Cutaneous hypothalamic-pituitary-adrenal axis homolog: regulation by ultraviolet radiation

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Submitted 4 May 2011; accepted in final form 7 June 2011


The hypothalamic-pituitary-adrenal (HPA) axis represents one of the main limbs of an adaptive system, which maintains the basal and stress-related homeostasis in vertebrates. Skin expresses all elements of the HPA axis including corticotropin-releasing hormone (CRH), proopiomelanocortin (POMC), ACTH, β-endorphin (β-END) with corresponding receptors, the glucocorticoidogenic pathway, and the glucocorticoid receptor (GR). To test the hypothesis that cutaneous responses to environmental stressors follow the organizational structure of the central response to stress, the activity of the “cutaneous HPA” axis homolog was investigated after exposure to ultraviolet radiation (UVR) wavelengths of UVA (320–400 nm), UVB (280–320 nm), and UVC (100–280 nm) in human skin organ culture and in co-cultured keratinocytes/fibroblasts. The level of stimulation of CRH, POMC, MC1R, MC2R, CYP11A1, and CYP11B1 genes was dependent on UV wave-lengths, with the highest effects observed for highly energetic UVC and UVB. ELISA and Western assays showed significant production of CRH, POMC, ACTH, and CYP11A1 proteins and of cortisol, with a decrease in GR expression only after UVB and UVC. However, β-END expression was also stimulated by UVA. Immunochemistry localized the deposition of the aforementioned antigens predominantly to the epidermis with additional accumulation of CRH, β-END, and ACTH in the dermis. UVR-stimulated CYP11A1 expression was seen in the basal layer of the epidermis and cells of adjacent dermis. Thus, the capacity to activate or change the spatial distribution of the cutaneous HPA axis elements is dependent on highly energetic wavelengths (UVC and UVB), implying a dependence of a local stress response on their noxious activity with overlapping or alternative mechanisms activated by UVA.

corticotropin-releasing hormone; proopiomelanocortin; stress; cortisol; glucocorticoid receptor

THE HYPOTHALAMIC–PITUITARY–ADRENAL (HPA) AXIS REPRESENTS ONE OF THE MAIN LIMBS OF AN ADAPTIVE SYSTEM, WHICH MAINTAINS THE BASAL AND STRESS-RELATED HOMEOSTASIS IN VERTEBRATES (6, 25, 28, 55). Thus, stress (psychological, physical, or biological) stimulates hypothalamic corticotropin-releasing hormone (CRH) production and release, which, after activation of CRH receptor type 1 (CRH-R1) in the anterior pituitary, stimulates production and release of proopiomelanocortin (POMC)-derived adrenocorticotropin ACTH (6, 25, 55). ACTH in the adrenal cortex activates MC2 receptors (MC2R, receptor for ACTH), stimulating secretion and production of glucocorticoids (GC), mainly cortisol [COR, (6)]. COR counteracts the effects of stressors and exerts powerful immunosuppressive effects.

The skin, the largest organ of the body, is continuously exposed to environmental factors, of which ultraviolet (UV) wavelengths of solar radiation represent the most prevalent stressor to humans and diurnal animals (17, 39, 53). Therefore, it has been proposed that the homolog of the HPA axis has developed in the integument (skin) as an efficient way to deal with environmental stressors (30, 41, 43). This concept is strengthened by the evidence that vertebrate skin expresses CRH and related peptides, POMC, which its further processed to β-endorphin (β-END), ACTH, and melanocyte-stimulating hormone (MSH) (reviewed in Refs. 40, 42). In addition, skin cells express the corresponding functional CRH-R1 (reviewed in Ref. 48), melanocortin (MC), and opiate receptors (reviewed in Refs. 2, 53). Furthermore, CRH, POMC, and their corresponding receptors are coexpressed in cultured skin cells, with this coexpression also being demonstrated in skin biopsies by in situ hybridization or immunocytochemistry (18, 20, 22, 36, 42, 58). Finally, production of corticosterone (CORT) and COR has been clearly demonstrated in cultured normal human melanocytes and fibroblasts (45–47) and in human hair follicles (18, 29). In fact, skin expresses cytochrome P-450scc (P450scc or CYP11A1) and is capable of initiating steroidogenesis from cholesterol with pregnenolone as an intermediate product (49), which undergoes further sequential transformation to progesterone, deoxycorticosterone (DOC), 18(OH)-DOC, CORT, and COR (18, 33, 34, 45–47). The expression of these elements appears to be nonrandom, with organization into functional, cell type-specific regulatory loops with a structural hierarchy similar to that found at the central level (18, 43, 45, 46).

