Acute and Chronic Ultraviolet Radiation Induction of Epidermal Ornithine Decarboxylase Activity in Hairless Mice

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ABSTRACT

The effects of acute, multiple, and chronic exposure of hairless mice to ultraviolet radiation (UVR) on induction of epidermal ornithine decarboxylase (ODC) (EC 4.1.1.17) activity were investigated. Acute UVR exposure results in a biphasic time course of induction of epidermal ODC activity. Enzyme activity maxima occur at 3 and 24 h postirradiation. The biphasic time course is observed in two different strains of hairless mice (Skh:HR-1 and Jackson HRS/J) when the UVR source is either UVB fluorescent tubes or a solar simulator. The ratio of 24-h/3-h postirradiation ODC activity increases with increasing UVR dose. UVR induction of ODC activity was not significant below the mouse minimum erythema dose (MED). The 3- and 24-h ODC activities have similar apparent K_Ms for ornithine (34 and 50 μM, respectively), and thermal stabilities at 52°C (t_50 = 23 and 18 min, respectively), and exhibit similar half-lives in vivo (t_1/2 = 15 and 18 min, respectively).

Multiple UVR exposure experiments showed 24-h ODC activity is sensitive to the preexposure history of the mouse, while 3-h ODC is not. Preexposure of hairless mice to several sub-MED levels of simulated solar radiation (SSR) specifically suppresses induction of 24-h ODC by a follow-up 2 × MED of SSR. Preexposure to a single 2 × MED of SSR specifically enhances induction of 24-h ODC induced by a second 2 × MED of SSR administered 48 h after the first. The 3-h ODC was not significantly affected by either preexposure regimen. Preexposure to a single high or low dose of UVA radiation did not affect epidermal ODC activity nor had an effect on ODC induction by UBV radiation. Several weeks of chronic exposure to UBV radiation elevated basal levels of epidermal ODC substantially (up to 350-fold). In these chronically irradiated mice, exposure to 2 × MED SSR resulted in a further 3.5-fold increase in ODC activity over the elevated basal level. These data reveal novel properties of epidermal cell expression of ODC activity in response to acute and chronic UVR insult. The results provide additional insight into the use of ODC as a marker for skin photodamage.

INTRODUCTION

The long time required for the progression of skin carcinogenesis in both humans and animals makes short-term markers for the disorder highly attractive for study. The epidermal enzyme, ornithine decarboxylase, is a potential marker. ODC catalyzes the rate-limiting step in spermidine and spermine biosynthesis, polyamines found ubiquitously in living systems and essential for cellular growth. Induction of ODC activity is a necessary component of tumor promotion by phorbol esters in mouse skin (1, 2). The relationship between ODC and neoplasia has led to ODC being used as a short-term marker of skin tumor promotion by chemicals. For example, acute induction of epidermal ODC activity by chemical tumor promoters has been used as a marker of tumor promotion in mice (3, 4). Alternatively, agents which block acute chemical induction of epidermal ODC activity also block chronic chemical promotion of skin tumors in mice (5). Pharmacological inhibition of phorbol ester–induced human epidermal ODC activity in vitro has been used to predict the drug dose and schedule needed for cancer chemoprevention (6, 7).

Long-term exposure to solar ultraviolet radiation is clearly the major etiological factor in skin carcinogenesis in humans. Analogous to chemical induction of epidermal ODC activity, acute doses of UVR also induce epidermal ODC activity in mice (8, 9) and humans (10). Agents which block acute UVR induction of ODC also show antitumor activity in long-term mouse photocarcinogenesis studies (11–13). Thus, ODC is a useful biological marker for photodamage as well as for chemical carcinogenesis in skin.

Our interest in skin photodamage led us to ODC as a possible marker for acute and chronic photodamage in both animal models and humans. In our early studies aimed at characterizing UVR induction of ODC in hairless mice, we noted that UVR induction of ODC was more complex than we had presumed, exhibiting a novel biphasic induction time course. We therefore decided to better characterize UVR induction of ODC activity in hairless mice and present the results here.

MATERIALS AND METHODS

Animals and Materials. Female albino hairless mice (Skh:HR-1 from Charles River, Portage, MI, and Jackson HRS/J hairless from The Jackson Laboratory, Bar Harbor, ME) were used at 10–15 weeks of age, unless indicated otherwise. Mice were housed five to a cage in a room with controlled temperature and humidity and with a 12–light, 12–dark cycle. They were given a standard diet and water ad libitum. L-[3-14C]Ornithine hydrochloride (specific activity 51.6 mCi/mmol) was from New England Nuclear (Boston, MA). All other chemicals were of analytical grade.

