Enhanced Prostaglandin Synthesis after Ultraviolet Injury Is Mediated by Endogenous Histamine Stimulation

A Mechanism for Irradiation Erythema

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Abstract

Acute ultraviolet light B (UVB) injury is associated with dermal mast cell histamine release. The possibility that histamine-stimulated prostaglandin (PG) synthesis could be a mechanism for irradiation erythema was therefore examined using human skin explants. Explants responded to UV irradiation (120 mJ/cm^2) with a fivefold increase in synthesis of prostaglandins E_2, $F_{2\alpha}$ and 6-keto $PGF_{1\alpha}$. Incubating explants with the H1 antihistamines brompheniramine (50 μM) or pyrilamine (30 μM) inhibited PG release from irradiated explants 63±4.9% (mean±SEM) 6 h after UV exposure. Antihistamines did not affect PG synthesis in control explants. Irradiation increased the histamine concentration in explant conditioned medium only 50% over basal values, suggesting that irradiation enhanced histamine responsiveness. Explants were therefore incubated with exogenous histamine. In irradiated explants, PG synthesis was stimulated threefold by 3 μM histamine. Unirradiated explants' PG synthesis was unaffected by histamine.

Enhanced histamine sensitivity was also examined in epidermal cell cultures. In irradiated cultures, histamine sensitivity was again markedly potentiated: as little as 1 μM histamine stimulated significant PGE_2 release and the response to 10–30 μM histamine was increased six to eight times compared with that of unirradiated cultures. These studies demonstrate that endogenous histamine stimulates PG synthesis in human skin after UV injury by potentiation of histamine-induced prostaglandin release. Potentiated agonist responses induced by UV exposure may contribute to the effects of UVB irradiation injury and in particular to irradiation erythema. (*J. Clin. Invest.* 1990. 86:566–574.) Key words: ultraviolet light • skin • injury • prostaglandin • histamine

Introduction

The biologic effects of ultraviolet light B (UVB)[1] on the skin are of particular interest because of the importance of UV-induced injury in the development of photoaging and skin cancer (1) and the role of UV irradiation in the therapy of skin disease (2). The precise mechanism(s) for the effects of UVB on tissue are poorly understood, but one of the most common manifestations of UVB injury of the skin is erythema.

Current evidence for a role for arachidonate metabolism in UV-induced erythema includes the correlation of increased metabolite formation with the development of erythema and erythema reduction by selective inhibitors. For example, significant elevations of arachidonic acid and its cyclooxygenase and lipoxygenase products are observed in dermal perfusates and in suction blisters raised on irradiated skin (3–5), and inhibition of cyclooxygenase-derived prostaglandins by indomethacin treatment blocks 50% of the erythema that develops during the first 24 h after UVB injury (3, 8–12). Studies of UV-irradiated keratinocytes suggest that the elevated levels of prostaglandins generated after irradiation might be due to increased synthesis and/or activity of the cyclooxygenase enzyme by 24 h after exposure (13). However, erythema in vivo is associated with increased quantities of prostaglandins 3–6 h after irradiation (1, 4, 5). Thus, the basis for increased prostaglandin synthesis during the early phase of erythema is uncertain.

One possible mechanism for the early increases in prostaglandin levels in UVB-induced erythema comes from the finding that dermal mast cells are also activated 3–6 h after irradiation, resulting in degranulation and histamine release (4, 14, 15). We speculated that histamine might in turn stimulate arachidonate metabolism in the skin, and furthermore that an increased response to histamine after UV irradiation might result in enhanced generation of prostaglandins.

The current studies were designed to determine if endogenous histamine release in UV injury induces prostaglandin synthesis in human skin. The histamine-dependent arachidonate metabolism of isolated human keratinocytes before and after UVB irradiation was also studied to assess whether UV injury alters the cellular response to histamine. Our finding that cells acquire the capacity to synthesize prostaglandin in response to endogenously released histamine after UV injury demonstrates that histamine-stimulated prostaglandin synthesis is an underlying mechanism in the early phase of the human erythema response.

Methods

Preparation of human skin explants. Explants were prepared from pale white full thickness human skin obtained from panniculectomies. To make explants, the dermis was trimmed using a scalpel, leaving tissue 1–1.5-mm thick and weighing ~ 0.1 g. Histologically, explants consisted of superficial dermis, superficial vascular plexus, and epidermis. All explant data was collected in quadruplicate. The wet weight of the tissue was measured to determine the protein content of the explants.

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1. *Abbreviations used in this paper:* AA, arachidonic acid, UVB, ultraviolet light.

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Because the trauma during preparation might produce mast cell degranulation, studies were begun 18 h after trimming, to allow mast cells to refill histamine stores (4, 15). After setting up explants, they were maintained at 37°C in a 5% CO_2 atmosphere. 18 h after the explants were cut, they were exposed to 120 mJ/cm^2 UV. This dose of light represents three to four times the minimal erythema dose of the average individual with pale white skin exposed to Westinghouse FS20 lamps (Westinghouse Electric Corp., Pittsburgh, PA) (2). In preliminary experiments, this dose of light was tested in individuals whose skin was pale white. The erythema reactions produced ranged in severity from erythema lasting only 24 h to deep erythema which persisted for 3 d, accompanied by transient edema at 24 h. Explants examined for morphologic effects of UV light were fixed in formalin, embedded in paraffin, cut into sections and stained with either hematoxylin and eosin or giemsa (for studies of mast cell number and morphology).

Preparation of keratinocyte cultures. Skin tissue was defatted and placed on gauze soaked in 0.25% trypsin in PBS. After incubation at room temperature overnight, the skin was rinsed in DME containing 5% FCS, 5 U/ml penicillin and 5 μg/ml streptomycin. The epidermis was separated from the dermis with forceps, and the epidermal cells scraped into DME containing 5% FCS, penicillin, streptomycin and 2.5 mM Hepes (pH 7.4). Isolated cells were placed into 22- or 100-mm dishes coated with collagen solution (Vitrogen; Flow Laboratories) diluted in PBS (1:3 vol/vol) at a density of 7.5×10^5 cells/cm^2. Cultures were used for experiments 3–4 d after reaching confluence. Experiments were also carried out using passaged keratinocytes. Passaged cells were maintained as described previously (16). These culture conditions do not promote the growth of melanocytes or Langerhans cells, so passaged keratinocytes are relatively free from contamination by other epidermal cell types (17).