Ultraviolet radiation (UVR) represents the electromagnetic energy covering wavelengths between 100 and 400 nm. It includes UVC (100–280 nm), which is absorbed by the atmosphere but when generated by artificial light sources has profound mutagenic and lethal effects (1). UVB (280–320 nm), although representing only ~5% of the UV spectrum of the solar radiation reaching the surface of the earth, is very efficient at stimulating cutaneous biological effects, including mutagenic and carcinogenic effects, induction of sunburns, stimulation of melanin pigmentation, and inducing transformation of 7-dehydrocholesterol to vitamin D3 (1, 10, 15, 17, 24, 38). It can penetrate to the level of the papillary dermis. UVA (320–400 nm), which has good cutaneous penetration, has...
lower ability to induce erythema and melanogenesis and is also less carcinogenic but having a profound effect on photoaging (1, 21). UV exerts many different biological actions on human and animal organisms utilizing different mechanisms of action including direct and indirect DNA damage, free radical production, and/or interaction with specific chromophores (1, 7, 10, 13, 35).

Studies on cultured isolated skin cells have demonstrated that UVB can stimulate the expression of CRH, POMC, and POMC-peptides (4, 23, 27, 32, 57), implying its involvement in the regulation of local neuroendocrine activities (41). Since UVR is a prevalent environmental stressor, we decided to clarify the nature of cutaneous neuroendocrine responses to UVR by testing wavelength-dependent changes in the expression pattern of crucial elements of the HPA axis. We used co-cultured human keratinocytes and melanocytes, because of the bidirectional communication between these cells, and full-thickness histo-cultured skin biopsies as the reliable ex vivo models of human skin.

MATERIALS AND METHODS

Approval of human protocols. All procedures adhered to the principles of the Declaration of Helsinki and were approved by the local Institutional Review Board with Exempt Protocol no. 4.

Co-cultures. Second passage of human neonatal epidermal keratinocytes (HEKn) and human neonatal epidermal melanocytes (HEMn) was used for co-cultures. Cells were seeded in a ratio 5:1; e.g., 5 × 10^5 HEKn and 1 × 10^5 HEMn per Petri dish (100 mm in diameter) in triplicate and maintained in 2 ml of serum-free medium (to remove all exogenous sources of POMC-derived peptides) composed of mixed KBM-2-MBM-4 (1:1) supplemented with insulin (5 μg/ml), human epidermal growth factor (2 μg/ml), human fibroblast growth factor (5 μg/ml), and 1% antibiotic antymycotic solution (AAS) (all from Lonza, Walkersville, MD). After 2 days of incubation time (37°C, 5% CO2), when the cells achieved 90% confluence, the media were discarded and cells washed 2× with PBS, and 3 ml of PBS was added to each flask. The cells were irradiated with the appropriate doses of UVA, UVB, or UVC (see Irradiation protocols below). The control cells were treated the same way, except that they were not exposed to UVR (sham-treated groups). During irradiation with UVA, both experimental and control (covered by aluminum foil) groups were placed on a cold (4°C) blanket to prevent heating. Eight skin fragments per dish (in duplicate cultures) were used for each condition. Next, the skin fragments were placed into six-well plates, eight per well, containing 1.5 ml of WILLIAM’S Medium E with L-glutamine and phenol red (Sigma, St. Louis, MO) and supplemented with insulin (5 μg/ml) and AAS (1%) for 6, 12, 24, and 48 h of incubation at 37°C, 5% CO2. The fragments were used separately for RNA, WB, ELISA, and immunohistochemical (IHC) studies.

