UV Irradiation and Topical Vitamin A Modulate Retinol Esterification in Hairless Mouse Epidermis*

HANS TÖRMÄ,[1] BERIT BERNE[1] and ANDERS VAHLQUIST[1,2]

[1]*Department of Dermatology, University Hospital, Uppsala, and* [2]*Department of Dermatology, University of Linköping, Linköping, Sweden*

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The formation of fatty acyl esters of retinol, the major form of vitamin A in epidermis, is catalysed by microsomal enzymes. To study the regulation of retinol esterification, we exposed hairless mice to ultraviolet (UV) irradiation which destroys vitamin A and to topical retinol treatment. Vitamin A (retinol and retinyl esters) in serum and epidermis was analysed by high-performance liquid chromatography at 0–12 days after a single irradiation with UVB (280–320 nm; 0.34 J/cm^2) or UVA (320–400 nm; 1.0 J/cm^2). The immediate vitamin A reducing effects of UVB and UVA were similar, but UVB elicited a more rapid replenishment of epidermal vitamin A with a corresponding transient depletion of serum retinol after 2–3 days. The activity of retinyl ester synthetase, measured by an in vitro radiochemical assay, was unaffected by the irradiations. By contrast, the acyl-CoA : retinol acyltransferase (ARAT; EC 2.3.1.76) activity increased to 167% on the 2nd day after UVB-irradiation and to 124% after topical retinol, but was otherwise quite constant. The UVB- and retinol-induced ARAT activity was less dependent on exogenous palmitoyl-CoA than that of control microsomes and experiments indicated that this might be due to an increased endogenous concentration of long-chain acyl-CoA in the microsomes. We conclude that extreme variations in the vitamin A supply to epidermis, such as a rapid influx of unesterified retinol, may modulate the epidermal ARAT activity. *Key words: Vitamin A; Ultraviolet radiation; Acyl-CoA : retinol acyltransferase; Palmitoyl coenzyme A.* (Received February 16, 1988.)

A. Vahlquist, Department of Dermatology, University of Linköping, S-581 85 Linköping, Sweden.

An adequate supply of vitamin A (retinol and its fatty acyl esters) is a requirement for normal epidermal cell differentiation (1). Esterification of retinol in epidermis, a prerequisite for local storage of the vitamin, is probably also of importance for the regulation of free retinol levels in the keratinocytes (2). Two esterifying enzyme activities have recently been characterized in microsomes from mouse epidermis: 1) an acyl-CoA : retinol acyltransferase (ARAT; EC 2.3.1.76) and 2) an acyl-CoA-independent retinyl ester synthetase (RES) which operates at a lower retinol concentration and a higher pH than ARAT (4). Although ARAT is considered to be a physiological regulator of retinol esterification in other tissues (5–9), a previous study showed that epidermal ARAT-activity after 5 weeks of restricted vitamin A intake was unchanged, despite a 65% drop in epidermal vitamin A (3). The results suggested that, in order to modulate the ARAT activity, more drastic changes in the epidermal vitamin A status would be required.

Abbreviations

ARAT: acyl-CoA : retinol acyl transferase; BSA: bovine serum albumin; HPLC: high performance liquid chromatography; RES: retinyl ester synthetase; UVA: ultraviolet (320–400 nm) radiation; UVB: ultraviolet (280–320 nm) radiation.

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We have previously shown that UV-irradiation induces an acute reduction of epidermal vitamin A followed, within a few days, by a rapid restoration of the vitamin levels, presumably from serum (10). In the present study, we have examined the vitamin A homeostasis in hairless mouse epidermis after UV irradiation and topical retinol with special reference to retinol esterification.