Irradiation of explants and keratinocyte cultures. Explants were exposed to a bank of eight FS-20 bulbs (Westinghouse), which emit light predominantly in the UVB range, 290–320 nm, but also emit some longer and shorter wavelengths (2). Irradiance was 1 mW/cm^2 per s at 30 cm as measured by a UVX-digital radiometer with a UVX 31 filter (Ultraviolet Products, San Gabriel, CA). The dose of light used was 120 mJ/cm^2 unless indicated otherwise, and the length of exposure was timed to yield the desired dose. The dose of light used to irradiate keratinocyte cultures was 30 mJ/cm^2 unless indicated otherwise. A smaller amount of light was used to irradiate cultures due to the impartial keratinization that occurs in tissue culture (18) and subsequent increased sensitivity to UV injury (2). A thin layer of PBS was placed over the cultures during the exposure period to prevent drying. Control cultures were treated in the same way without UV exposure.

Measurement of products of arachidonic acid metabolism. Product formation by explants and cultures was determined by specific RIA of conditioned supernatants. In each case, explants or cultures irradiated with UV light were compared to unirradiated controls. Experiments using explants were conducted up to 8 h after irradiation. 1-h collections were made 6 h after irradiation, unless otherwise noted. Antihistamines were added at the time the skin was prepared. Explant agonist responsiveness was assessed 6 h after UV irradiation by incubation with histamine (1–10 μM) for 60 min.

Stimulation of cells in culture was carried out on cells labeled with [^{3}H]arachidonic acid to assess product identity by HPLC, and on unlabeled cells in experiments analyzed by specific RIA. For experiments with labeled cells, cultures were incubated for 6 h in DMEM containing 0.5% FCS, 40 μCi/ml [^{3}H]arachidonic acid (100 Ci/mmol; New England Nuclear, Wilmington, DE), and 0.5% DMSO. Cultures were exposed to UV light just before the labeling period. After labeling, cultures were washed twice with serum-free DME and then stimulated with histamine in DME containing 0.05% fatty acid-free bovine serum albumin (Calbiochem, San Diego, CA). Labeled cultures did not release detectable quantities of product without histamine stimulation. Unlabeled cultures were incubated with histamine (1–30 μM) for 0.5 to 15 min at 1–24 h after UV irradiation to assess concentration response and time course of histamine responsiveness.

High-pressure liquid chromatography. Products of [^{3}H]arachidonic acid oxygenation were extracted from culture supernatants using prostaglandin B_2 as an internal standard as previously described (19–21), and extracts were reconstituted in chromatographic solvent for analysis by reverse-phase HPLC. The chromatograph was fitted with a 4.6 × 100-mm analytical column packed with 3 μM octadecylsilane-coated particles (Dynamax; Rainin Instruments, Woburn, MA), and run at 1.0 ml/min using a solvent program set at 34% B for 0–9 min, 56% B for 10–35 min and 85% B for 36–43 min where A was water/acetic acid (100:0.01; vol/vol) and B was acetonitrile/acetic acid (100:0.01). The HPLC eluate was monitored using a diode array spectrophotometer 1040; Hewlett-Packard Co., Palo Alto, CA, and the outflow from the spectrophotometer was routed to a Radiomatic Flo-One-beta detector for concurrent measurement of ^{3}H. Authentic standards for PGE_2, $PGF_{2\alpha}$, PGD_2, and PGB_2 were obtained from Sigma Chemical Co. (St. Louis, MO) and HETEs (12-hydroxy-(5Z,8Z,10E,14Z)-eicosatetraenoic acid (12-HETE); 15-hydroxy-(5Z,8Z,11Z,13E)-eicosatetraenoic acid (15-HETE); and 5-hydroxy-(6E,8Z,11Z,14Z)-eicosatetraenoic acid (5-HETE) were from Bio-mol, Philadelphia, PA and Cayman Chemical, Ann Arbor, MI.

Determination of cyclooxygenase activity. The cellular capacity for cyclooxygenation was tested in control and irradiated cultures by incubation with exogenous arachidonic acid under conditions that resulted in maximal generation of prostaglandin. In preliminary experiments, arachidonic acid (1–30 μM) in DME containing 0.5% DMSO was incubated with cells for 1–30 min at 37°C to arrive at conditions for maximal product generation. 20 μM arachidonic acid was sufficient to saturate cyclooxygenase, and maximal product formation occurred in both conditions after incubation for 15 min.

Radioimmunoassays. PGE_2, $PGF_{2\alpha}$, PGD_2, TXB_2, 6-keto-$PGF_{1\alpha}$, 12S-HETE, and 15-HETE were determined in the supernatants from cultured cells by RIA as previously described (22). Antibodies to PGE_2, 6-keto-$PGF_{1\alpha}$, LTC_4, and TXB_2 were the gift of Philip Needleman (Washington University). Antibody to $PGF_{2\alpha}$, PGD_2, 12S-HETE and 15-HETE was purchased from Advanced Magnetics, Cambridge, MA. The limits of detection by RIA were: 2 pg for PGE_2, 74 pg for PGD_2, 2 pg for $PGF_{2\alpha}$, 1.5 pg for TXB_2, 10 pg for 6-keto-$PGF_{1\alpha}$, 17.2 pg for LTC_4, 300 pg for 12-HETE, and 24 pg for 15-HETE. Cross-reactivities of the antibodies were < 1%, except for the 12S-HETE antibody, which cross-reacts 10% with 12R-HETE, and for the PGD_2 antibody, which cross-reacts 2.75% with 6-keto-PGE_1. Treatment of the explants with 1 μg/ml indomethacin at the time of preparation completely inhibited prostaglandin synthesis. Histamine was assayed by competition of acylated histamine and ^{125}I-acylated histamine (AMAC, Inc., Westbrook, ME). The limit of detection of the assay is 1 nM.