Irradiation protocols. Doses of irradiations were as follows: UVA, sham control (C) = 0, 10, 20, 50 J/cm²; UVB, C = 0, 50, 100, 200 ml/cm²; and UVC, C = 0, 1, 5, 10 ml/cm² (Table 1). Real-time RT-PCR (RT-PCR) assay. RNA from cell pellets was extracted using the Absolutely RNA Miniprep kit (Stratagene La Jolla, CA), and TRIzol reagents (Invitrogen, Carlsbad, CA) were used to isolate RNA from skin. The RNA concentration was quantified, and 3 μg of total RNA (either from cells or from the skin) was reverse-transcribed with SuperScript First-Strand Synthesis System (Applied Biosystems, Foster City, CA). Primers used for PCR amplification are listed in Table 2 and were designed with the Universal Probe Library (Roche, https://www.roche-applied-science.com) and synthesized by Integrated DNA Technologies (Coralville, IA). The reaction was performed in triplicate with SYBR Green I Master (Roche, Manheim, Germany). The subcutaneous fat was removed, and skin was cut into 0.5 × 0.5-cm pieces. Skin fragments were placed dermis down onto humid (PBS) Waltham paper in a 60-mm Petri dish and rinsed with PBS and harvested by trypsinization, centrifuged into pellets as described previously (32, 37), and frozen separately at −80°C for RNA, Western blot (WB), and ELISA assays.

ELISA assays. Cell pellets were lysed by vortexing while the skin fragments were homogenized with Brinkmann homogenizer (Brinkmann, Dallas, TX) with 2 ml of ice-cold RIPA buffer [PBS containing 1% Nonidet P-40, 0.1% SDS supplemented with 1% proteinase inhibitor cocktail (PIC, 10 μl/1 ml, Sigma, St. Louis, MO)]. The homogenates were kept on ice for 20 min and then centrifuged for 25 min (13,000 g, 4°C). Supernatants were col-

Table 2. List of primers used for real-time RT-PCR

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Primer Location</th>
<th>Accession no.</th>
<th>Forward Sequence, 5'-3'</th>
<th>Reverse Sequence, 5'-3'</th>
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<tr>
<td>CRH</td>
<td>Exon 2</td>
<td>NM_000756</td>
<td>ACTCGAGAAGCGAAGTCGA</td>
<td>CTTCCAGAGGTGGTCTGAGGT</td>
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<tr>
<td>POMC</td>
<td>Exon 3</td>
<td>NM_000939</td>
<td>CTCGAGGGGTTTCAATGACTT</td>
<td>GCTGACTCGCCCTGTTCTTTG</td>
</tr>
<tr>
<td>MC1R</td>
<td>Exon 1</td>
<td>NM_002386.3</td>
<td>ACTCTGCTGCTGCTGAAAGC</td>
<td>AGAGCTGGAGAAGAGATG</td>
</tr>
<tr>
<td>MC2R</td>
<td>Exon 2</td>
<td>NM_000529</td>
<td>TCTTCAGGTCCTGGAGTGA</td>
<td>GGCACAGGATGAAGAGCC</td>
</tr>
<tr>
<td>CYP11A1</td>
<td>Exon 4</td>
<td>NM_001099773</td>
<td>GGCCACCTGCTGGCTGTGG</td>
<td>AAAATCACTGGCTGAGGAG</td>
</tr>
<tr>
<td>CYP11B1</td>
<td>Exons 2/3</td>
<td>NM_000497.3</td>
<td>AGGTGAGAAGCTGCGTGA</td>
<td>CAACTTACGCGCAGTACG</td>
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<tr>
<td>β-Actin</td>
<td></td>
<td>NM_001101.3</td>
<td>CCAACCGCGAGAAGATGAG</td>
<td>CACAGAGCGGAGAACAGTTAC</td>
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</tbody>
</table>
lected into fresh tubes and pellets discarded. Peptides and cortisol concentrations were measured with ELISA kits (Table 3) and normalized for total protein content in cell lysates (2.5 μg/μl, Bradford assay). The amount of peptides and cortisol was calculated from the standard curve (according to the manufacturer’s instructions) and presented as nanograms or picograms per milliliter of cell/tissue extract from organ culture.