UV Irradiation. The UVB source was a bank of 4 Westinghouse FS–40 sunlamps with a spectral emission between 280 and 400 nm and peak emission at 317 nm. Spectral emission was measured with an International Light 791 radiometer (Newburyport, MA). The relative spectral output profile of the sunlamps is shown in Fig. 1a. Mice were irradiated in individual wire cage chambers (7.5 × 7.5 × 7.5 cm) in which their backs were 35 cm from the fluorescent tubes. UBV irradiance at this distance was 0.23 mW/cm² as measured with an International Light 700 radiometer. The UVA radiation source was a bank of 4 General Electric F–40 sunlamps with a spectral emission between 340 and 400 nm. The mouse UVA irradiations were as described for UVB irradiation. UVA irradiance was 0.8 mW/cm². The SSR source was a solar simulator (Kratos, Ramsey, NJ) equipped with a 1000-W xenon arc lamp and dichroic filter. The relative spectral irradiance profile of the solar simulator is shown in Fig. 1b. Mice were individually irradiated with SSR so that their backs were 10 cm from the dichroic filter. Irradiance at this distance was 18 mW/cm².

Assay of ODC Activity. The assay of ODC activity is similar to that described by Binder et al. (14). At the indicated times after irradiation, mice were killed by cervical dislocation. The UVR–exposed dorsal skin (≈2 × 3 cm) was removed and immediately placed dermal side down on an ice–cold glass plate. The epidermis was scraped off by using a single–edged razor blade until the shiny basement membrane was clearly visible. The epidermis was transferred to a tube containing 0.6 ml cold homogenization buffer (50 mM sodium phosphate (pH 7.0), 0.1 M}

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2 The abbreviations used are: ODC, ornithine decarboxylase; UVR, ultraviolet radiation; UBV, ultraviolet B radiation (290–320 nm); UVA, ultraviolet A radiation (320–400 nm); SSR, simulated solar radiation (290–400 nm); 3-h ODC, ODC activity induced 3 h postirradiation; 24-h ODC, ODC activity induced 24 h postirradiation; MED, minimum erythema dose; UVC, ultraviolet C radiation (200–290 nm); TPA, 12-O-tetradecanoylphorbol-13-acetate.

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1.25 mM EDTA, 2.5 mM dithiothreitol, and 0.1 mM pyridoxal 5'-phosphate] and homogenized on ice with a Tekmar Tissumizer (Cincinnati, OH) at setting 8 for 20 s. The homogenate was then centrifuged at 16,000 × g for 10 min at 4°C to obtain a soluble epidermal fraction.

ODC activity in the soluble fraction was determined by measuring the release of [14CO2] from L-[^14C]ornithine hydrochloride. The assay mixture (0.125 ml) contained 40 mM sodium phosphate (pH 7.0), 1 mM EDTA, 2 mM dithiothreitol, 0.2 mM pyridoxal 5'-phosphate, 0.4 mM L-ornithine, 0.5 μCi of L-[^14C]ornithine hydrochloride, and 100 μl epidermal soluble fraction. The reaction was started by the addition of substrate and incubated at 37°C for 30 min (unless stated otherwise) in 15 × 18-mm culture tubes equipped with a rubber stopper and center well assemblies (Kontes, Vineland, NJ). The reaction was stopped by injecting 0.25 ml of 2 M citric acid (pH 1.5) through the rubber stopper by using a syringe and needle. Reactions were allowed to postincubate for at least 1 h at 37°C to ensure complete absorption of [14CO2] by the methylbenzethonium hydroxide (0.1 ml; 1 M in methanol) contained in the center well. Center wells were then transferred to vials containing 10 ml ScintiVerse II scintillation fluid (Fisher Scientific, Pittsburgh, PA) and radioactivity was measured with a Beckman LS8100 scintillation counter.

ODC activity was assayed in triplicate for each mouse. Blank assays contained homogenization buffer instead of the epidermal soluble fraction and these values were subtracted from test samples. Experimental group sizes were 3–5 mice. Protein in the soluble fraction was quantitated by using the Bio-Rad protein assay (Bio-Rad, Richmond, CA) with bovine serum albumin as protein standard. ODC activity (expressed as nmol CO2/h/mg protein) was linear with time during the range in which measurements were made.

