Exclusive androgenic effect of dehydroepiandrosterone in sebaceous glands of rat skin

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Abstract

In order to analyze the hormonal effects of dehydroepiandrosterone (DHEA) in skin sebaceous glands, the precursor steroid was administered to ovariectomized (OVX) female Sprague–Dawley rats at a dose of 30 mg applied on the dorsal skin, twice daily, for 3, 6 and 12 months. In a parallel experiment, female OVX rats were treated with DHEA at the same daily percutaneous dose of 30 mg, alone or in combination with the antiandrogen Flutamide or the pure antiestrogen EM-800, for 12 months, in order to determine the androgenic and/or estrogenic components of DHEA action. Treatment of female OVX rats with DHEA resulted in a similar mild to moderate hyperplasia of the sebaceous glands of both dorsal (site of application) and ventral skin, as illustrated by an increase in the number and size of the acini. The above-indicated effects were observed at all time intervals studied, beginning at 3 months of treatment, and they were not further increased after longer term administration of DHEA (for 6 and 12 months). The addition of Flutamide to DHEA treatment completely prevented the DHEA-induced changes in the sebaceous glands, whereas the antiestrogen EM-800 had no effect. The present data indicate an exclusive androgenic stimulatory action of DHEA on the sebaceous glands, thus pointing out the importance of local intracrine DHEA transformation into androgens for skin anatomical integrity and function, while showing that estrogens, if active in rat skin, do not originate from DHEA.

Journal of Endocrinology (2000) 166, 455–462

Introduction


There is already convincing evidence that DHEA of adrenal origin may have an important androgenic influence on sebaceous gland activity. In fact, transformation of DHEA into testosterone and other metabolites has been observed in the skin (Cameron et al. 1966, Gallegos & Berliner 1967, Faredin et al. 1969, Chrousos et al. 1982, Voigt et al. 1984, Martel et al. 1992, 1994). Nevertheless, although the steroids synthesized in various skin compartments could possibly have some systemic effects, it is more likely that the steroids synthesized locally in each appropriate cell type from adrenal precursors exert their effects in the same cells in which they are produced, without being released outside the cells of origin; this new area of endocrinology is called intracrinology (Labrie et al. 1988, Labrie 1991).

Abnormalities of androgen action are frequent and cosmetically important findings. These abnormalities include acne, seborrhea, hirsutism and androgenic
alopecia. Such androgen excess results from either increased androgen production in the ovary, overproduction of DHEA or androstenedione by the adrenal gland, excess local formation of androgens or a combination of these types of mechanisms (Lookingbill et al. 1985). In fact, increased skin 5α-reductase (Sansone & Reisner 1971, Kuttner et al. 1977, 1979) and 3β-HSD (Thomas & Oake 1974) activities have been reported in hirsutism and acne. On the other hand, several studies describe elevations in serum testosterone and/or dehydroepiandrosterone sulfate (DHEA-S) in women with acne (Lucky et al. 1974) activities have been reported in hirsutism and acne.

Moreover, changes in androgen receptor levels could be involved (Choudry et al. 1992, Randall et al. 1992, Hibberts et al. 1998). In the present study, we used the ovariectomized female Sprague–Dawley rat as a model to evaluate the effect of DHEA (given percutaneously for 3, 6 or 12 months) on the histomorphology of the skin and its appendages and on the sebaceous glands in particular. In addition, we administered a combination of DHEA and the pure antiandrogen Flutamide (Neri et al. 1986) or the pure antiestrogen EM-800 (Gauthier et al. 1997, Luo et al. 1997a,c, Simard et al. 1997, Luo et al. 1998) for 12 months in order to analyze the androgenic and/or estrogenic component(s) of the action of DHEA in rat skin.

Materials and Methods

Animals

Adult female Sprague–Dawley rats (Crl:CD(SD)Br) (Charles River Laboratory, St-Constant, Canada), at 3 months of age and weighing 230–310 g at the start of the study, were used. The animals were acclimated to the environmental conditions (temperature at 22 ± 2 °C, 14 h light, 10 h dark cycles, lights on at 0715 h) for at least one week before the start of the experiment. The animals were housed two per cage and were allowed free access to tap water and a commercial pellet diet (Agway ProLab R–M–H 4018, Syracuse, NY, USA). The experiment was conducted in a facility approved by the Canadian Council on Animal Care and in accordance with the CCAC Guide for Care and Use of Experimental Animals.