**Western blot analyses.** Cell pellet was lysed in 100 μl of ice-cold RIPA buffer, while the skin scraps (2 per condition) were homogenized with 1 ml of the same lysis solution a using homogenizer. Next, homogenates were kept on ice for 20 min and centrifuged for 20 min. Supernatants were collected into fresh tubes, and pellet or tissue was discarded; 2.5 μl of each sample was used for Bradford protein assay. Extracts (30 μg of protein per line) were suspended in the loading Laemmli buffer (4× concentrated), denatured, and separated by SDS-15% PAGE. Next, separated proteins were transferred to a 0.2-μm PVDF membrane (Millipore, Bedford, MA). Membranes were blocked for 2 h at room temperature in skim milk (5% wt/vol) and Tris-buffered saline with Tween 20 (TBS-T). Membranes were then washed and incubated with primary antibody (Table 4) in 5% skim milk diluted (TBS-T) or nonimmune rabbit serum overnight (4°C). Membranes were then washed (TBS-T) and incubated with goat anti-rabbit IgG-conjugated HRP (0.1 μl/ml; Santa Cruz Biotechnology, Santa Cruz, CA) for 1 at room temperature. Blots were rinsed with TBS-T/TBS and exposed to the chemiluminescent substrate (SuperSignal West Pico, Thermo Sci, Rockford, PA). Bands were visualized by exposure to a classic blue autoradiography film BX (MidSci, St. Louis, MO) for 1–10 min. Membranes were stripped in Restore Plus Western Blot Stripping Buffer (Thermo Sci). The positive signals were standardized to the β-actin antibody (1:10,000, Sigma). Expression levels of proteins are the ratio of primary antibody to β-actin signal, respectively.

**Immunofluorescent staining of co-cultures in chambers.** HEKn/HEMn were maintained and treated similarly as described earlier, except they were seeded at chamber slides (Thermo Sci). Following a 24-h incubation, cells were washed in PBS and fixed with 4% buffered PFA for 30 min. Afterward, cells were permeabilized and blocked in a solution comprising 0.2% Triton-X 100, 0.1% BSA, and 5% donkey serum for 30 min. Primary rabbit antibody directed against CRH, PC1, ACTH, β-END, and P450scC (details in Table 4) were mixed separately with mouse monoclonal MEL-5 antibody diluted in the same blocking solution and incubated for 3 at room temperature. After an extensive washing in PBS, cells were incubated for 1 h in a mixture of species-specific secondary antibody conjugated with appropriate fluorophore, e.g., donkey anti-rabbit IgG conjugated-CY3 (red) and donkey anti-mouse IgG conjugated-FITC (green) (both from Jackson ImmunoResearch West Grove, PA). Cells were further washed, dried out, and mounted with mounting medium (Sigma). Negative controls were performed including omission of primary antibody, replacement with nonimmune rabbit serum, and exclusion of secondary antibodies. The positive control was performed on AtT-20 cell lines treated similarly. Stained cells were viewed with a fluorescent microscope equipped with a digital camera (Leica Digital DM4000B, Bannockburn, IL) and photographed under ×200 or ×400 magnifications. At least three chambers from each specimen were stained in each condition.

**Immunohistochemistry.** Following a 24-h incubation with UV irradiation, the skin was fixed in 4% PFA (12 h, 4°C), rinsed several times in PBS, cryoprotected with 18% sucrose in PBS for 10 days (4°C), and cryosectioned (Leica, Bannockburn, IL). Ten-micrometer sections were mounted on siliconized slides (Dako, Carpinteria, CA), rinsed several times in PBS, and maintained for 1 h at room temperature in a blocking solution (5% donkey serum, 0.1% BSA, 0.2% Triton X-100 in PBS), rinsed in PBS and incubated for 16 h at room temperature with some rabbit polyclonals listed in Table 4. To visualize the immunocomplexes, the secondary donkey anti-rabbit biotinylated IgG (1:1,000) and then with CY3-conjugated streptavidin (0.2 μg/ml) as a fluorophore (both from Jackson ImmunoResearch, West Grove, PA) were applied. All slides were finally counterstained with DAPI. At least six slides from each specimen were stained and assessed. The immunoreactive (IR) signals were examined under fluorescent microscope (Leica Digital DM4000B) equipped with a digital camera. In addition, the intensity of fluorescent signals inside the epidermis layer was evaluated using ImageJ software (National Institutes of Health) and statistically compared between conditions with the controls. Positive-control staining was performed on human pituitary gland and uterus tissues, which were treated similarly to the skin. The negative controls consisted of tissues incubated without primary antibody or with nonimmune rabbit serum or with antibodies preabsorbed with 0.1 mmol/l concentrations of CRH (Sigma, St. Louis, MO), β-END, and ACTH (Peninsula Laboratory, Torrance, CA).

