GDX males significantly reduced sebaceous gland size by 23% ($P < 0{\cdot}01$) when compared with the GDX males treated with DHEA alone. In females, the reduction caused by E_2 was measured at 16% ($P < 0{\cdot}05$) when compared with the GDX group that received DHEA alone.

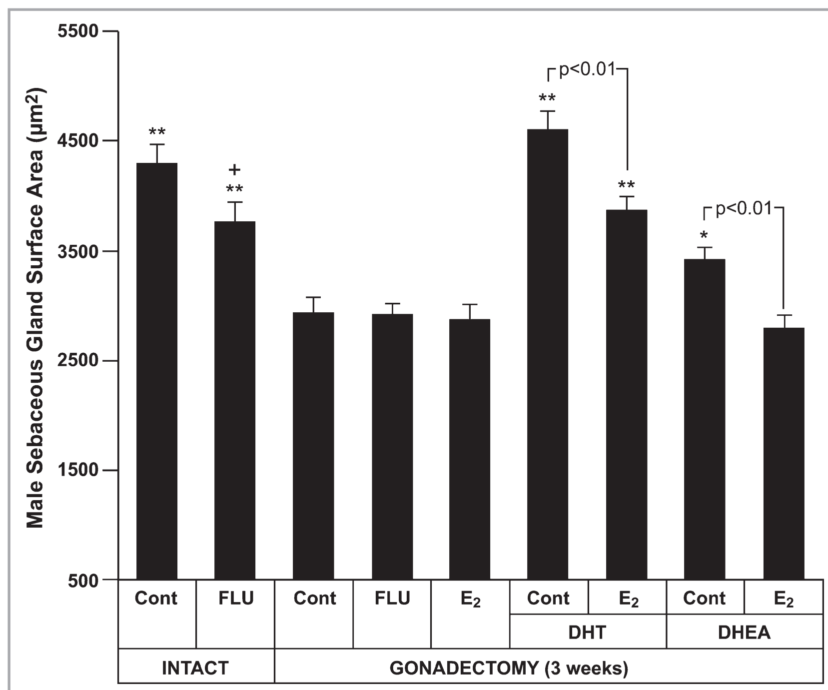

Fig 3. Sebaceous gland area of dorsal skin of male mice. The effects of 3 weeks of gonadectomy (GDX) and the administration of the indicated compounds on the size of the glands are presented. Cont, control; FLU, flutamide; E_2, 17β-estradiol; DHT, dihydrotestosterone; DHEA, dehydroepiandrosterone. Values are expressed as mean ± SEM. +$P < 0{\cdot}05$ experimental vs. intact control; *$P < 0{\cdot}05$, **$P < 0{\cdot}01$, experimental vs. GDX control (Duncan–Kramer multiple range test).

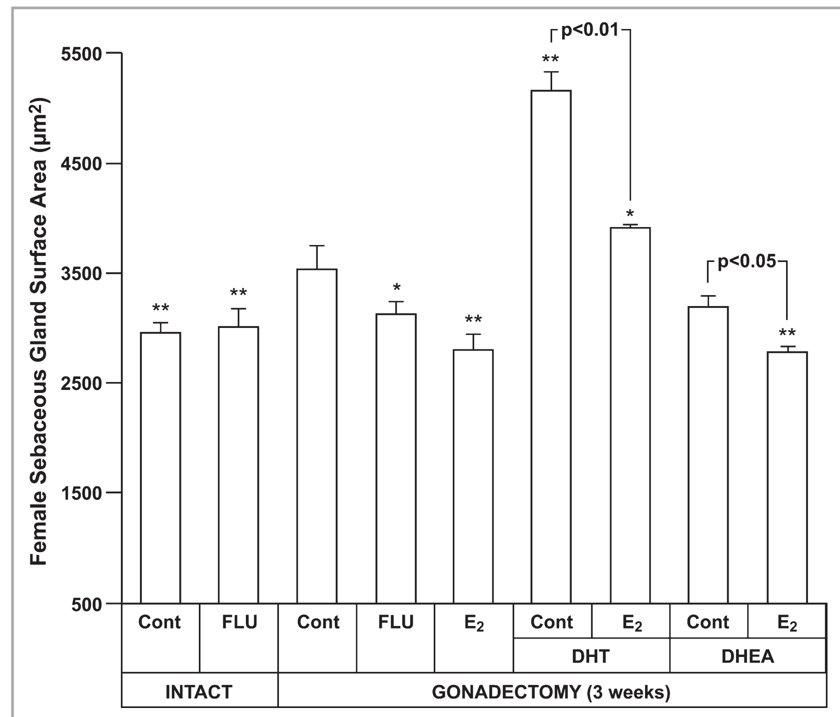

Fig 4. Sebaceous gland area of dorsal skin of female mice. The effects of 3 weeks of gonadectomy (GDX) and the administration of the indicated compounds on the size of the glands are presented. Cont, control; FLU, flutamide; E_2, 17β-estradiol; DHT, dihydrotestosterone; DHEA, dehydroepiandrosterone. Values are expressed as mean ± SEM. *$P < 0{\cdot}05$, **$P < 0{\cdot}01$, experimental vs. GDX control (Duncan–Kramer multiple range test).