Protein determinations. Metabolite levels were expressed per amount of total cell protein. The quantity of protein was determined by the fluorescamine method using BSA as a standard (23). UV irradiation of the cultures did not result in a significant change in their protein content.

Results

UV irradiation enhances release of eicosanoid metabolites from skin explants. In vivo, the erythema induced by UV exposure is dose-related and dependant upon skin color (1, 2). Initial studies were done to correlate the UV-stimulated prostaglandin release in explants with the pattern of UV erythema in vivo. UV exposure (30–180 mJ/cm^2) induced a dose-dependant increase in cumulative PGE_2 release from explants during the first 6 h after UV exposure (Fig. 1). The effect of skin color on UV-stimulated prostaglandin synthesis by explants was also studied in several experiments using black skin. When black skin explants were irradiated, no significant stimulation of PGE_2 synthesis was observed (6.2±1.3 ng/g per h in controls versus 6.7±1.5 ng/g per h after UV irradiation), con-

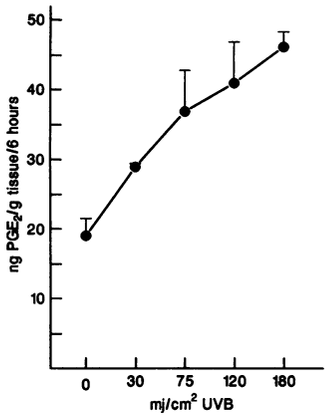

Figure 1. UV irradiation of human skin explants produces a dose-dependent increase in PGE_2 synthesis. Human skin explants were irradiated with 30–180 mJ/cm² UV. The cumulative PGE_2 synthesis of the explants during the next 6 h is shown. The average values from three experiments ± the SEM are shown.

sistent with the observation that black individuals can tolerate exposure to 120 mJ/cm² UV without developing erythema (2).

Radioimmunoassay was used to determine the range of eicosanoids released. PGE_2, $PGF_{2\alpha}$, and PGI_2 (measured as its stable metabolite 6-keto-$PGF_{1\alpha}$) were the prostaglandins synthesized in the greatest quantity (Table I). PGE_2 release by explants 6–7 h after irradiation was increased 5.5±0.83-fold over that of unirradiated tissue. An even greater increase in $PGF_{2\alpha}$ release was induced by irradiation (7.3±1.4-fold), whereas release of 6-keto $PGF_{1\alpha}$ was stimulated only 3.4±1.4-fold. Exogenous prostaglandin was added to indomethacin-treated cultures to determine the effect of UV exposure on prostaglandin degradation. No difference was observed in the quantity of material degraded over a 2-h interval, as determined by RIA. The increase in prostaglandins detected was therefore due to new synthesis, and not decreased breakdown of prostaglandin. 12-HETE was also detected in some experiments, but was present at levels near the limit of detection of the assay, so it was not possible to determine the effect of UV exposure on the synthesis of this metabolite. LTC_4, PGD_2, TXA_2 (measured as TXB_2) and 15-HETE were not detected. Therefore, in subsequent experiments, PGE_2, $PGF_{2\alpha}$, and 6-keto $PGF_{1\alpha}$ were the metabolites monitored to document the changes in prostaglandin release induced by UV exposure.

Table I. Eicosanoid Metabolites Detected by RIA in Human Skin Explants

Metabolite	Fold increase after UV injury
PGE_2	5.5±0.8*
6-Keto $PGF_{1\alpha}$	3.2±1.4
$PGF_{2\alpha}$	7.3±2.0
PGD_2	ND
12-HETE	ND
15-HETE	ND
LTB_4	ND
LTC_4	ND
TXB_2	ND

UV-irradiated explant eicosanoid release into conditioned medium was determined 6 h after exposure and compared to that of unirradiated tissue. The mean±SEM from five experiments is shown. ND, not detected. * $P \leq 0.015$, paired t test.

H1 receptor blockers decrease UV-induced prostaglandin synthesis in human skin. The possible stimulation of explant prostaglandin synthesis by endogenous histamine release was examined by incubation of the explants with H1 and H2 histamine receptor blockers. Pretreatment of the explants with the H1 receptor antagonist brompheniramine (50 μM) resulted in a 63±4.3% inhibition of prostaglandin release from the explants (mean±SEM, $n = 6$, $P \leq 0.02$) (Fig. 2). A 30% inhibition of the UV-stimulated prostaglandin release also occurred in explants incubated with 30 μM pyrilamine, an H1 antagonist from a different chemical class. Despite the significant inhibition of prostaglandin synthesis by H1 antagonists in irradiated explants, no effect on prostaglandin synthesis of control explants was observed. The suppression of prostaglandin release produced by H1 receptor blockers slowly increased over the 8-h period of observation (Fig. 2). The effect of antihistamines on explant prostaglandin synthesis was further examined by incubating explants with doses of brompheniramine ranging between 5 and 100 μM. A dose-dependent inhibition of PGE_2, $PGF_{2\alpha}$, and 6-keto $PGF_{1\alpha}$ release was again observed only in irradiated explants (Fig. 3). Treatment of irradiated explants with as little as 10 μM brompheniramine resulted in a 50% inhibition of prostaglandin release. The decrease in prostaglandin release induced in antihistamine-treated explants was not a toxic effect, since prostaglandin release could be stimulated by 1 μM bradykinin, but not 10 μM histamine (Fig. 4). In contrast to the effectiveness of the H1 antagonists in suppressing prostaglandin synthesis, incubation of the explants with 30 μM of the H2 antagonist cimetidine did not change prostaglandin synthesis (Fig. 5).