**Statistical evaluation.** Data are presented as means ± SD and were analyzed with Student’s t-test (for 2 groups) or (for more than 2 groups) with one-way ANOVA Dunnett’s multiple comparison post hoc test using Prism 4.00 (GraphPad Software, San Diego, CA). Statistically significant differences are denoted by black (t-test) and white or gray (ANOVA) asterisks, where ***P ≤ 0.001, **P ≤ 0.005, and *P ≤ 0.05.

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**Table 3. List of ELISA kits used**

<table>
<thead>
<tr>
<th>Name</th>
<th>Cat. No.</th>
<th>Vendor</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRH</td>
<td>EK-019-06</td>
<td>Phoenix Pharmac., USA</td>
</tr>
<tr>
<td>ACTH</td>
<td>21-ACTH-E01</td>
<td>Alpco Immuno., USA</td>
</tr>
<tr>
<td>β-END</td>
<td>S-1170</td>
<td>Peninsula Lab., USA</td>
</tr>
<tr>
<td>CORTISOL</td>
<td>KGE008</td>
<td>R&amp;D Systems, UK</td>
</tr>
</tbody>
</table>

**Table 4. List of primary antibody used for Western blotting or immunohistochemistry**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Host</th>
<th>Titer</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corticotropin-releasing hormone, CRH*</td>
<td>Rabbit</td>
<td>1:2000</td>
<td>Prof. Wylie Vale, Salk Inst., USA</td>
</tr>
<tr>
<td>Proconvertase-1, PC1</td>
<td>Rabbit</td>
<td>1:1000</td>
<td>Dr. Iris Lindberg, USA</td>
</tr>
<tr>
<td>Adrenocorticotrophic hormone, ACTH*,#</td>
<td>Rabbit</td>
<td>1:500</td>
<td>Dr. Allen, USA</td>
</tr>
<tr>
<td>Adrenocorticotrophic hormone, ACTH</td>
<td>Rabbit</td>
<td>1:1000</td>
<td>Dr. Parlow, NIDDKD, USA</td>
</tr>
<tr>
<td>β-Endorphins, β-END*</td>
<td>Rabbit</td>
<td>1:2000</td>
<td>Dr. Allen, USA</td>
</tr>
<tr>
<td>Cytochrome P-450 side-chain-cleavage, P450hcc</td>
<td>Rabbit</td>
<td>1:1000</td>
<td>Prof. Robert Tuckey, Univ. of Western Australia</td>
</tr>
<tr>
<td>Glucocorticoid receptor, GR (sc-8992)</td>
<td>Rabbit</td>
<td>1:500</td>
<td>Santa Cruz Biotech., USA</td>
</tr>
<tr>
<td>MEL-5 (MA02026)</td>
<td>Mouse</td>
<td>1:300</td>
<td>Signet, USA</td>
</tr>
<tr>
<td>β-Actin-HRP, β-actin* (A3854)</td>
<td>Mouse</td>
<td>1:10,000</td>
<td>Sigma, USA</td>
</tr>
</tbody>
</table>

*In general, cross-reactivity of antibodies against β-END and ACTH with noncorresponding POMC peptides has been reported to be <1% (1, 33, 40). Anti CRH antibody had very low cross-reactivity to other hypothalamic peptides (25). #Antibody used exclusively for Western blot.
RESULTS