Determination of ODC Thermal Stability and Substrate Km. Mice were irradiated with 1.6 J/cm² SSR and soluble epidermal supernatant solution was prepared at either 3 or 24 h postirradiation. Each supernatant solution was adjusted to 0.65 mg/ml total protein with homogenization buffer. For determination of thermal stability, enzymes were incubated at 52°C and ODC activity was measured at 0, 5, 10, 15, 20, 30, and 40 min of incubation. To linearize the relationship between ODC activity and time, the log transformation on ODC activity was performed. The transformed data were statistically analyzed by using General Linear Models techniques. For determination of the Michaelis constant for ornithine, enzyme assays were performed with variable amounts of ornithine (0.02 to 2 mM) and Km were determined from a Lineweaver–Burk plot of the data. The relationship between reaction velocity and substrate concentration was statistically analyzed by using nonlinear regression techniques (15).

RESULTS

Time Course and Dose Response of Acute UVR Induction of Epidermal ODC Activity. The time course of acute UVR induction of epidermal ODC activity by various UVR doses was determined. As shown in Fig. 2, exposure of Skh:HR-1 or Jackson HRS/J hairless mice to variable UVR doses resulted in a biphasic time course of induction. The UVR source was either a solar simulator (Fig. 2A) or UVB fluorescent tubes (Fig. 2B). In either mouse strain and with either UVR source, enzyme activity maxima occurred at approximately 3 and 24 h postirradiation. At low UVR doses, epidermal ODC activity returned to basal levels by 48 h postirradiation. However, at the higher UVR doses, epidermal ODC activity remained slightly elevated over basal levels for at least 72 h postirradiation. At a fixed UVR dose, induction of epidermal ODC activity was greater in the Jackson HRS/J versus the Skh:HR-1 strain of mice.

Fig. 2 also shows the times for maximum ODC activity (3 and 24 h postirradiation) remained constant with changing UVR dose. However, the relative fold induction of 3-h versus 24-h ODC was UVR dose dependent. To more easily illustrate this result, the 3- and 24-h time data in Fig. 2 were replotted

![Fig. 1. Relative spectral distribution of radiation emitted from (A) UVB fluorescent sunlamps (Westinghouse FS-40), and (B) the 1000 W xenon arc lamp (Kratos).](image-url)
to yield the UVR dose–response curves shown in Fig. 3A (SSR) and 3B (UVB). Using either UVR source, maximum induction of 3-h ODC activity occurred at a lower UVR dose compared to 24-h ODC activity. Additionally, the 24-h/3-h ODC activity ratio increased with increasing UVR dose. Interestingly, significant UVR induction of epidermal ODC activity did not occur until the UVR dose approaches or exceeds the mouse MED, determined in separate experiments to be 55–70 mJ/cm² UVB and 0.5–0.8 J/cm² SSR in the two mouse strains tested.  

When we compared UVB versus SSR exposure at the same MED, we noted UVB induced a higher 24-h/3-h postirradiation ODC activity ratio [compare 1.08 J/cm² SSR (2 × MED, Fig. 2A) versus 112 mJ/cm² UVB (2 × MED, Fig. 2B)]. This result was observed in both Skh:HR–1 and Jackson HRS/J hairless mice. To determine if the UVA wavelength component (320–400 nm) of SSR has an effect on UVB induction of epidermal ODC activity, we sequentially exposed mice to UVA then to UVB fluorescent tubes. Exposure to a single high (10.8 J/cm²) or low (1 J/cm²) dose of UVA immediately before exposure to a high dose (2 × MED) of UVB had no effect on induction of epidermal ODC activity relative to control mice exposed to UVB alone (data not shown). Exposure of mice to these single doses of UVA alone also had no effect on epidermal ODC activity measured either 3 or 24 h postirradiation.  

Influence of Multiple UVR Preexposure on Acute UVR Induction of Epidermal ODC Activity. Since tumor development requires repeated exposure to the tumor promoter, we determined if multiple exposure of mice to UVR influenced induction of epidermal ODC activity. Jackson HRS/J mice were exposed to two high doses (2 × MED) of SSR spaced 48 h apart. As shown in Fig. 4A, the first SSR exposure induced ODC with activity maxima at 3 and 24 h postirradiation. Exposure to the second SSR dose also induced ODC activity 3 h postirradiation (51 h after the first exposure) of similar magnitude to the first 3-h peak. However, relative to a single irradiation, the second irradiation induced 6.5-fold more ODC activity 24 h postirradiation (72 h after the first irradiation) relative to the first SSR exposure. Therefore, ODC activity induced 24 h postirradiation was enhanced by preexposure to 2 × MED SSR, whereas ODC activity induced 3 h postirradiation was not.  