Treatment

In the first experiment, the animals were randomly divided into three groups each containing eight rats as follows: a) intact; b) ovariectomized (OVX) control; and c) OVX+DHEA. In the second experiment, the animals were divided into five groups each containing eight animals, as follows: 1) intact control; 2) OVX control; 3) OVX+DHEA; 4) OVX+DHEA+Flutamide (FLU); 5) OVX+DHEA+EM–800.

On the first day of the experiment, the animals of the appropriate groups were bilaterally OVX under isoflurane-induced anesthesia. DHEA was administered percutaneously on an area 2 × 2 cm in 0·050 ml 50% ethanol/50% propylene glycol (v/v) on shaved dorsal skin at a dose of 30 mg, twice daily, for 3, 6 and 12 months; in the second experiment, the duration of treatment was 12 months. The dose of DHEA chosen was based on our previous study, in which different doses and routes of administration were compared (Labrie et al. 1996a).

The antiandrogen Flutamide (FLU, 4′-nitro–3′-trifluoromethylisobutyranilide; 7·5 mg) was administered by s.c. injection, twice daily, while the antiestrogen EM–800 (4′/(+)-7-pivaloyloxy-3-((4′-pivaloyloxyphenyl)-4-methyl-2-(4′-2″′–piperidinoethoxy)phenyl)-2H-benzopyran) (Gauthier et al. 1997, Luo et al. 1997b, Simard et al. 1997) was administered orally at a dose of 250 μg, once daily. Treatment was initiated on the morning of day 1 of the experiment. FLU and EM–800 were administered in 4% ethanol, 4% polyethylene glycol-600, 1% gelatin and 0·9% NaCl. Flutamide was generously supplied by Dr Rudi Neri (Schering-Plough Research Institute, Kenilworth, NJ, USA) while DHEA was purchased from Diosynth Inc. (Chicago, IL, USA). EM–800 was synthesized in the medicinal chemistry division of our laboratory as described elsewhere (Gauthier et al. 1997).

Histological procedures

At the end of the experiment, the animals were killed (by decapitation) and sections of dorsal skin (from the site of application and the adjacent area 1–3 cm away from the application site) as well as ventral skin from each animal were carefully excised, after shaving, flattened and then fixed in 10% buffered formalin solution. Tissue sections were routinely processed in a tissue processor and 5 μm-thick sections were mounted and stained with hematoxylin–eosin. Four longitudinal sections from both dorsal– and ventral skin specimens were examined. Histopathological examination was performed using light microscopy.

Sebaceous gland histomorphometry

Measurements of dermal sebaceous glands were obtained from four longitudinal sections. Images were captured with a DC-330 3 CCD color camera (Dage-MTI, Michigan City, IN, USA) and quantified using IMAGE-PRO PLUS 3.0 software (Media Cybernetics, Silver Spring, MD, USA). Using a × 5 objective (Leica Microsystems, Willowdale, Ont., Canada), both the number and area of all sebaceous gland acini were collected on five luminal fields from each of the four sections of dorsal or ventral skin, for a total of 20 luminal fields analyzed per animal.
Additionally, the total length of each luminal field analyzed was measured at the internal limit of dermis reached by the sebaceous glands.

Results

In control, OVX, untreated animals 3, 6 or 12 months after ovariectomy, the sebaceous glands of both the dorsal and ventral skin are composed of small cells each showing poor staining of the nucleus and cytoplasm, a small number of acidophilic granules and an absence of lipid droplets. The acini have a small lumen in the immediate vicinity of the duct of the acinus (Fig. 1). The appearance was not different from that of intact female rats of the same age.

After 3, 6 or 12 months of DHEA administration to OVX animals, a mild to moderate increase in the number and size of the sebaceous glands is seen, to a similar degree, in both dorsal and ventral skin. This change is due to the enlargement and budding of the acini as well as an increase in the size of the individual sebaceous cells (Fig. 1). Interestingly, no differences were observed in the degree of the above-indicated histological changes of the sebaceous glands after either 3, 6 or 12 months of DHEA administration.