Changes in the expressions of CRH, POMC, MC1R, MC2R, CYP11A1 and CYP11B1 genes. UVR significantly changed the expression of the HPA-related genes in both co-cultured human keratinocytes and melanocytes (the two main cell populations of the epidermis) and in full-thickness skin biopsies incubated ex vivo, in time-, dose-, and wavelength-dependent manners (Fig. 1). The most pronounced induction of the expression was observed at 12 and 24 h after irradiation. Of the doses tested, the one causing the highest stimulation was variable, particularly for UVA. For UVB the highest stimulation was at a dose of either 100 or 200 mJ/cm² and for UVC was either 1 or 5 mJ/cm². The highest increase of CRH mRNA expression was observed after 1 mJ/cm² UVC [507 ± 2.15-fold change (fc)] and was also enhanced after UVA (10 J/cm², 5.6 ± 0.19 fc) and UVB (100 mJ/cm², 3.5 ± 0.6 fc) for skin biopsies and 200 mJ/cm², 8 ± 0.6 fc for co-cultures (Fig. 1). Stimulation of POMC followed the same trend, with the highest effect seen after UVC and UVB with the highest stimulation at 5 and 100 mJ/cm², respectively. There was also a moderate effect after UVA, seen only at 10 J/cm². The stimulation of MC1R expression was the greatest after UVC (all doses) and was nearly 100× higher than with the UVB (highest at 100 mJ/cm²), whereas UVA had no effect. Although MC2R expression was very low in untreated samples, its mRNA expression was induced by UVR, with the highest stimulation after UVB (200 mJ/cm²; 820 ± 0.24 fc) and a moderate

![Fig. 1. Comparison of the expression level of selected HPA axis genes (CRH, POMC, MC1R, MC2R, CYP11A1, CYP11B1) after UVA, UVB, or UVC irradiation followed by 12 h of cultured human skin (A), and co-cultured HEKs/HEMns after UVB (B). See text for definitions. Values on the x-axis are doses of irradiation: mJ/cm² for UVB and UVC and J/cm² for UVA. On the y-axis are provided the mRNA expression levels as a fold change using a comparative ΔΔCT method with β-actin. Real-time RT-PCR presented as means ± SD.](http://ajpendo.physiology.org/)

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stimulation after UVA (at 50 J/cm²), but only a minimal effect was seen after UVC (at 5 mJ/cm²). Expressions of the CYP11A1 and CYP11B1 genes encoding the GC synthesis pathway enzymes were noticeably increased after UVC exposure, especially with 1 mJ/cm² as well as after UVB. UVA stimulated the expression of CYP11A1 at 10 and 50 J/cm², but it inhibited CYP11B1 gene expression (Fig. 1A). In co-cultured melanocytes and keratinocytes, the UVB-induced expression of HPA related genes expression consistently showed a dose-dependent stimulation, with the highest increases observed for either 100 or 200 mJ/cm² after 12 h of irradiation (Fig. 1B).

Effect of UVR on CRH, ACTH, β-END, and COR production. The most remarkable effects were obtained after 24 h of irradiation. CRH production from the skin and co-cultures was stimulated in a dose-dependent manner by UVB and UVC (highest stimulation at 100 and 5 mJ/cm², respectively) but not by UVA (Fig. 2). Interestingly, the highest doses of UVC slightly but significantly decreased the CRH levels. The basal levels of ACTH were very low (below or at the border of detection). However, the ACTH concentration was significantly increased in a dose-dependent manner by UVB and UVC irradiation, which was highest at 100 and 5 mJ/cm², respectively. Again, UVA had no effect on ACTH concentration (undetectable). β-END was endogenously produced both in skin and co-culture and its levels significantly increased after exposure to UVA, UVB and UVC in a dose-dependent manner with maximum stimulation at 20 J/cm², 100 mJ/cm² and 5 mJ/cm², respectively. COR was also produced endogenously, and its levels increased after UVB and UVC irradiation in a dose-dependent manner, with UVA being without any effect.

Changes in protein levels for CRH, POMC, P450scc (CYP11A1), and GR measured by western blotting. To complement gene expression and ELISA assays, we performed WB analyses on extracts from skin and co-cultures after UVB followed by 24 h of incubation (Fig. 3). The antibody against CRH recognized the 23-kDa pro-CRH protein, whose concentration was enhanced in a dose-dependent manner after UVB (Fig. 3A). Increased levels of POMC-derived 33-kDa protein

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**Fig. 2. UVB irradiation enhances CRH, ACTH, β-END, and cortisol (COR) production in the skin (A) and in HEKn/HEMn co-culture (B) followed by 24 h of culture. ELISA study presented as means ± SD.**
were also seen after UVB by using an antibody directed against ACTH (Fig. 3B). In addition, a dose-dependent increase in the concentration of P450scc was evident after UVB irradiation (Fig. 3C). Remarkably, the same doses of UVB downregulated the levels of GR (Fig. 3D).

