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Skin Exposure to Ultraviolet B Rapidly Activates Systemic Neuroendocrine and Immunosuppressive Responses†

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Abstract

The back skin of C57BL/6 mice was exposed to a single 400 mJ/cm² dose of ultraviolet B (UVB), and parameters of hypothalamic-pituitary-adrenal (HPA) axis in relation to immune activity were tested after 30–90 min following irradiation. Levels of brain and/or plasma corticotropin releasing hormone (CRH), β -endorphin, ACTH and corticosterone (CORT) were enhanced by UVB.

Hypophysectomy had no effect on UVB-induced increases of CORT. Mitogen induced IFN γ production by splenocytes from UVB-treated mice was inhibited at 30, 90 min and after 24 h.

UVB also led to inhibition of IL-10 production indicating an immunosuppressive effect on both Th1 and Th2 cytokines. Conditioned media from splenocytes isolated from UVB-treated animals had no effect on IFN γ production in cultured normal splenocytes, however IFN γ increased with conditioned media from sham-irradiated animals.

Sera from UVB-treated mice suppressed T cell mitogen-induced IFN γ production as compared to sera from sham-treated mice. IFN γ production was inhibited in splenocytes isolated from UVB-treated animals with intact pituitary, while

stimulated in splenocytes from UVB-treated hypophysectomised mice. Thus, cutaneous exposure to UVB rapidly stimulates systemic CRH, ACTH, β -endorphin, and CORT production accompanied by rapid immunosuppressive effects in splenocytes that appear to be independent of the HPA axis.

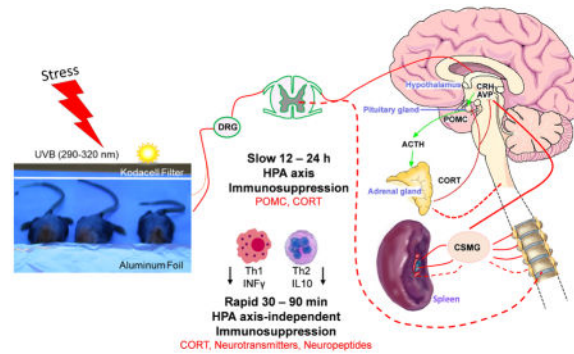
Graphical Abstract

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AUTHOR CONTRIBUTION

CS performed experiments, contributed to the study design, analysis, interpretation, and writing of this manuscript. AS was involved in the study design, analysis, interpretation, writing of this manuscript, and contributed to the experiments. AP performed, designed, and analyzed experiments on splenocytes and was involved in writing of the manuscript



INTRODUCTION

Skin covers and protects the body from the external environment, while maintaining internal homeostasis (1–4). Keratinocytes and melanocytes, representing the major component of the epidermis, have a common neuroectodermal origin and, therefore, are the source of neuroendocrine factors, which are either capable of entering the blood circulation and/or activating ascending neuronal pathways (5, 6). Specifically, skin expresses all elements of the hypothalamic-pituitary-adrenal (HPA) axis (7). This local “equivalent” of the HPA axis follows the same activatory pattern as the central HPA (8, 1). The noxious stimuli encoded as stressors sensitize skin cells and/or enhance cutaneous production or release of corticotropin releasing hormone (CRH) from nerve endings (8, 9). This stressocortin, upon binding to a specific receptor (CRH-R1), triggers the release of ACTH, and increases expression of proopiomelanocortin (POMC), which undergoes enzymatic cleavage yielding several important neuropeptides that in addition to ACTH include β -endorphin (β -END) and α -melanocyte stimulating hormone (α -MSH) (8, 10–12). Pituitary ACTH reaches the adrenal cortex, via blood, and induces release of glucocorticosteroids (GC) with stimulation of glucosteroidogenesis from cholesterol with involvement of steroidogenic acute regulatory protein (StAR) and steroid 11 β -hydroxylase (CYP11B1) which are encoded by *StAR* and *Cyp11b1* genes, respectively (13, 14). In addition, peripheral organs such as skin can produce regulatory GC (15, 16) including cortisol (COR) in humans (15, 17–22) and corticosterone (CORT) in rodents (23, 24). This cutaneous steroidogenesis can also proceed from cholesterol (25, 26).

Recent evidence showed that ultraviolet B (UVB), when applied to the skin, triggers both local - cutaneous (20, 23) and central HPA axis (27) with the global consequences manifested by elevated levels of CRH, urocortin, ACTH, β -END, and CORT in plasma and brain (27) in mice. Thus, UVB-stimulated CRH in the paraventricular nucleus (PVN) is followed by pituitary release of ACTH and increases in CORT plasma levels, with the latter effects requiring an intact pituitary (27). Similar activation of the central hypothalamic-pituitary (HP) axis leading to increased levels of α -MSH in the serum has been reported after exposure of the eye to UVB energy (28, 29). Therefore, it has been proposed that UVB-related activation of either the central HP and/or HPA axis, through overlapping but distinct mechanisms, operates in order to maintain peripheral neuroimmune homeostasis (30). Most recently, we have also shown that UVB can also activate the POMC signaling system in the

arcuate nucleus (Arc) of the hypothalamus (31). These findings emphasize the central role of the brain in coordination and translation of UVB signals into the systemic responses (30).

