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The Induction of Epidermal Ornithine Decarboxylase Following UV-B Irradiation Is Inhibited by Estriol

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The induction of epidermal ornithine decarboxylase (ODC) can be partially blocked by corticosteroids, retinoic acid or active vitamin D_3. The influence of the other members of this so-called "steroid hormone receptor superfamily", namely the sex-steroids and thyroid hormone, is unknown in epidermis, but they enhance ODC induction in certain other tissues. Here we investigated whether topical estriol leads to a spontaneous and/or enhanced epidermal ODC induction 8 h after UV-B irradiation of 6 postmenopausal women. Contrary to expectation, estriol did not stimulate induction but reduced induction by 44%. This observation raises the possibility that all members of the steroid hormone receptor superfamily may share a common AP-1 binding site.

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Ornithine decarboxylase (ODC) is the rate-limiting enzyme in the production of polyamines, which are essential for DNA duplication. Hence, ODC is used as "marker-enzyme" for proliferation. Under physiological circumstances, epidermal ODC is biochemically not detectable but can be induced following standardized injuries such as tape stripping of the stratum corneum (activity peak after 8 h) (1) or irradiation with UV-B (peak after 24 h) (2). This induction can be partially blocked by certain members of the so-called "steroid hormone receptor superfamily", namely corticosteroids (1), retinoic acid (3) and active vitamin D_3 (4). The influence of other members, the sex-steroids (progesterone, estrogens, testosterone) and thyroid hormone, has not yet been investigated in the epidermis, although they enhance ODC induction is other tissues (5–7). Since corticosteroids, retinoic acid and calcipotriol are potent antipsoriatic drugs, it seems worthwhile to study the effects of the other members of this superfamily on epidermal ODC induction.

Human keratinocytes contain estrogen receptors in varying concentrations, apparently depending on location and age (8–10). However, exact information is not available due to limitations in detection techniques. In the epidermis estrogens have once been reported to increase the mitotic rate (11), but this single early report has remained unconfirmed. They have negative effects on epidermal adnexal structures, i.e. by reducing the size and activity of sebaceous glands (12) and the rate of hair growth (13). In the dermis estrogens stimulate the synthesis, maturation and turn-over of collagen (14), increase the synthesis of hyaluronic acid (15) and increase the vascularization (16).

Here we have addressed the following question: Does topical application of estriol lead to a spontaneous and/or enhanced induction of ODC in human epidermis in vivo? ODC measurements were carried out in vehicle- and estriol-treated epidermis 8 h after irradiation with UV-B, when ODC activity is still suboptimal, and in estriol-treated epidermis only.

METHODS

Subjects

Six postmenopausal women (age range 57–69 years) without estrogen substitution therapy (tablets, plasters or vaginal creams) and without a previous history or signs of uterine-/breastcancer, liver pathology or thrombo-embolic processes participated in this project. Every woman had skin type II or III. Informed consent was obtained from all the subjects and the study had the approval of the Medical Ethics Committee.

The minimal erythema dose (MED) for each individual was determined, and two sites on their backs (3 cm^2) were each irradiated with 3 MEDs of UV-B according to MacKenzie. Immediately after irradiation the left site was treated with 200 mg cremor lanette (per g: lanette wax cream 150 mg, cetiol 200 mg, sorbitol in water), the right side with the same amount of estriol (Sigma) in cremor lanette (1 mg/g), and this estriol cream was also applied on an unirradiated site on the right side. All three areas were occluded with a non-toxic plastic foil for 8 h. After this period the skin was cleaned with 70% ethanol, and biopsies (approximately 1 cm^2) were taken from the centre of each site using a Castroviejo keratotome (Storz) under local anaesthesia produced by cooling the skin with ethyl chloride. Biopsies were washed briefly in cold phosphate-buffered saline and snap-frozen in liquid nitrogen prior to analysis.

ODC and protein measurements

These have been described previously (1); the ODC assay was modified by reducing the amount of "cold" ornithine in the reaction mixture from 40 nmol to 4 nmol to improve the sensitivity.

Statistics

The Wilcoxon signed rank test for matched pairs (two-tailed) was used for statistical analysis.

RESULTS

Although all women received the standardized dose of 3 MED of UV-B, the range of ODC induction varied from 8 to 65 pmol/min/mg protein, as shown in Table I. These findings were in accordance with previous observations that an increase in age is accompanied by an increased spread in individual values (unpublished data). Therefore we normalized all ODC activities to 100% (see Table I, column 6).

At the time of biopsy no differences in erythema were visible in the vehicle- and estriol-treated irradiated areas.

Table I. *ODC levels following UVB only, UVB plus estriol and estriol only*

The figures in column 6 are calculated as (column 4/column 3)×100%.

Subject No	Age	ODC (pmol/min/mg protein)			Effect of estriol
		UV-B only	UV-B + estriol	Estriol only	
1	69	08	03	<1	38%
2	63	17	05	<1	29%
3	62	49	34	<1	69%
4	57	38	31	<1	82%
5	60	65	55	<1	85%
6	62	29	10	<1	34%
Mean	62	34	23	<1	56%
SEM	1.6	8.5	8.4	–	10%

Nevertheless, as seen in Table I, ODC activities in the estriol-treated irradiated sites of all 6 women were lower than in the vehicle-treated irradiated areas, averaging 56% ± 10% (mean ± SEM; $p = 0.03$) of this control, almost in accordance with the effects of a potent corticosteroid (1). ODC activities in the estriol-treated sites without irradiation were not detectable in any of the 6 samples.

DISCUSSION

It is clear from our results that topical application of estriol does not lead to a spontaneous or enhanced induction of ODC in human epidermis in vivo. On the contrary, this member of the steroid hormone receptor superfamily seems to share the capacity to antagonize ODC transcription with its corticosteroid-, retinoic acid- and vitamin D_3-relatives.

Activated corticosteroid receptors reduce ODC induction by formation of a heterodimer with the ODC transcription factor AP-1 (17), probably subsequent to binding onto their specific response elements immediately upstream of the coding region of the DNA. Similar mechanisms are thought to be responsible for ODC inhibition by retinoic acid and vitamin D_3 (18). Could it be that this AP-1 binding site already existed in the phylogenetically oldest steroid receptor molecule and has remained constant during evolution, adding new hormone- and DNA-binding regions at different sites (19)? But how could the opposite effects of estrogen on ODC induction in epidermis, as compared to other tissues, be explained? An explanation may be that in these latter tissues ODC transcription is directly regulated by the activated estrogen-receptor, without participation of the c-jun/c-fos complex.

Much research still has to be done. For example, it would obviously be of value to quantify and characterize estrogen-binding sites; unfortunately, using current techniques, this requires more material than can conveniently be obtained from biopsies. Secondly, modern flowcytometric methodology offers the possibility to re-investigate the early claim regarding the influence of estrogens on epidermal cell cycle kinetics. Finally, localization, isolation and synthesis of this common AP-1 binding site may eventually lead to a strongly anti-proliferative drug without hormonal side-effects.

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