**Immunofluorescent in situ detection of CRH, proconvertase-1, ACTH, β-END, P450scc, and GR expressions followed by UVR.** The basal expressions of CRH, proconvertase-1 (PC1), β-END, and P450scc antigens in control samples were low and limited mainly to basal or suprabasal epidermal layers, while only single ACTH-immunoreactive (IR) cells were seen (Fig. 4). However, there were significant and dose-dependent increases in CRH-, ACTH-, and β-END-IR signals after UVB, which were located in the cytoplasm of keratinocytes distributed through all layers of the epidermis (Fig. 4A). UVC also remarkably stimulated expression of all above with UVA having stimulatory effect mainly on CRH-IR and β-END-IR (Fig. 4B). The spatial distribution of these neuropeptides was similar to that of the samples treated with UVB. The expression of GR-IR was high in nuclei of control epidermal keratinocytes, being seen in all layers of the epidermis. Exposure to a high dose of UVB (100 mJ/cm²) or UVC (5 mJ/cm²), but not to UVA, significantly decreased the immunopositive signal. The calculated values of immunopositive signal intensity for appropriate antigens expression are shown on inset graphs to Fig. 4, A and B. In contrast, P450scc-IR, while being low in control samples, significantly increased after UVB and UVC but only slightly after UVA. The antigen was localized in cytoplasm of basal keratinocytes, dermal fibroblasts, and other nonepithelial cells of the dermis (after UVC) (Fig. 4B).

The chamber double staining of co-cultured melanocytes and keratinocytes showed that there were increases in immunofluorescence intensities for CRH, PC1, ACTH, β-END, and P450scc (localized to the cytoplasm), especially after UVB and UVC irradiation, with UVA lacking a visible effect on ACTH-IR (Fig. 4B). Double staining with melanocyte-specific MEL-5 antibody (FITC-green) demonstrated that not only keratinocytes but also melanocytes (yellow, as a result of digital overlapping of green and red fluorophors) expressed CRH, PC1, ACTH, β-END-IR, and P450scc after UVR (Fig. 4B).

**DISCUSSION**

This is the most comprehensive study yet on the effects of UVR on the cutaneous HPA axis. It shows wavelength-dependent changes in cutaneous expression of almost all of the main regulatory elements of the HPA axis at the levels of gene expression, protein, and final hormone (CRH, ACTH, β-END, and COR) concentrations. This indicates that UVR, a prevalent environmental stressor, can trigger local neuroendocrine stress responses, with CRH, POMC, and GC acting as coordinators of phenotypic responses aimed at stabilization or preservation of local homeostasis.

These studies are in agreement with previous reports on UVB induction of CRH (32, 57), POMC, and MC1R (4, 5, 23, 27) expressions in cultured in vitro normal and malignant melanocytes and keratinocytes. There is also a striking resemblance between the range of biologically active UVB doses seen in this study with those demonstrated by Pawelek’s group as the most effective for induction of melanin pigmentation (5, 24). The significance of our study is the demonstration of the wavelength-dependent capability of UVR to stimulate the expression of the crucial regulatory genes of the HPA axis. In general, the more energetic and shorter the wavelength, the stronger the effect, with UVC > UVB > UVA. Nevertheless, there are some exceptions; for example, UVB was the most efficient in induction of the MC2R gene, whereas UVC had only a minor effect, and UVA inhibited CYP11B1 expression. Since UVC and UVB, but not UVA, show direct and prominent effects on DNA, which serves as a chromophore for both wavelengths (1), it is likely that the observed gene responses could occur via an SOS-like mechanism following DNA damage, similar to that which induces melanin pigmentation (13, 14). This conclusion is further substantiated by the protective role of the products of genes tested as in the following cases. First, CRH in addition to triggering the HPA axis, also has protective properties at tissue levels (16, 48). Second, POMC is processed to β-END, ACTH, and MSH peptides, which have respective, antinociceptive, anti-inflammatory,
melanogenic, and protective activities in the skin (38, 40, 53). Third, the increased expression of steroidogenic genes leading to enhanced production of COR amplifies the above-mentioned protective properties to maintain the homeostasis in the skin. The above UV-induced, keratinocyte-derived growth factors can also regulate the activity of epidermal melanocytes in a context-dependent fashion as described above (38, 41). Finally, MC2R is crucial for ACTH-induced steroidogeneic activity (2, 6), while MC1R has antimutagenic and antiapoptotic activities, which are separate from melanin pigmentation (2, 19).