Increased prostaglandin release results primarily from increased agonist sensitivity, not increased histamine release. Since endogenous histamine release was apparently responsible for stimulating increased prostaglandin release in the first 8 h after UV injury, the quantity of histamine present in the conditioned media of the explants was measured by radioimmunoassay. 6 h after UV exposure 35.7±4.5 nM histamine was detected in the medium from irradiated explants, while

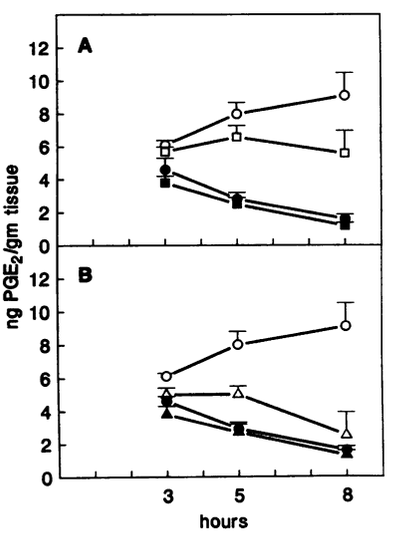

Figure 2. Time course of antihistamine effect on prostaglandin release from control and UV irradiated explants. Explants were incubated with either 30 μM pyrilamine (*A*) or 50 μM brompheniramine (*B*). The quantity of PGE_2 synthesized by the explants was determined at intervals after irradiation. Only the irradiated explants' prostaglandin synthesis was suppressed by antihistamine treatment. Control, closed symbols; UV, open symbols. (○) no addition, (□, ■) pyrilamine, (△, ▲) brompheniramine. Quadruplicate determinations from a representative experiment are shown. The experiment was repeated with similar results.

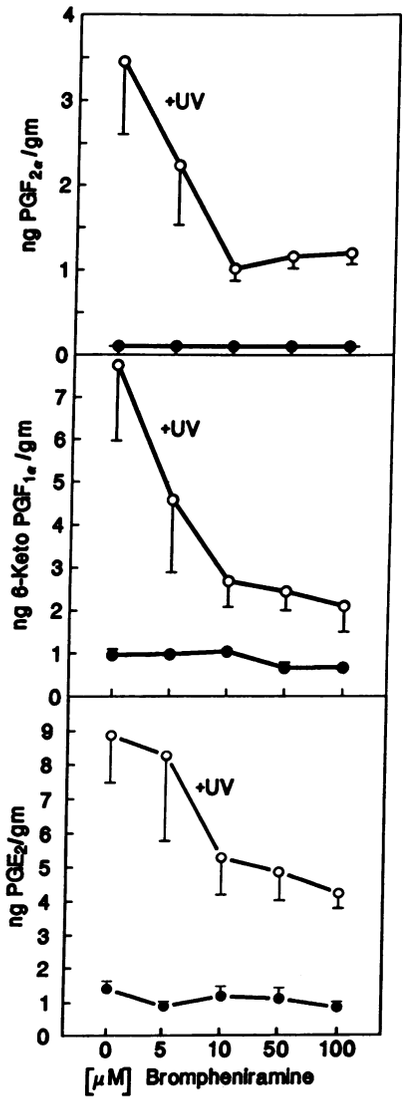

Figure 3. Brompheniramine suppresses prostaglandin synthesis in irradiated explants. Explants were incubated with 5–100 μM brompheniramine. Prostaglandin synthesis was determined 6 h after irradiation. Quadruplicate determinations from a representative experiment are shown. The experiment was repeated with similar results.

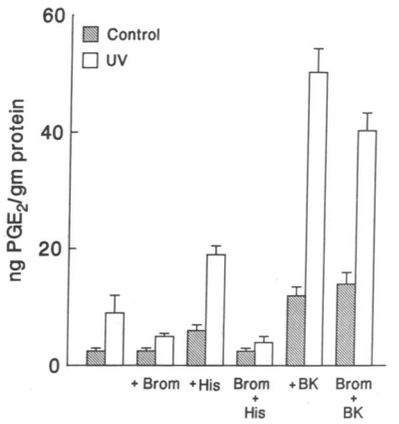

Figure 4. Brompheniramine-treated explants synthesize PGE_2 in response to bradykinin, but not histamine. Control and irradiated explants were incubated with 50 μM brompheniramine (*Brom*) from the time of preparation. 6 h after irradiation, explants were stimulated with either 10 μM histamine (*His*) or 1 μM bradykinin (*BK*), and the ng PGE_2/g tissue determined. Quadruplicate determinations from a representative experiment are shown. The experiment was repeated with similar results.

23.4±2.8 nM was detected in medium from controls (n = 6). Although some increase in the quantity of histamine released from explants did occur, most intriguing was the finding that despite a significant concentration of histamine in the medium of control explants, their prostaglandin synthesis was not increased by histamine.

This observation was further examined by incubating explants with 1 to 10 μM exogenous histamine then determining the quantity of prostaglandin released. Prostaglandin release in irradiated explants incubated with 3 μM histamine was increased threefold, whereas control explants release of prostaglandin increased only minimally when incubated with 10 μM histamine (Fig. 6). Thus, while endogenous histamine release is important to stimulate prostaglandin synthesis, an increase in sensitivity to histamine also contributes to the increased prostaglandin synthesis that occurs.

UV irradiation causes increased histamine responsiveness in epidermal cultures. Since the majority of the light entering the skin is absorbed by the epidermis, epidermal cell cultures were prepared to study the mechanism of enhanced histamine responsiveness that appeared in irradiated explants. Analysis of supernatants from histamine-stimulated keratinocytes by HPLC and RIA demonstrated that PGE_2 was the predominant metabolite: PGE_2 release was markedly increased after UV irradiation, as was observed in irradiated explants. In unirradiated keratinocyte cultures, significant release of PGE_2 was detected only after stimulation with the highest concentration of histamine (30 μM) and the level of release was so low that it was detectable by RIA but often not by HPLC (Figs. 7 and 8). In contrast, keratinocyte cultures exposed to 30 mJ/cm^2 of UV light became responsive to lower concentrations of histamine and generated larger amounts of PGE_2 at all concentrations tested (Fig. 8). As little as 1 μM histamine caused significant release of PGE_2 from irradiated keratinocytes, and 10–30 μM histamine caused irradiated cells to generate six to eight times