MATERIAL AND METHODS

Chemicals

[11,12(n)-^{3}H]All-trans retinol (TRK 646 : 60 Ci/mmol) was purchased from The Radiochemical Centre, Amersham, Bucks, England. All-trans retinol, dithiothreitol, palmitoyl-CoA, bovine serum albumin, butylated hydroxytoluene, hydroxylaminehydrochloride and cycloheximide were purchased from Sigma (St Louis, Mo.). Retinyl palmitate was synthesized as described by Huang & Goodman (11). All solvents were spectrograde reagents from Merck AG, Darmstadt, FRG, or Rathburn Chemicals, Peeblesshire, Scotland.

Animals

Female hairless mice (hr/hr) were purchased from Bomholtsgården (Ry, Denmark) at the age of 8–10 weeks and were maintained in our own colonies. They were fed standard pellets, containing 6 600 IU vitamin A/kg, and water ad lib. Before starting the experiments they were allowed to adapt to our diet for at least 3 weeks.

Irradiations

The animals were irradiated once with UVB (280–320 nm) or UVA (320–380 nm) using Ultravitalux (Osram, West Germany) or UV 800 (Waldmann GmbH & Co, West Germany) as UV sources. Irradiations were performed by keeping the cages uncovered under the source at a distance of 30 cm (UVB) or 20 cm (UVA). The irradiation doses were 0.11–0.34 J/cm^2 UVB and 0.5–5.0 J/cm^2 UVA, corresponding to maximum irradiation times of 6 min 45 s (UVB) and 12 min (UVA), respectively.

Other treatments

All-trans retinol (0.2 µmol in 0.2 ml ethanol) was applied once on the backs of the animals. Cycloheximide (0.7 µmol in 0.1 ml acetone) was applied three times on the backs of the animals (for details, see Table II).

Sampling

The animals were anaesthetized with CO_2, whereafter they were decapitated and serum collected. Epidermis and epidermal microsomes were prepared as described before (3).

Vitamin A analysis

Retinol was analysed in hydrolysed serum and tissue extracts by high performance liquid chromatography (HPLC) as previously described (12). Reversed-phase HPLC, using a Waters M-45 pump (Waters Assoc Inc, Milford, Mass.) in combination with a UV-monitor D (LDC/Milton Roy, Riviera Beach, Fla.) was performed on a Nucleosil 5 µ PEAB-ODS column (4.6×200 mm) eluted with water : acetonitrile (14 : 86) at a flow rate of 1.2 ml/min.

Determination of retinol esterifying activities

The ARAT activity was analysed as before (3). Briefly, epidermal microsomes were incubated in a test tube together with [^{3}H]retinol and palmitoyl-CoA. The reaction was terminated after 20 min by addition of ethanol and an excess of unlabelled retinyl palmitate. The retinoids were extracted with hexane and subjected to HPLC using an Altex Model 110 pump (Altex Scientific Inc, Berkeley, Calif.) in combination with a UV-monitor 1203 (360 nm) (LDC, Riviera Beach, Fla.). Reversed-phase chromatography was performed on a Nucleosil 5 µ PEAB-ODS column (4.6×200 mm) eluted with ethylacetate : methanol (15 : 85) at a flow rate of 1.6 ml/min. The eluate was subjected to liquid scintillation counting (Philips, Eindhoven, The Netherlands), and based on the specific activity of the tracer the formation of retinyl palmitate could be calculated. The data are expressed as pmol ester/mg microsomal protein/min.

The RES activity was analysed as described earlier (4). The assay tubes contained 0.1 µM [^{3}H]retinol, 5 mM dithiothreitol, 1 mg BSA and 25–50 µg microsomal protein in a 0.2 M potassium phosphate buffer, pH 8.0, final volume 0.5 ml. Incubation, analysis and calculation were performed as for the ARAT activity (see above).