The striking UVB- and UVC-restricted effects are best illustrated when one analyzes the levels of the final products. For both wavelengths, but not UVA, the boosted production of CRH, ACTH, and COR, with UVA being able to increase only β-END, was shown in whole skin extracts and in situ in the epidermis by IHC. The spatial pattern of UV-induced CRH, ACTH, and β-END expression in epidermis is consistent with previous studies on increased expression of POMC in the upper layers of the epidermis (3) and prodifferentiation effects of CRH (44, 48, 56). This could contribute to building the biological barrier in the outermost layer of skin to protect against environmental or biological insults (9, 31, 36, 41, 52). Increased expression of CYP11A1 in basal layers of the epidermis suggests strategic production of pregnenolone that could be used for steroid synthesis in either epidermal or superficial dermal compartments with multiple biological implications (49–51). Colocalization of those antigens in co-cultured melanocytes and keratinocytes indicates that both cell types play a role in building this barrier. In contrast, expression of GR was inhibited by UVB and UVC, which may indicate an epidermal mechanism designed to attenuate the long-term immunosuppression caused by cortisol throughout the downregulation of its receptor. At the present stage of our knowledge, a possible molecular mechanism regulating this process is speculative; however, it indicates a linkage to pathways activated by UVB and UVC but not UVA. Also, in many autoimmune skin disorders, the glucocorticoid resistance appears to be associated with a qualitative or quantitative deficiency in GR activity (11, 26). Thus, further careful studies including defining a role for GRα and GRβ isoforms and a mechanism regulating their expression and activity are required to uncover a biological and clinical significance of the above findings.

POMC-derived peptides will also generate an immunosuppressive environment (2, 23) weakening the epidermal barrier, which however is compensated for by their induction of melanin (a factor protecting skin from environmental stress) production (38). The UV-induced stimulation of the expression of P450scc (CYP11A1), which is localized predominantly in the basal layer of epidermis and dermal fibroblasts, is consistent with the skin cell expression of this enzyme previously described by us (49). Furthermore, increased cleavage of cholesterol or its precursor by this enzyme in the upper layer of the epidermis would disrupt proper barrier formation, which requires cholesterol and its derivatives (8). The noticeable stimulation of β-END by all wavelengths of UV tested is consistent with the nociceptive action of this peptide and perhaps may explain the phenomenon of UV-induced “opioid-like” effects described in the literature (12, 54). Thus, the described differential expression of HPA axis elements induced by diverse UV wavelengths offers distinct but overlapping mechanisms by which local neuroendocrine pathways maintain the protection of cutaneous homeostasis. This extends far beyond the visionary concept that proposed a critical role of MSH
receptor activity for UV-induced melanin pigmentation (24), a recognized protector against environmental stress. These mechanisms include different layers of local HPA axis activities, which could have developed in the integument (30) and which are independent of regulation of melanin pigmentation or represent reaction to sub- or lethal insults, for which the testing model has been UVC.

**Conclusion**

In summary, we have shown differential susceptibility of the cutaneous HPA axis following UV irradiation at diverse wavelengths. The most remarkable effects were mediated by highly energetic UVC and UVB, implying a dependence on a local stress response for their noxious activity. Stimulation of CRH and β-END within the epidermis by UVA indicates an overlapping (with the equivalent hypothalamic-pituitary axis) or alternative mechanisms induced by this wavelength. These differential responses of “cutaneous HPA” axis elements are consistent with differences in the mechanism of action of different UV wavelengths and their pathological consequences.

**ACKNOWLEDGMENTS**

We thank Dr. Zorica Janjetovic and Tae-Kang Kim for technical assistance.

**GRANTS**

This study was supported by grants from National Science Foundation (IOS-0918934) and National Institutes of Health (AR-052190) to A. Slomin-ski.
REFERENCES

No conflicts of interest, financial or otherwise, are declared by the author(s).

19. Slominski A, Wortsman J, Pisarchik A, Zbytek B, Linton EA, Ma-