Discussion

The AR-positive immunostaining supports the evidence which demonstrates the predominant role of androgens in the sebaceous glands. A particularly interesting finding of the present study is that the pattern of expression of AR is markedly different in male and female sebocytes. AR is strongly and exclusively expressed in the nuclei of male sebocytes, while in the female the receptor is expressed at a lower level and is diffuse in both cytoplasmic and nuclear compartments. Moreover, following androgen depletion by GDX in the male, the expression of AR changes to a pattern similar to that observed in intact females.

The localization of ligand-free AR is somewhat controversial. Depending upon the cell type, both cytoplasmic and nuclear localizations of AR have been reported in the absence of hormones.[18] The present data clearly demonstrate the cytoplasmic localization of AR in the absence of androgens, while its transfer to the nuclear compartment is highly androgen specific.

The strong and exclusive nuclear localization of AR in both intact and GDX male and female mice treated with Flu shows that the antiandrogen does not act by inhibiting the nuclear transfer of AR. After Flu treatment of GDX animals of both sexes for 3 weeks, AR shifts from the cytoplasm to the nuclei. A similar observation was reported in a study investigating the effect of antiandrogens on AR localization.[18] These authors observed that antiandrogens in cell cultures led to the nuclear localization of AR without activating transcription of androgen-regulated reporter genes, thus indicating that the transfer of AR to the nucleus occurred independently from activating the transcription of androgen-regulated genes.

It is of special interest that in GDX males and females treated with DHEA, AR was exclusively localized in nuclei of sebocytes, a localization pattern similar to that observed in intact males and in DHT-treated GDX animals of both sexes. In order to interact with AR, DHEA must be transformed in target cells by the enzymes 3β-hydroxysteroid dehydrogenase (HSD), 17β-HSD and 5α-reductase into active androgens.[5,6,19] These enzymes are present in the skin,[5,6,20–22] thus enabling the effect of DHEA on AR expression. A previous study has shown that DHEA has an exclusive androgenic effect and that rat sebaceous glands and human sebocytes are able to convert DHEA into potent androgens.[11,23]

It is well recognized that oestrogens suppress human sebaceous gland secretion, this therapeutic effect being applied in the treatment of acne.[24] The present data establish clear gender differences in the expression of ERα in the sebaceous glands where it is detected exclusively in the nuclei of basal female sebocytes and is absent in the male. Recently, in a study of human skin it was observed that ERα is widely distributed in sebaceous glands of scalp skin of both men and women,[25] thus indicating differences between human and mouse skin. When GDX female mice received DHT treatment, ERα completely disappeared, as observed in male sebocytes, thus suggesting an inhibitory effect of DHT on ERα expression. In agreement with this finding, West *et al.*[26] described a similar inhibitory effect of androgens on ER level of the seminal vesicles of the rhesus monkey.

E_2 treatment of GDX female animals induced a marked increase in the expression of ERα in basal sebocytes. Moreover, in GDX males treated with E_2, ERα appeared for the first time in the nuclei of basal sebocytes. Such data clearly suggest that the presence of E_2 stimulates the expression of

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ERα in basal sebocytes, independently of the sex of the animals.

In male mice the present data show that GDX reduces sebaceous gland size, while a small, but opposite, effect is observed in females. Removal of androgens by GDX in the male mouse affected sebaceous glands by reducing their size by 46% within 3 weeks while, in GDX females, the lack of oestrogens induced a 19% increase in sebaceous gland size. Such data indicate important gender differences in the regulation of sebaceous gland size. In addition to androgens and oestrogens, other factors are well known to affect sebaceous gland activity, namely thyroid hormone, insulin-like growth factors, growth hormone, and adrenocorticotropic hormone.[27,28]

In GDX males E_2 did not cause an additive suppressive effect on sebaceous gland size in addition to the atrophy caused by androgen ablation, while in females E_2 significantly reduced sebaceous gland size which became similar to the value found in intact females.

On the other hand, without gender distinction, concomitant administration of DHT and E_2 to GDX animals significantly decreased sebaceous gland size when compared with animals treated only with DHT. A similar effect has been reported in the rat where the administration of testosterone and E_2 caused an inhibition of sebaceous gland size and sebum secretion despite an increased mitotic rate.[29] It has also been reported that oestrogens suppress androgen-stimulated sebaceous gland activity at particularly low doses.[30]

DHEA induced an increase in sebaceous gland size in the GDX males, an effect probably resulting from the transformation of DHEA into androgens. In agreement with these data, the local transformation of DHEA into sex steroids having potent androgenic activity has been reported in humans[31] and in the sebaceous glands from hamster flank organs and ears.[32]

In conclusion, although the present findings in mice remains to be confirmed in the human, the present data show that androgens and oestrogens exert specific and sometimes antagonistic effects in the mouse sebaceous glands, these effects being generally associated with changes in AR and ERα expression. Steroid receptors can cross-talk and interact with each other,[33] while both androgens and oestrogens can activate nonclassical genomic pathways.[34] The present data thus show for the first time that AR and ERα expression in mouse sebaceous glands is gender specific and is dependent upon the specific and sometimes opposite effects of sex steroids.

Acknowledgments

We thank Dr Céline Martel and Mrs Louise Berger for their much appreciated help. This study was supported by Endorecherche.

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