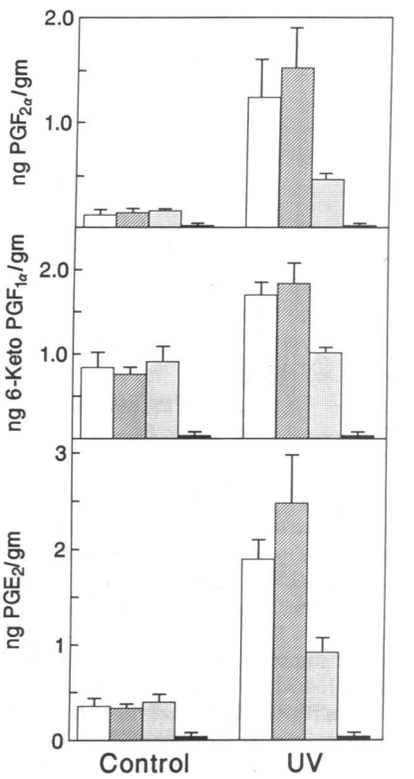

Figure 5. Brompheniramine, but not cimetidine suppresses irradiated explant prostaglandin synthesis. Control or irradiated explants were incubated with 50 μM brompheniramine (□), 30 μM cimetidine (▨) or 1 μM indomethacin (■) or no addition (□). The average release of prostaglandin was then determined 6 h after irradiation. Quadruplicate determinations from a representative experiment are shown. The experiment was repeated with similar results.

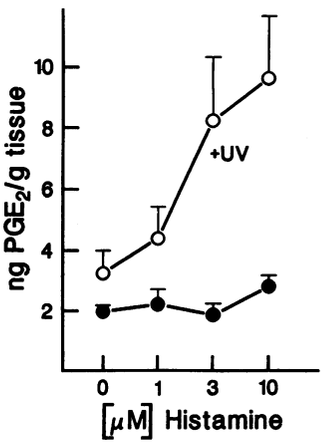

Figure 6. UV irradiation increases the response of skin to exogenous histamine. Control (•) or UV-irradiated (○) explants were incubated with 1–10 μM histamine and the synthesis of PGE_2/g tissue determined. The average values from three experiments ± the SEM are shown.

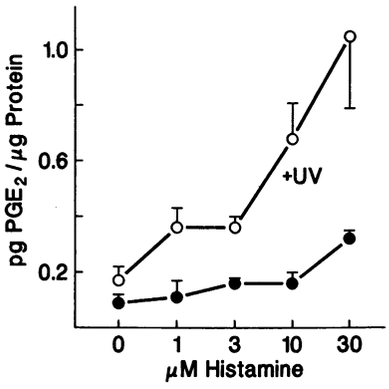

Figure 8. Enhanced synthesis of PGE_2 by ultraviolet light (UV)-irradiated keratinocyte cultures in response to histamine. Confluent keratinocyte cultures were irradiated with 30 mJ/cm² of UV, and 6 h later were incubated with 1–30 μM histamine for 15 min. Unirradiated controls showed synthesis of PGE_2 in response to 30 μM histamine, but UV irradiated cultures synthesized significant quantities of PGE_2 in response to only 1 μM histamine. UV (○), Control (•). Each point represents the mean±SEM for three experiments. $P < 0.05$ by paired t test for all values.

higher levels than the maximal amounts from unirradiated control cultures (Fig. 8). This pattern of increased histamine sensitivity was identical to that observed in histamine-stimulated irradiated explants. Despite the increased sensitivity to histamine, no significant increase in the basal accumulation of PGE_2 was detected during the 6 h between irradiation and histamine stimulation.

Reverse-phase HPLC analysis of the radiolabeled metabolites released by irradiated keratinocytes demonstrated that, in addition to PGE_2 the largest peak of activity corresponded to arachidonic acid itself and that the quantity of both was increased by UV irradiation (Fig. 7). The degree of increase for PGE_2 determined by HPLC was similar to the change detected by RIA, and the increase in the level of arachidonic acid was consistent at a mean value of 20% above control (n = 3). Analysis of supernatants from irradiated keratinocytes also revealed a smaller peak of radiolabeled material which corresponded to the retention time of $PGF_{2\alpha}$, but no evidence of generation of HETEs, leukotrienes, or epoxides (Fig. 7). The presence of $PGF_{2\alpha}$ and its increase after UV irradiation were substantiated by determinations using RIA, which exhibited a mean increase of five times in irradiated compared to control cultures (data not shown). The absence of detectable HETEs on HPLC was confirmed by the failure to detect significant amounts of 12- or 15-HETE by RIA (data not shown).

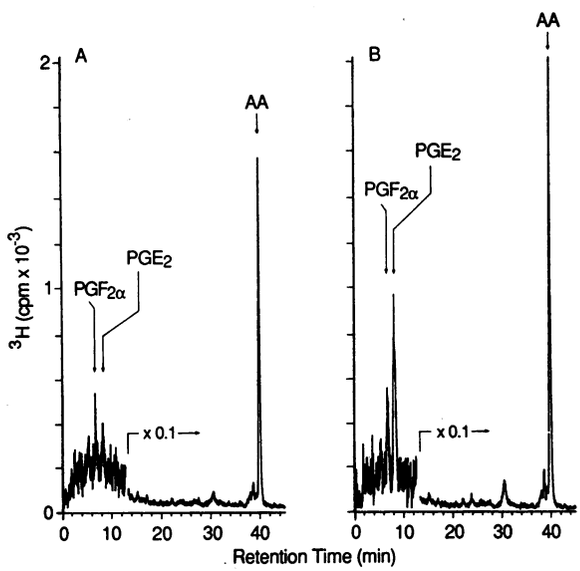

Figure 7. Analysis by reverse-phase HPLC of ^{3}H-labeled products released from keratinocytes in response to histamine. Control (*A*) or UV-irradiated (*B*) confluent cultures in 100-mm dishes were incubated for 6 h in DMEM containing 0.5% FCS and 40 μCi/ml [^{3}H]arachidonic acid. At the end of the labeling period, cultures were stimulated with 10 μM histamine in 0.5% fatty acid free BSA in DMEM for 15 min. Labeled cultures not stimulated with histamine did not release detectable quantities of product. Each tracing represents material released from four 100-mm plates. Major peaks of radioactivity coeluted with PGE_2 and arachidonic acid (*AA*). The peak at 30 min was not identified and was not present in all tracings.