The skin with subcutis represents the largest organ in the body that is endowed with neuroendocrine activities that regulate local and global homeostasis [reviewed in (1, 6, 32)]. UVB is a major cutaneous stressor that, on the one hand, induces multiple skin pathologies including cancer (33–35), while on the other is necessary for vitamin D production (36, 37), activation of local neurohormones (1, 8) and stimulation of melanin pigmentation (38, 39, 4, 34, 40). UVB has also local and systemic immunosuppressive effects, the mechanisms of which are still being investigated (41–43, 40, 44, 45). The above information [as well as strong evidence for reciprocal communication cross-talk between skin, brain, endocrine and immune systems (1)] prompted us to test the effects of a single exposure of back skin to the UVB on the systemic neuroendocrine parameters and immune functions.

MATERIALS AND METHODS

Animals

All procedures involving mouse tissue collection were approved by the Institutional Animal Care and Use Committee at the University of Tennessee Health Science Center (UTHSC) and were adherent to the Animal Welfare Act and Regulations. Regular (n=24) and hypophysectomised (hypox, n=24) six week old C57BL/6 females from Taconic Biosciences, Inc. were maintained at the UTHSC animal facility under specific pathogen-free conditions for a week to avoid transportation stress. The animals had free access to standard laboratory chow and water and were maintained on a 12:12 light/dark cycle with room temperature ranged from 20° to 24° C. At the age of 7–8 weeks, when the hair cycle was in telogen phase (46), and a day before irradiation, all backs of mice were shaved with animal clippers. The back skin of the mice was then irradiated with UVB wavelength (under isoflurane anesthesia) with their eyes covered by aluminum foil to prevent any signal transmission through the optic nerve (27) or sham-irradiated (as a control). All animals were sacrificed under deep anesthesia in the early morning (around 7 am) to control for their circadian rhythm, and then plasma, skin, brain, adrenals and spleen were collected for further examination.

UV radiation

Details regarding specific lamp set up, wavelength, and dosimetry are provided in (47, 48, 27, 49). After brief isoflurane anesthesia, mice were irradiated with a single dose of 400 mJ/cm² of UVB (290–320 nm), which corresponds to a 4.1 Standard Erythema Dose (SED) and left for 30, 60, 90 min, and 12, 24 h survival time. One SED is equivalent to an erythema effective radiant exposure of 100 J/m² (MKS) or 0.01 J/cm² (CGS) (48). Kodacel filter (Kodacel™, Eastman Kodak, Rochester, NY), which acts as a cut-off filter of UVC allowing sole transmission of wavelengths longer than 290 nm, was applied. A preliminary study showed that smaller doses of UVB [25, 50, and 100 mJ/cm² (27, 31)] or shorter time (15 min, not shown) were not effective in stimulating the central neuroendocrine responses in mice. Furthermore, as previously reported (27), 400 mJ/cm² of UVB does not damage cutaneous structures that would be manifested as detectable epidermal necrosis, marked

keratinocyte apoptosis, or acute inflammatory responses (Fig. 1). The UVB irradiation was performed exclusively during early mornings when the activity of rodent HPA axis is the lowest (the opposite to humans) based on a circadian rhythm (50).

Experiments with lymphocytes isolated from spleen

The sham- (control) or UVB-treated animals were sacrificed 30 min, 90 min, 12 h, and 24 h following the irradiation, spleens were collected and processed as described (51). Briefly, lymphocytes (splenocytes) were collected after erythrocyte lysis by hypotonic shock and set up at 4×10^6 cells/mL in triplicate of 48-well tissue culture plates (Corning@Costar@ Life Sciences, Tewksbury, MA) in RPMI 1640 medium supplemented with 9% FCS, antibiotics, and Glutamax (Thermo Fisher Sci., Waltham, MA). T cells were stimulated with $0.2 \mu\text{g/mL}$ hamster anti-mouse CD3e monoclonal antibody [(MoAb), BD Pharmingen, San Jose, CA] or PBS as vehicle (control). Plates were cultured for 48 h at 37°C in a humidified incubator with atmosphere containing 5% CO_2 . Next, supernatants were harvested and subjected to ELISA for measurement of $\text{IFN}\gamma$ and IL-10.