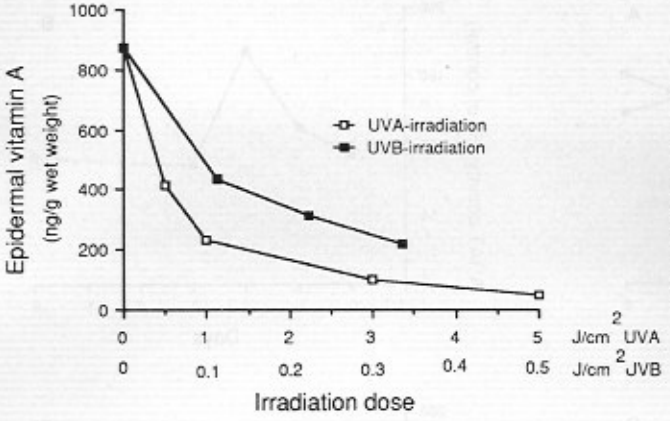

Fig. 1. Effects of UV irradiation on epidermal vitamin A concentration. Animals were irradiated once with different doses of UVB or UVA and killed half an hour later. Epidermal vitamin A was analysed as described under Materials and Methods. Values represent the mean of duplicate determinations of one animal.

RESULTS

Dose–response for UV-induced lowering of epidermal vitamin A

Fig. 1 shows the concentration of vitamin A in epidermis 30 min after irradiation with different doses of UVB or UVA. It can be seen that UVB reduced the vitamin A content more efficiently than did UVA. Thus, a UVB dose of 0.34 J/cm^2, equivalent to 1 J/cm^2 of UVA, reduced the epidermal vitamin A concentration by approximately 70–80%. These UV doses, which produced no (or only minimal) erythema, were selected for further studies on the effect of UV radiation on epidermal vitamin A metabolism.

Variations in vitamin A concentrations (serum and epidermis) and retinol esterification (epidermal microsomes) after a single exposure to UVB or UVA

UVB: Fig. 2 A shows that, in addition to producing an 80% decrease in epidermal vitamin A, UVB irradiation caused a slight fall in serum retinol within the first 30 min of the experiment. The serum retinol concentration continued to fall for another 2 days and a normalization was not obtained until day 6. By contrast, the level of epidermal vitamin A started to rise quite soon after irradiation and was normalized within 3 days. The composition of retinyl esters and free retinol was virtually constant (88–95% retinyl esters) during the experiment (not shown in Fig. 2 A). The rapid regeneration of epidermal vitamin A coincided with a 67% increase in the ARAT activity at day 2 (Fig. 2 B). By contrast, the RES activity on day 2 did not differ from the control value (not shown).

UVA: Fig. 2 C shows the effect of UVA irradiation, which produces an initial decrease in epidermal vitamin A of 70%. The much smaller decline in serum retinol and the slower regeneration of epidermal vitamin A relative to that in Fig. 2 A is noteworthy. The ARAT activity remained virtually constant over the 12-day-period required to normalize epidermal vitamin A (Fig. 2 D). Similarly, the RES activity was unaffected by UVA (data not shown).

It is conceivable that UVB, in contrast to UVA, elicits a process that rapidly replenishes epidermis with retinol from serum and that the rapid influx of retinol to the epidermis after UVB may induce ARAT activity. To test this possibility we examined the effects of topically applied retinol on the epidermal ARAT activity in non-irradiated animals.

Effects of a single topical retinol application

As can be seen from Fig. 3, topical retinol treatment produced peak ARAT activity on day 2 of the experiment. Although the increase was less marked than after UVB irradiation (cf. Fig. 2 B), the patterns clearly resemble one another.