To exclude the possibility that the melanocytes and langerhans cells present in primary epidermal cultures were the source of enhanced prostaglandin release, the histamine responsiveness of serially passaged keratinocytes was examined. The cultures were exposed to 30 mJ/cm², then stimulated with doses of histamine between 1 and 10 μM. The UV potentiation of the histamine response in the passaged cells was the same as that of primary epidermal cell cultures (data not shown).

Dose-response and time course of UV effect on histamine responsiveness. Enhancement of histamine responsiveness after varying amounts of UV irradiation exhibited a steep dose-response with a marked increase in responsiveness at 30 mJ/cm² (Fig. 9). Doses of light lower than 30 mJ/cm² did not affect histamine-stimulated PGE_2 release, but cultures exposed to 30–60 mJ/cm² exhibited a marked increase in release: supernatants from irradiated cultures contained three to four times as much PGE_2 as cultures exposed to lower amounts of UV light. The development of histamine responsiveness after exposure of 30 mJ/cm² was observed as early as 1 h after irradiation and was maximal by 3–6 h (Fig. 10), in a pattern identical to the brompheniramine-suppressible prostaglandin release of the explants. Increased responsiveness was still present up to 72 h after exposure to UV light.

UV-induced morphologic changes. Despite the marked enhancement of histamine-stimulated arachidonate metabolism after UV exposure, neither explants nor irradiated keratinocyte cultures showed evidence of morphologic changes by phase microscopy. When cultures were examined 6 h after UV irradiation with 30 mJ/cm², the appearance of irradiated cul-

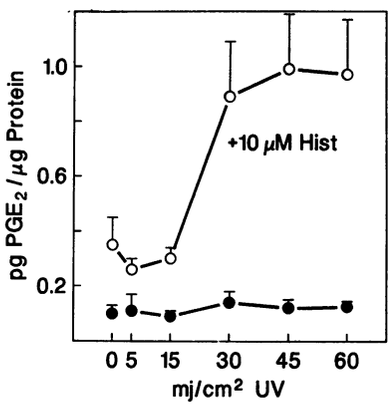

Figure 9. Effect of quantity of UV light on histamine-induced PGE_2 release. Confluent keratinocyte cultures were irradiated with 5–60 mJ/cm^2 UV light. 6 h after UV exposure, they were stimulated with 10 µM histamine for 15 min. Doses of light 30 mJ/cm^2 or greater enhanced the histamine response in the cultures. UV (●), UV + histamine (○). Each point represents the mean±SEM for three experiments.

tures was indistinguishable from that of control cultures. However, by 24 h after exposure to this dose of UV light, the irradiated cells exhibited mild cytoplasmic vacuolization and swelling, without loss of adherence, cell lysis or dyskeratosis (Fig. 11). Explants fixed in formalin, sectioned and stained with either hematoxylin and eosin or Giemsa showed no morphologic change or change in mast cell number or granularity.

Although the changes in histamine-stimulated PGE_2 release from cultured keratinocytes were similar at 30, 45, and 60 mJ/cm^2, only the two highest doses of UV light caused patchy areas of necrosis by 24–48 h after irradiation. Even at the highest doses of light, no changes in cell morphology were evident 6 h after irradiation.

H1 receptors mediate the histamine response. Inhibition with selective antagonists suggested that the receptor that mediated the histamine response in cultured keratinocytes was of the H1 type. Cultures incubated with the H1 antagonist pyrilamine no longer responded to histamine, but those that were pretreated with the H2 antagonist cimetidine exhibited no significant change in histamine-stimulated PGE_2 release (Table II). Thus, H1 receptors are linked to release and oxygenation of arachidonic acid in human keratinocytes.

Cyclooxygenase activity is not increased by UV irradiation. The possibility that the increase in PGE_2 synthesis after UV-irradiation was due to an increase in cyclooxygenase activity was examined by assaying control and irradiated cells for maximal cyclooxygenase activity. Cells that were UV-irradiated 6 h before study generated no more PGE_2 when incubated with exogenous arachidonic acid (20 µM for 15 min) than did unirradiated cultures.

Protein synthesis is required for UV-potentiated PGE_2 release. To further examine the mechanism of the enhanced responsiveness to histamine, the effect of inhibiting protein synthesis with cycloheximide was examined. Addition of cycloheximide 5 µg/ml immediately after irradiation blocked increased synthesis of PGE_2 in response to histamine but did not significantly alter basal PGE_2 release (Table III). [^{3}H]Leucine incorporation into acid-precipitable protein was monitored to document the effectiveness of cycloheximide treatment. An 80% inhibition of protein synthesis occurred using this dose of drug. Of note is the observation that UV exposure did not alter the total incorporation of [^{3}H]leucine into protein after 6 h, so the protein synthesis required for the increased response to histamine is not accompanied by an overall increase in cellular protein synthesis.

Discussion

The results show that UV irradiation increases prostaglandin synthesis in human skin explants through endogenous histamine stimulation. This implies an important role for histamine in mediating UV erythema, since the role of prostaglandins in mediating UV erythema is well established (3, 8–12). Several aspects of the data collected support this conclusion. Suppression of prostaglandin synthesis was specific and receptor mediated since blockade of histamine receptors using drugs from two different chemical classes was effective in decreasing prostaglandin release in irradiated explants, while the H2 antagonist cimetidine was ineffective. In addition, bradykinin-stimulated prostaglandin release could occur in the presence of the H1 receptor blocker brompheniramine. This antihistamine-suppressible component of UV-stimulated prostaglandin synthesis also occurs at the same time that UV-induced mast cell degranulation takes place, and increased quantities of histamine have been measured in suction bullae raised on irradiated skin (4). Taken collectively, this evidence implicates histamine as an important mediator of UV-stimulated prostaglandin synthesis in vivo.