The quantitative analysis performed after 6 months of treatment demonstrates that DHEA caused increases of 170 and 175% in the number of glands in the dorsal and ventral skin, respectively (Fig. 2A and B). The total surface area of the sebaceous glands was similarly stimulated by DHEA at both sites, showing increases of 225 and 260% in the dorsal and ventral skin, respectively (Fig. 3A and B).

In order to differentiate between androgenic and/or estrogenic effects of DHEA, the pure antiandrogen Flutamide and the pure antiestrogen EM-800 were administered concomitantly with DHEA. Concomitant administration of DHEA and Flutamide abolished the effects of DHEA on the number and surface of sebaceous glands in both dorsal and ventral skin (Figs 2–4). In the group of animals that received DHEA and Flutamide, the histological pattern was similar to that seen in OVX control animals (Fig. 4). It is noteworthy that the effect of Flutamide was somewhat more striking on ventral skin glands. In contrast, the addition of EM-800 to DHEA had no influence on the effects of DHEA on skin histomorphology (Figs 2–4), the values not being significantly different from those obtained for animals that received only DHEA.

Discussion

The present data show that treatment with DHEA results in a significant stimulation of the sebaceous glands, characterized by an increase in both their number and their size. Since the skin is a tissue rich in steroidogenic enzymes and as it is the largest organ in the body, such data strongly suggest that the skin should be considered as an important site of sex-steroid formation. As mentioned above, the skin possesses all the enzymes required for the transformation of steroid precursors of adrenal origin, namely DHEA and its sulfate, DHEA-S, into active androgens and estrogens (Baillie et al. 1966, Pochi & Strauss 1969, Luu-The et al. 1989, Labrie 1991, Labrie et al. 1992, Luu-The et al. 1994).

The sebaceous gland itself has been shown to actively convert testosterone into DHT and other 5α-reduced steroids, such as 5α-androstane-3β, 17β-diol and 5α-androstane-3α,17β-diol in vitro, through the action of 5α-reductase, in both rats and humans (Sansone & Reisner 1971, Bingham & Shaw 1973, Hodgins & Hay 1973, Lutsky et al. 1974, Bowden et al. 1976, Cooper et al. 1976). In addition, human skin and rat preputial glands have been shown to actively metabolize DHEA to various compounds including androstenedione, 5α-androstanedione, androsterone, androst-5-ene-3β,17β-diol, 7α-hydroxy-DHEA and 7-keto-DHEA, whereas the conversion of DHEA to androstenedione, testosterone and DHT has been described as occurring in the sebaceous glands of human facial skin (Hay & Hodgins 1973, Thomas & Oake 1974, Hodgins & Hay 1976, Takayasu 1979, Hsia et al. 1983).

Androgens are well known for causing enlargement of the sebaceous glands in rats (De Graaf 1942, Ebling 1948, Haskin et al. 1953, Lasher et al. 1954), rabbits (Montagna & Kenyon 1949), hamsters (Hamilton & Montagna 1950) and mice (Lapiere 1953). Moreover, testosterone is known to prevent the reduction in volume of the sebaceous glands following castration in male rats, the effect of the androgen resulting from increased cell proliferation and reduced cell turnover (Ebling 1963). In contrast, estrogens have been reported to inhibit the growth, and reduce the size, of the sebaceous glands (Hooker & Pfieffer 1943, Ebling 1948).

The superimposable stimulatory effect of DHEA on the sebaceous glands at distant and local sites of DHEA application indicate that DHEA is well absorbed in the general circulation. This situation is analogous to that in humans, in which DHEA secreted by the adrenal gland reaches the peripheral target sites via the circulation (Labrie et al. 1988, Labrie 1991). The intracrine formation of androgens and/or estrogens thus depends upon the expression of androgen- and/or estrogen-forming steroidogenic enzymes.

Interestingly, as indicated by the effect of Flutamide in the present study, the influence of DHEA and DHEA-S on sebaceous gland activity is mediated by testosterone and DHT in the skin. It should be mentioned that a significant correlation has been reported between the activity of 3β-HSD in human sebaceous glands and their secretory activity (Cameron et al. 1966, Oertel & Treiber 1969, Hay & Hodgins 1973, Simpson et al. 1983). The presence of androgen receptors (Kimura et al. 1993) in several
structures of the skin, including the sebaceous glands, suggests that androgens regulate the activity of the sebaceous glands. This intracrine steroidogenic activity provides an explanation for the observation that the sebaceous glands develop fully in both boys and girls in utero (Serri & Huber 1963, Sharp et al. 1976).