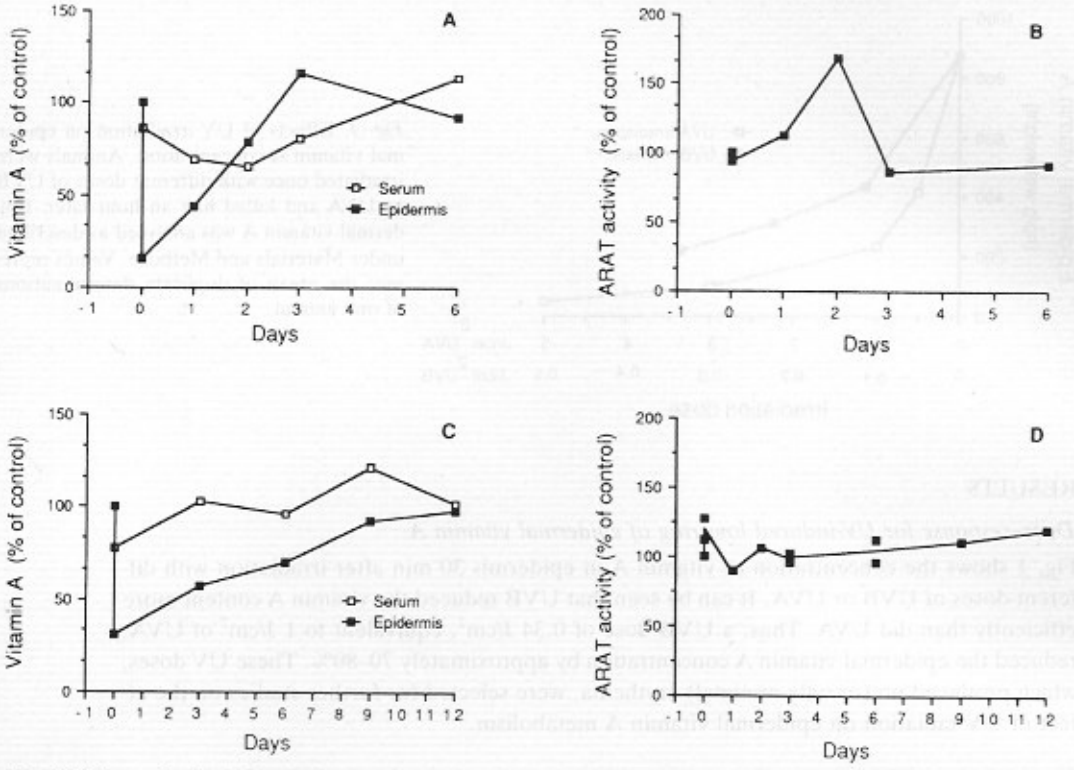

Fig. 2. Effects of a single irradiation with UVB or UVA on the vitamin A concentration in epidermis and serum and retinol esterification in epidermis as a function of time. Experimental details are outlined under Material and Methods. A. Epidermal (■) and serum vitamin A (□) as a function of time after UVB (0.34 J/cm^2). B. ARAT activity after irradiation with UVB. C. Epidermal (■) and serum vitamin A (□) as a function of time after UVA (1 J/cm^2). D. ARAT activity after irradiation with UVA. Values (A–C) represent mean of duplicate determinations of pooled samples from 2 animals. Values (D) represent single determinations from two experimental series, one identical with the UVB-experiment (Fig. 2B) and one with extended observation time. All values are expressed as percentages of the control value.

Characterization of the increased ARAT activity after UVB and topical retinol

The ARAT results described above were obtained by a standard assay which included 50 μM of exogenous palmitoyl-CoA. Surprisingly, when ARAT activities were measured without addition of palmitoyl-CoA (basic conditions), the difference between UVB/retinol-treated microsomes and control microsomes was even more conspicuous. We therefore studied the palmitoyl-CoA titration curves for the different types of microsomes. Fig. 4A, B shows that, while the optimal ARAT activities were similar, the titration curves of UVB- and retinol-treated microsomes were shifted to the left compared with normal. It can also be seen that the palmitoyl-CoA concentration used in the standard assay (50 μM) was slightly less than the optimum for the control microsomes, but close to that for the other two types of microsomes. This difference may largely explain the increase in ARAT activity recorded after both UVB and retinol treatment.