Another important observation is the discovery that UV exposure decreases the threshold for histamine stimulation in irradiated skin. Irradiation enables the explant to respond to the concentration of histamine actually present in the tissue. This is demonstrated by the observation that the prostaglandin synthesis of unirradiated explants bathed in nearly the same concentration of histamine as irradiated explants is not affected by the presence of the agonist. An irradiation-induced decrease in the threshold and increase in the magnitude of histamine-stimulated PGE_2 synthesis was also found in cul-

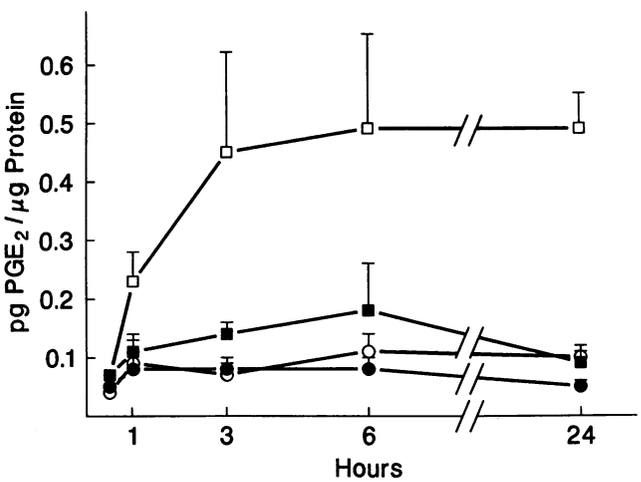

Figure 10. Time course of the UV effect on histamine responsiveness. At 1–24 h after irradiation with 30 mJ/cm^2 UV, cultures were incubated with 10 µM histamine for 15 min and PGE_2 release was determined. UV irradiated cultures acquired enhanced responsiveness to histamine within 1 to 3 h of irradiation. UV (○), UV + histamine (□), control (●), control + histamine (■). Each point represents the mean±SEM for three experiments.

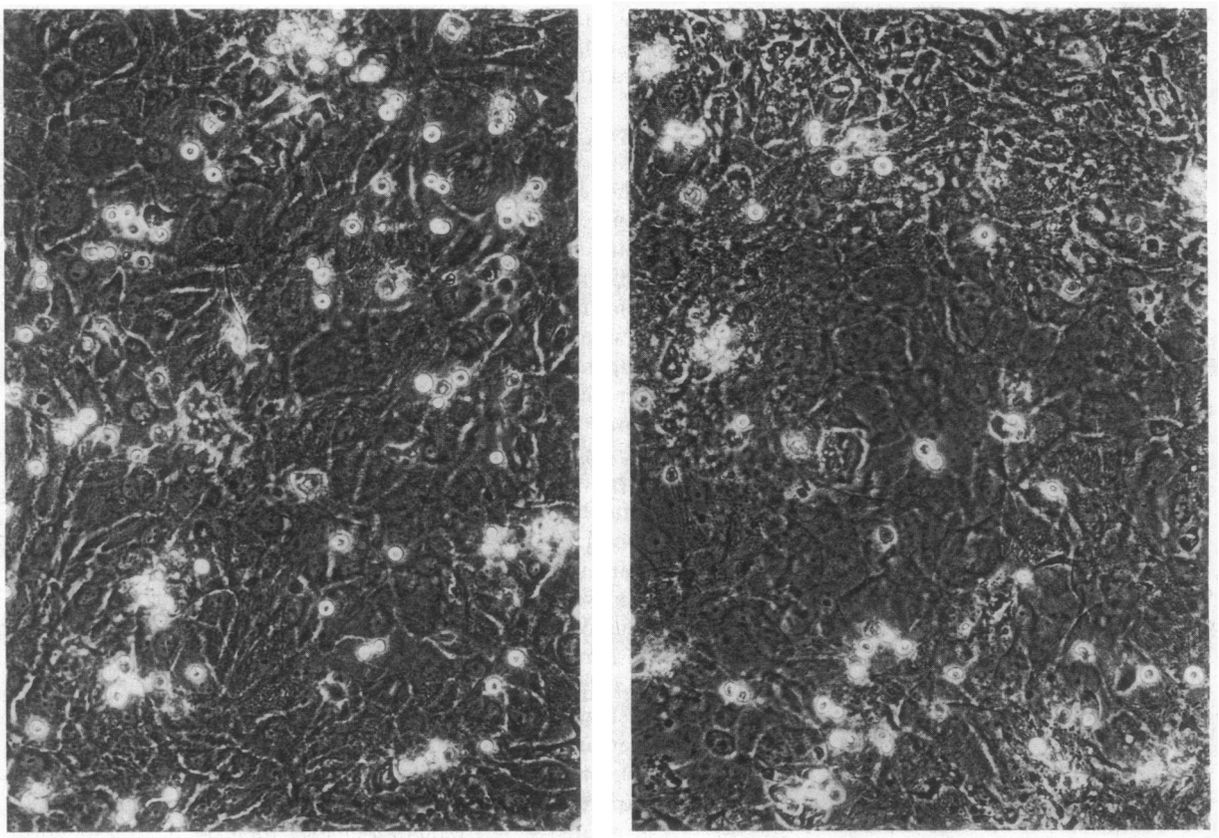

Figure 11. Morphology of control and UV-irradiated keratinocyte cultures 24 h after exposure to light. Photomicrograph of control (*left*) or UV-irradiated (*right*) cells 24 h after exposure to 30 mJ/cm^2 UVB. Irradiated cells appear swollen with decreased cytoplasmic filamentous material. × 150.

tured human keratinocytes. Therefore, the change induced by UV exposure is not just mediator release, but an increase in the capacity of irradiated tissue to respond. The time course of the onset of increased histamine sensitivity in the epidermal cultures corresponds well with the observed onset of UVB erythema (3–6 h after UVB irradiation) (1, 4, 5), and with the onset of brompheniramine-suppressible prostaglandin synthesis by irradiated explants. Taken collectively, these observations strongly support the role of histamine-induced prostaglandin synthesis in the response to UV light in vivo.