We also preexposed Jackson HRS/J mice to three 0.5 × MEDs of SSR (irradiations spaced 48 h apart) prior to irradiation with a high dose (2 × MED) of SSR 72 h after the last sub–MED exposure. As shown in Fig. 4B, preexposure to 3 sub–MEDs of SSR significantly reduced ODC activity measured 24 h after the 2 × MED exposure compared to control mice. Sub–MED preexposure had no significant effect on ODC activity measured 3 h after the 2 × MED of SSR. In control experiments (not shown), three sub–MED exposures alone, spaced 2 days apart, had no detectable effect on epidermal ODC activity. Therefore, SSR induction of 24 h postirradiation ODC activity was depressed by multiple low dose UVR preexposure, whereas 3 h postirradiation activity was not.  

Influence of Chronic UVB Exposure on Basal ODC Activity. The influence of chronic UVB exposure of hairless mice on the basal level of epidermal ODC activity is shown in Fig. 5. Exposing Skh:HR–1 hairless mice to UVB radiation (55 mJ/cm²) 3 times/week for up to 27 weeks increased basal ODC activity up to 350-fold (relative to chronologically aged-matched control animals). In this experiment, ODC activity was determined 3–5 days after the last irradiation to ensure acutely induced levels of ODC activity had returned to basal level. We also note that skin papillomas were induced in this irradiation protocol at a total cumulative UVB dose of about 2 J/cm². When tumors were present, basal ODC activity was determined only in noninvolved UVR–exposed skin, to avoid the tumor area. In a parallel experiment, chronically irradiated...
mice (exposed 27 weeks to UVB as above) were challenged with a single exposure to 2 x MED SSR 24 days after the last chronic UVB irradiation. Even with elevated basal ODC activity (2.66 ± 0.65 (SE) nmol/h/mg protein) these mice responded with a further 3.5-fold increase in ODC activity (to 9.05 ± 0.82 nmol/h/mg protein) measured 3 h post-SSR irradiation (24 h ODC was not measured).

Fig. 5 also shows the increase in basal ODC activity induced by chronic UVB exposure was not linear with total cumulative dose. Rather, significant basal activity induction did not occur until a UVB total cumulative dose of 2 J/cm², concurrent with visible tumor formation (about 15 weeks UVB irradiation). Parallel experiments (not shown) showed that for mice removed from irradiation at the threshold dose (15 weeks total exposure), basal epidermal ODC activity remained low (0.1 nmol/h/mg protein) for 12 weeks postirradiation but increased to 1 nmol/h/mg protein by 22 weeks postirradiation. Similarly, in mice removed from irradiation after 27 weeks UVB exposure, basal epidermal ODC activity remained high (2.7 nmol/h/mg protein) for at least 4 weeks postirradiation. Therefore, the increased basal level of ODC activity associated with chronic UVB exposure does not appear to reverse with cessation of irradiation.

We also noted that basal epidermal ODC activity decreased significantly in the chronologically aged-matched unirradiated control mice from approximately 0.2 to 0.01 nmol/h/mg protein, as the animals aged from 3 to 15 weeks of age (data not shown). Chronic UVA exposure for 30 weeks (total dose of 1290 J/cm²) appeared to maintain basal ODC activity at 0.06 ± 0.01 nmol/h/mg protein relative to chronologically aged controls (0.01 ± 0.005 nmol/h/mg protein).

Tissue Activity Half-Lives. To determine if 3- and 24-h ODC results from either de novo protein synthesis or activation of latent enzyme, Skh:HR–1 mice were exposed to 3 × MED of SSR. At various times prior to harvest at either 3 or 24 h postirradiation, cycloheximide was injected (i.p., 30 mg/kg body weight) to inhibit protein synthesis. Mice were killed at either 3 or 24 h postirradiation and epidermal ODC activity was determined. As shown in Fig. 6, ODC activity at both 3 and 24 h postirradiation was rapidly inhibited by cycloheximide. Therefore, most of the UVR-induced ODC activity at 3 and 24 h postirradiation resulted from synthesis of new enzyme. A least squares fit of the log-transformed data showed 3- and 24 h ODC have a \( K_{\text{app}} \) for ornithine of 34 and 50 μM, respectively.