The left-hand shift of the palmitoyl-CoA titration curve relative to the normal curve may be attributed to: 1) ARAT is more sensitive to palmitoyl-CoA, or 2) the endogenous concentration of palmitoyl-CoA is increased in the microsomes. The latter possibility was examined by trapping of endogenous acyl-CoA with hydroxylamine. Table I shows that hydroxylamine reduced the ARAT activity (basic conditions) of UVB-treated microsomes by at least 60%.

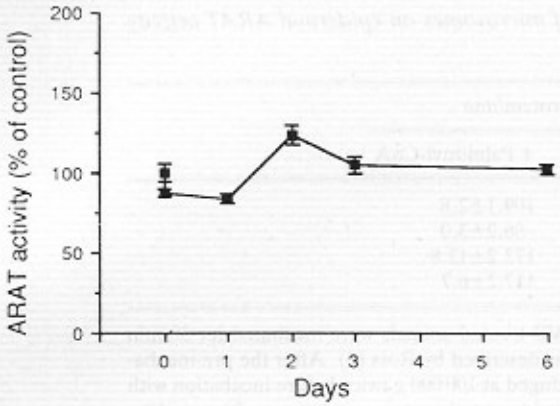

Fig. 3. Variations in retinol esterification after a topical application of retinol (200 nmol) to the back skin. Two animals were killed at each time and epidermal microsomes were prepared and pooled. Values represent means ± SE of four determinations (from two different series of animals) expressed as percentages of control values.

Replenishment of palmitoyl-CoA (standard conditions) restored much of the ARAT activity, showing that hydroxylamine had not adversely affected the enzyme.

Effects of cycloheximide on epidermal ARAT activity

Cycloheximide is an inhibitor of protein synthesis (13). Table II shows that, whereas cycloheximide treatment of the skin had little effect on the ARAT activity (standard assay) in control animals, it completely inhibited the ARAT elevation in UVB-irradiated animals. It is also worthy of note that, when assayed under basic conditions (minus palmitoyl-CoA), cyclo-

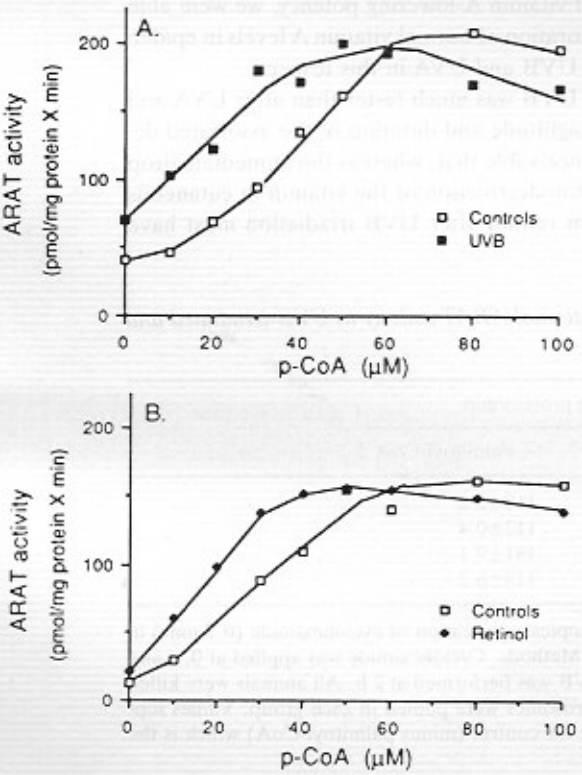

Fig. 4. Effect of increasing concentrations of palmitoyl-CoA on retinol esterification. Epidermal microsomes were prepared from animals which, 2 days before, had been subjected to UVB irradiation (see Methods) or retinol-application (see caption to Fig. 3). A) UVB-irradiated (■) vs. control animals (□); B) retinol-treated (◆) vs. control animals (□).