The underlying mechanism of the increased agonist responsiveness induced by UV injury remains to be defined. New protein synthesis is required, because cycloheximide blocked the increase in histamine sensitivity. This suggests an increase in the quantity of the enzymes leading to synthesis of prostaglandin, or in the receptor-linked signal transduction apparatus regulating their function. A change in the synthesis or degradation of cyclooxygenase is not implicated, since cyclooxygenase activity was not increased in the irradiated cultures. Enhanced prostaglandin synthesis due to increased phospholipase activity is likely since increased release of labeled arachidonic acid in histamine-stimulated irradiated cultures was present. UV-induced lipid peroxidation (24, 25) may contribute to this increase in phospholipase activity since phospholipase stimulation and inhibition of fatty acid reacylation have been demonstrated in tissues suffering from peroxidation injury (26–28).

Cytokines such as IL-1, tumor necrosis factor, and IL-6, which are synthesized by epidermis and which are released in skin by UV exposure (29) may play a role in mediating irra-

Table II. Effects of H1 and H2 Receptor Antagonists on Histamine-stimulated PGE_2 Release

	Control		UV			
		+Histamine		+Histamine	+Histamine +Pyrilamine	+Histamine +Cimetidine
Exp. 1	0.12	0.13	0.11	0.58	0.08	0.6
Exp. 2	0.04	0.09	0.11	0.46	0.06	0.48

UV-irradiated cultures were preincubated with 10 μM pyrilamine (an H1 antagonist) or 30 μM cimetidine (an H2 antagonist) for 30 min, then stimulated with 10 μM histamine and the resulting synthesis of PGE_2/μg protein was determined. Pyrilamine, but not cimetidine, was able to completely inhibit prostaglandin synthesis by the cultures. The average values obtained from two experiments are shown.

Table III. New Protein Synthesis Is Required for an Enhanced Keratinocyte Prostaglandin Synthesis in Response to Histamine

	Basal		+Histamine		[^{3}H]Leucine	
		+CHX		+CHX		+CHX
	pg PGE_2/μg protein				*cpm/culture*	
Control						
Exp. 1	0.06	0.06	0.06	0.06	17,380	3550
Exp. 2	0.03	0.05	0.05	0.05	19,088	3886
Exp. 3	0.01	0.04	0.03	0.03	34,620	4680
UVB						
Exp. 1	0.09	0.07	0.36	0.06	12,725	3280
Exp. 2	0.05	0.07	0.17	0.05	15,528	2258
Exp. 3	0.04	0.02	1.27	0.05	34,120	3732

Control or UV-irradiated cultures were incubated with 5 μg/ml cycloheximide (CHX) during the 6-h interval between UV irradiation and stimulation with histamine. Cycloheximide treatment of the cultures eliminated their responsiveness to histamine, but did not affect basal prostaglandin release.

diation erythema. However, use of recombinant IL-1, IL-6, and tumor necrosis factor did not reconstitute enhanced histamine sensitivity in cultured keratinocytes (Pentland, A., unpublished observations). Other polypeptide mediators not yet identified may still induce the keratinocyte response. In addition, these cytokines may be very important at later time points in the irradiation response.

Our results differ in one aspect from previous studies of UVB-induced injury carried out in vivo in that we did not find evidence of 12-HETE generation in the cultured cells in response to UV irradiation. Cultured keratinocytes exhibit little evidence of the monooxygenase pathway responsible for 12-HETE generation (unpublished observations by Holtzman and Pentland), which is expressed at a high level in freshly isolated epidermal cells (21). Explanations for differences between freshly isolated and cultured cell oxygenation pathways include the loss of a subset of epidermal cells during culture or an alteration in the expression of keratinocyte oxygenation pathways during growth in artificial media. Determinations of factors that regulate expression of oxygenation enzymes are currently under investigation. The absence of consistently detectable quantities of 12-HETE in the explants is most likely due to the poor sensitivity of the RIA available. More work will be needed to define whether the synthesis of this metabolite is also stimulated by endogenous agonists in skin.

The data also clarify previous work examining histamine responses in the skin. While increased prostaglandin synthesis has been demonstrated in histamine-stimulated whole skin (30), the capacity of isolated keratinocytes to synthesize prostaglandins in response to this agonist has not been examined. The presence of histamine receptors in epidermis has been shown by earlier work demonstrating that keratinocyte adenylate cyclase is stimulated via an H_2 receptor (31, 32). To our knowledge, the capacity of keratinocytes to release prostaglandins through stimulation of H1 receptors is clearly demonstrated here for the first time.

A role for histamine in the erythema response to UVB has been established in several animal models but not in humans. In studies of guinea pigs, the early phase of erythema is suppressible with antihistamines (12, 33), suggesting it is mediated by histamine. In rats, a histamine-suppressible component of UVB-induced erythema occurs during the later, peak erythema phase (33). In humans, evidence demonstrating a role for histamine in UV erythema is equivocal. In one study by Partington, no effect was found, but the effectiveness of the antihistamine was documented only by its ability to suppress histamine-induced vasodilation (34). In this series of experiments, the histamine receptor of epidermis may not have been adequately blocked. An extended preincubation with antihistamine was found to be useful in our experimental system (data not shown). In contrast to these findings, a trial of triprolidine to reduce UVB erythema in patients with several photosensitivity dermatoses produced a significant benefit (35). The data presented here suggest the importance of histamine-stimulated prostaglandin release as an endogenous mediator of UV erythema in humans. The realization that increased sensitivity of irradiated tissue to histamine occurs after UV exposure will facilitate design of rational drug therapy for UV injury.

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