**DISCUSSION**

We have investigated the effect of acute, multiple, and chronic UVR exposure on epidermal ODC activity in hairless mice. Our studies show a single exposure to UVR, greater than the mouse MED, delivered from either UVB fluorescent tubes or a solar simulator, results in a novel biphasic time course of induction of epidermal ODC activity. ODC activity maxima occur at 3 and 24 h postirradiation (Fig. 2). We observed biphasic ODC induction in two strains of mice tested (Skh:HR–1 or Jackson HRS/J).

At least 5 other studies have reported on the time course of induction of epidermal ODC activity in hairless mice by a single exposure to UVR without observing significant biphasic induction (8, 9, 16–18). These studies reported maximum ODC activity occurs only at 24–28 h postirradiation. Results reported here reveal two reasons which can explain why the 3 h postirradiation peak of ODC had not been previously observed. First, 3-h ODC is short-lived, most readily detected only at 3 h postirradiation, a time point not measured in the aforementioned studies. Second, the relative induction of 3- and 24-h ODC is dependent on both the UVR source and UVR dose (Fig. 3). At low UVR doses, maximum induction of ODC occurs at 3 h postirradiation. However, at high UVR doses, maximum induction of ODC activity shifts to 24 h postirradiation, somewhat masking the peak of activity at 3 h postirradiation. This was most apparent when the radiation source was UVB fluorescent tubes (Fig. 3B). Previous studies used mice irradiated with high UVR doses (>90 mJ/cm²) delivered from UVB fluorescent tubes, conditions favoring observation of only the 24 h postirradiation ODC activity.

Interestingly, the in vitro ODC studies of Lichti et al. (19, 20) using mouse epidermal cell cultures are very similar to our in vivo findings. Exposure of mouse epidermal cell cultures to UVC radiation causes a biphasic time course of induction of ODC activity, with activity maxima at 4–6 h and 14–17 h postirradiation (19). Also, the dependence of the time of maximum ODC induction on high versus low doses of UVR pre-
presented in this paper is similar to the response of epidermal cell cultures to high versus low doses of TPA (20). Low doses of TPA induce maximum ODC activity 3–6 h posttreatment, whereas at high doses of TPA, maximum induction shifts to 9 h posttreatment.

The dependence of the time course of ODC induction on the tumor promoter dose may explain the different induction time courses observed with different chemical promoters (4, 5, 14) and actinic exposure. Topical treatment of mice with chemical promoters such as TPA provide epidermal keratinocytes with much longer exposure to the promoter (several hours) compared to the very short exposure (seconds) possible with UVR. The chemical stability and skin penetration properties of the chemical promoter will also undoubtedly influence ODC induction.

Multiple UVR exposure experiments showed 24–h ODC was dependent on the preexposure history of the animal while 3–h ODC was not. For example, preexposure to a high dose of SSR specifically enhanced 24–h ODC activity induced by a second high dose of SSR (Fig. 4A). We also found preexposure to several sub-MED doses of SSR specifically inhibited induction of 24–h ODC activity by a follow-up high dose of SSR (Fig. 4B). This result is similar to that found in the multiple exposure studies of Lowe et al. (18). In that work, repeated daily UVB irradiation of hairless mice was found to shift maximum ODC induction from 24 to 6 h after the final irradiation. The apparent inhibition of ODC activity induced 24 h postirradiation may manifest from a thickened stratum corneum due to repeated UVR exposure (21). Since relative UVR induction of 24–h/3–h ODC decreases with decreasing UVR dose, a thickened stratum corneum would reduce the UVR insult favoring maximum ODC induction at 3 h postirradiation. Similar results are observed during benzoyl peroxide induction of epidermal ODC activity in SENCAR mice (14). Multiple exposure to benzoyl peroxide shifts the time course for maximum induction from 25 to 3 h after treatment.

The biological significance of biphasic UVR induction of epidermal ODC activity is not understood at this time. However, the data are consistent with the existence of two epidermal keratinocyte cell populations, each with different sensitivities to UVR. Keratinocytes sensitive to UVR may express ODC 3 h postirradiation. The less sensitive keratinocytes would express ODC 24 h postexposure. Immunohistochemical localization of ODC in both the basal and suprabasal keratinocyte layers of human epidermis (22) support this notion.

Other investigators have used the inhibition of tumor promoter–induced epidermal ODC activity as a short–term screen for antitumor efficacy (5–7, 11–13). Our finding regarding the dependence of the time course for ODC induction on the UVR dose suggests a measure of 3–h ODC as well as 24–h ODC be taken during screening. Finally, actinic induction of epidermal ODC has the potential to be a useful short–term system for investigating tumor promotion and its pharmacological inhibition in human skin in vivo.

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