Table I. *Effects of hydroxylamine preincubation of microsomes on epidermal ARAT activity in UVB-irradiated and control animals*

	ARAT activity, pmol/mg protein/min	
	− Palmitoyl-CoA	+ Palmitoyl-CoA
Controls	16.8±0.3	109.1±2.8
+ hydroxylamine	10.9±1.2	86.2±3.0
UVB (Day 2)	101.8±8.9	172.2±12.8
+ hydroxylamine	42.1±0.4	117.2±6.7

Microsomes (1.7 mg protein) from both control and UVB-treated animals were incubated for 30 min (32°C) with buffer or hydroxylamine (0.5 M) produced as described by Ross (3). After the pre-incubation, the microsomes were washed with buffer and centrifuged at 100 000 *g* twice before incubation with [^{3}H]retinol as outlined under Methods. The recovery of microsomal protein was between 28 and 35%. Values represent means ± SE of triplicate test tubes.

heximide markedly reduced the esterifying activity in both control and UVB-treated epidermis, thereby abolishing the difference in palmitoyl-CoA dependence between the two.

DISCUSSION

In agreement with previous studies in rabbits (10), the present study shows that UV irradiation of hairless mice reduces the vitamin A concentration in epidermis in a dose-dependent manner. UVB was more potent in this respect than UVA, and presumably the wavelength-range of the former was closer to the absorption spectrum of retinol. By applying doses of UVB and UVA that were equivalent in terms of vitamin A-lowering potency, we were able to examine the processes leading in vivo to a restoration of normal vitamin A levels in epidermis and to compare the biological responses to UVB and UVA in this respect.

The restoration of epidermal vitamin A after UVB was much faster than after UVA and this difference apparently correlated with the magnitude and duration of the associated decrease in serum retinol (see Fig. 2 A, C). It is conceivable that, whereas the immediate drop in serum retinol can be directly attributed to photodestruction of the vitamin in cutaneous blood vessels, the continued depletion of serum retinol after UVB irradiation must have

Table II. *Effects of topical cycloheximide on epidermal ARAT activity in UVB-irradiated and control animals*

	ARAT activity, pmol/mg protein/min	
	− Palmitoyl-CoA	+ Palmitoyl-CoA
Controls	29.3	118±2.2
+ cycloheximide	8.4±2.6	112±0.4
UVB (Day 2)	95.0±0.8	181±9.1
+ cycloheximide	6.1±1.3	118±6.2

Two animals in each group were treated either with topical application of cycloheximide (0.7 µmol in acetone) or with UVB irradiation as described under Methods. Cycloheximide was applied at 0, 4 and 26 h after start of the experiment. Irradiation with UVB was performed at 2 h. All animals were killed at the same time, 50 h after start. The epidermal microsomes were pooled in each group. Values represent means ± SE of triplicate determinations except for control (minus palmitoyl-CoA) which is the mean of duplicate determinations.

another explanation. We hypothesize that, in order to restore epidermal vitamin A levels, a rapid uptake of retinol by the epidermis must take place. Retinol circulates bound to retinol-binding protein (RBP) for which keratinocytes have specific receptors. An abrupt increase in the mobilization of serum retinol-RBP to the epidermis will probably cause a transient decrease in serum retinol as there is probably some delay before the liver increases its secretion of retinol-RBP. At present we cannot explain why UVB and UVA doses, normalized for the acute effects on epidermal vitamin A, elicit different responses in terms of retinol replenishment. In several unpublished experiments, variations in the minimal levels of vitamin A obtained after irradiation (20–30%) did not seem to influence the regenerative process.

Although vitamin A is supplied to the keratinocytes as retinol, about 90% of mouse epidermal vitamin A is in the form of retinyl esters (1, 2) and this percentage was virtually constant throughout the UVB-experiment. Thus, intracellular esterification of retinol must be important for the maintenance of vitamin A homeostasis in epidermis. The esterification of retinol is catalysed by two enzymes. The reverse process, the de-esterification of retinol, is catalysed in rat liver by retinyl palmitate hydrolase (14) but it is not known whether this enzyme also operates in epidermis. A primary aim of this study was to establish whether depletion and restoration of retinol in some way modulated the epidermal esterifying activities.