In agreement with the present data, the histomorphological changes induced by DHEA in the skin of OVX female rats, especially in the sebaceous glands, could result from the intracrine local transformation of DHEA into steroids having androgenic activity. Pochi & Strauss (1969) and Drucker et al. (1972) have reported that the administration of 4-dione and DHEA stimulates sebaceous gland secretion in humans, while Chen and coworkers (Chen et al. 1996a) have demonstrated that the administration of DHEA and 4-dione, constantly released from silastic implants, are potent stimulators of the sebaceous glands of the flank organs and ears in the hamster.

At puberty, the increase in the secretion of DHEA, and especially of DHEA-S (de Peretti & Forest 1978), is

![Figure 1](image1.png)

**Figure 1** Stimulation of sebaceous glands of ventral skin of OVX female rats by DHEA administered at a distant site (dorsal skin). The histology of ventral skin after 3 (D), 6 (E) or 12 (F) months of treatment with DHEA is shown. A similar mild to moderate increase in both the number and the size of the sebaceous glands (→) was seen, at all time intervals studied. Compare with OVX controls 3(A), 6(B) or 12(C) months after ovariectomy. Bar=50 µm.

![Figure 2](image2.png)

**Figure 2** Effects of DHEA administered alone or in combination with Flutamide or EM-800 on the number of sebaceous gland acini in dorsal skin (A) and ventral (B) skin of OVX rats. The results are expressed as means ± S.E.M. ***P<0.001, **P<0.01 OVX controls versus all the other experimental groups (eight animals per group). The group receiving DHEA is significantly (P<0.05 for dorsal skin and P<0.001 for ventral skin) different from the group that received both DHEA and Flutamide.
Figure 3 Effects of DHEA administered alone or in combination with Flutamide or EM-800 on the surface area of sebaceous glands in dorsal skin (A) and ventral (B) skin of OVX rats. The results are expressed as the means ± S.E.M. ***P<0.001, OVX controls versus all the other experimental groups (eight animals per group).

Figure 4 Antagonism of the effect of DHEA on ventral skin by the antiandrogen Flutamide. The histology of ventral skin in intact rats (A), OVX rats (B) and OVX rats treated with DHEA alone (C) or with a combination of DHEA and Flutamide (D) or DHEA and EM-800 (E) for 12 months is shown. The stimulatory effects of DHEA on the growth and size, as well as the secretory activity, of the sebaceous glands of ventral skin, seen after 12 months of treatment of OVX animals (C), were completely abolished by the concomitant administration of Flutamide (D). The addition of EM-800, however, had no effect on the DHEA-induced histological changes in the skin (E). Compare with intact (A) and OVX controls (B). Bar=50 µm.
associated with an increase in sebaceous gland size and sebum production (Ramasar et al. 1970, Pochi et al. 1977) which frequently leads to problems of acne (Milne 1969). The activity of 5α-reductase in genital skin has been found to be higher in hirsute than in normal women, the values being intermediate between those of normal women and men (Mowssowicz et al. 1983, Serafini & Lobo 1985, Serafini et al. 1985). In addition, it has been found that sebaceous gland size is increased in patients with hirsutism and acne (Burton et al. 1972, Plewig 1974) while a higher level of DHEA metabolism has been described in the skin of hirsute women relative to that of normal women (Thomas & Oake 1974).

Although the presence of estrogen receptors has been described in both human skin and mouse skin (Hasselquist et al. 1980) and estrogen has been reported to increase vascularization of skin in rodents (Reynolds & Foster 1940), the effect of DHEA on rat skin dermis appear to result exclusively from androgenic action, as demonstrated by the reversal of the above-described effects by the addition of the specific antiandrogen Flutamide. In contrast, the co-administration of the specific antiestrogen EM-800 did not alter the histological changes induced by DHEA, thus excluding significant estrogenic action by DHEA on skin.

The present study provides morphological evidence that DHEA secreted by the adrenal cortex has an exclusive androgenic influence on the histomorphology and function of the sebaceous glands, probably via its intracrine conversion into testosterone and DHT. The development of inhibitors acting locally in the skin to block the intracrine formation of androgens offers the potential for an effective treatment of common skin diseases, such as acne and hirsutism, without systemic effects.

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