Our results show that a single dose of UVB increased the ARAT (but not RES) activity after a few days, while UVA had no consistent effects at all. Because topical retinol and UVB irradiation elicited similar variations in ARAT activity, we assumed that, in both cases, a rapid influx of retinol to epidermis, would lead to increased esterification as a result of substrate-stimulated ARAT activity. However, it soon emerged that the enzyme from UVB- or retinol-treated animals behaved anomalously.

We have previously reported that epidermal ARAT is critically dependent on the concentration of palmitoyl-CoA in the reaction mixture (2, 3); thus, whereas appropriate amounts of exogenous palmitoyl-CoA yield a 5–10-fold stimulation of the activity, higher concentrations are inhibitory. The palmitoyl-CoA concentration (50 μM) used in our standard assay was slightly less than the optimum for microsomes from normal adult animals, but is suitable for several other types of microsomes (2). However, microsomes from UVB- or retinol-treated epidermis differed from normal in that optimal ARAT activity was recorded at a lower concentration of exogenous palmitoyl-CoA. Indirect evidence suggests that this abnormality was due to an increased concentration of endogenous palmitoyl-CoA. Thus, the UVB-induced ARAT activity was abnormally high when assayed without exogenous palmitoyl-CoA (cf. Fig. 4) and hydroxylamine-trapping of palmitoyl-CoA markedly reduced this activity (Table I). Since our attempts to quantitate palmitoyl-CoA in the epidermal microsomes did not yield reproducible results (not shown), we can only hypothesize that an increase in endogenous long-chain acyl-CoA is responsible for the elevated ARAT activity both after UVB irradiation and after retinol treatment.

The biosynthesis of palmitoyl-CoA from palmitate is catalysed by an enzyme, fatty acyl-CoA synthetase (EC 6.2.1.3), which is located in microsomes and mitochondria (15). Our results with cycloheximide (inhibitor of protein synthesis) provide indirect evidence for the involvement of an increased acyl-CoA synthetase activity in murine epidermis 2 days after UVB irradiation. Thus, topical cycloheximide treatment markedly reduced the high basal esterification rate (minus exogenous palmitoyl-CoA) in UVB-stimulated microsomes, but had little effect on the optimal ARAT activity (plus exogenous palmitoyl-CoA). Since only the former activity is critically dependent on endogenous long-chain acyl-CoA as substrate, we believe that the altered activity after cycloheximide is due to a reduced microsomal content of the endogenous substrate. Fatty acyl-CoA synthetase, because of a hypothetical rapid turnover, might be readily inhibited by cycloheximide, whereby endogenous CoA levels would rapidly decline. ARAT, on the other hand, appears to have a slow turnover and the

epidermal concentration of this enzyme was apparently only marginally reduced by the cycloheximide treatment. Indeed, the optimal ARAT activity in different tissues appears to be constant under a variety of extreme conditions (16, 17). The unravelling of these putative events would help to establish whether palmitoyl-CoA plays a key role in the in vivo regulation of retinol esterification, thus explaining the changes in ARAT acitivity after UVB and topical retinol treatment.

It may be argued that the observed modulation of the ARAT activity is not a direct consequence of the influx of retinol to epidermis. For example, UVB irradiation by causing a slight inflammation and epidermal hyperproliferation might unspecifically trigger retinol esterification. However, in preliminary experiments using tape-stripping and application of croton oil, powerful stimulators of inflammation and proliferation, we found no effect on the epidermal ARAT activity. Also, the response to topical retinol in non-irritating concentrations was specific in the sense that several other non-alcoholic retinoids did not produce this effect when applied topically to the animals (Törmä & Vahlquist, unpublished observations).

In conclusion, we propose that the physiological regulator of epidermal retinol esterification is not just ARAT but also the supply of endogenous long-chain acyl-CoA. Verification of this hypothesis and of its possible applicability to other situations where acyltransferases are involved requires further studies.

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