Effects of cultured splenocyte supernatants from UVB- and sham-treated mice on $\text{IFN}\gamma$ production by anti-CD3e T cell mitogen-stimulated splenocytes from normal untreated mice

Ninety minutes after brief UVB exposure, mice were sacrificed, spleens were isolated, and cultured as described above with-or-without hamster anti-mouse anti-CD3e MoAb (BD Biosciences-Pharmingen, San Jose, CA) ($0.2 \mu\text{g/ml}$ in $500 \mu\text{l}$ RPMI 1640 medium supplemented with 1% FCS, antibiotics, and Glutamax in 48-well tissue culture plates). 48 h later, supernatants from each of the sham-treated mice were pooled together and from the UVB-treated mice were pooled together. To prepared cultures of normal C57BL/6 splenocytes at a concentration of 2×10^6 cells/wells of RPMI 1640 medium supplemented with 1% FCS, antibiotics and Glutamax and to triplicate wells was added 0%, 3.125%, 6.25%, 12.5%, or 25% (volume:volume) of either the above-described, anti-CD3e-stimulated cultures of splenocytes from sham-treated or UVB-treated mice. After 24 h incubation at 37°C , 5% CO_2 humidified atmosphere, $0.2 \mu\text{g/ml}$ hamster anti-mouse CD3e was added to each culture well and incubation continued for 48 additional hrs. The cultured supernatants were then harvested and levels of $\text{IFN}\gamma$ determined by a commercial ELISA test (R&D Systems, Minneapolis, MN).

Effect of serum from UVB- and sham-treated mice on $\text{IFN}\gamma$ production by anti-CD3e T cell mitogen-stimulated splenocytes from normal untreated mice

After 90 min, sham- (control) ($n=5$) and UVB-treated ($n=6$) of C57BL/6, as described above, were sacrificed and sera collected and pooled together regarding treatment, and added at a final concentration of 0.1% by volume to triplicate wells of 48 well tissue culture plates containing per well 2×10^6 splenocytes from untreated normal C57BL/6 mice in $450 \mu\text{l}$ RPMI 1640 medium containing 1% FCS and supplemented with Glutamax and antibiotics. After 2 h incubation, $0.2 \mu\text{g/ml}$ hamster anti-mouse CD3e was added to 3 wells containing 0.1% serum from sham-treated mice and to 3 wells containing 0.1% serum from UVB-treated mice. As a control, PBS was added to three wells. The final volume of all wells was $500 \mu\text{l}$ and then plates were incubated for 48 h. Cultured supernatants were then harvested

and analyzed for levels of IFN γ by ELISA kit (R&D Systems, Minneapolis, MN). Repeated measures ANOVA (SigmaStat, San Jose, CA) was used to analyze for differences in IFN γ concentration in cultures supplemented with different percentages of splenocyte-cultured supernatants or mouse sera from sham-treated vs. UVB-treated mice. P values ≤ 0.05 were considered statistically significant.

Quantitative real time RT-PCR (qRT-PCR)

The adrenals were dissected out, and total RNA was extracted with TRIZOL[®] (Invitrogen, Carlsbad, CA) as described (31). One microgram of RNA was reverse transcribed into cDNA with a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA). Primers for murine *Star* (NM_011485.4; F: ttggcactactcaacaacca, R: actctgctccccgttctcc) and *Cyp11b1 exon 2/3* (NM_000497.3; F: aggtggacagcctgcatc, R: ccattcaggccattcag) were used for qRT-PCR amplification. The PCR reactions were performed in triplicate with KAPA SYBR[®] Fast Master Mix (Kapa Biosystems, Inc. Woburn, MA) and data were collected on a Light Cycler 480 from Roche. The amount of amplified product for each gene was compared to that of the reference gene (β -actin) using a comparative CT method and presented as a fold change \pm SD.

Enzyme-linked immunosorbent assays (ELISA) of CRH, ACTH, β -END and CORT

Blood, following centrifugation (1,600 g, 5 min, 4°C) yielded plasma samples which were diluted in a ratio 1:10 with 0.9% NaCl and processed with commercially available kits to measure CRH (#FEK-019-06, Phoenix Pharm., Burlingame, CA), ACTH (#21-ACTHU-E01, Alpcos Immunoassays, Salem, NH), β -END (#S-1245, Peninsula Lab., San Carlos, CA), and CORT (ADI-900-097, Enzo Life Sci., Plymouth Meeting, PA), according to the manufacturer's directions [details are in (27, 31)]. Brain was removed and hypothalamic area was dissected out with the use of Zivic brain slider (27) according to the <http://mouse.brain-map.org/> and homogenized using the homogenizer (Polytron PT-MR2100, Swiss) in ice-cold T-PER[®] buffer (Thermo Sci., Rockford, IL) supplemented with protease inhibitor cocktail (10 μ l/1mL; Sigma, St. Louis, MO), and then centrifuged at 12,000 g for 25 min at 4°C. The supernatants were collected with the following protein concentration calculated with BCA assay (Thermo Sci., Rockford, IL) and adjusted to the same value (20 μ g/ μ L). For CRH examination, the peptide extraction was performed with equilibrated SEP-COLUMN containing 200 mg of C18 (#RK-SEPCOL-1, Phoenix Pharmaceuticals Inc., Burlingame, CA) according to the manufacturer's recommendations. Eluent was dried with N₂ and extracted peptide reconstituted in ELISA buffer. All assays were run in triplicate, the OD's were read with a spectrometer SpectraMax M2 and the concentrations were calculated from the 4 parametric standard curve with SoftMax Pro software (Molecular Devices, Sunnyvale, CA) and presented either in pg/mL, ng/mL, or recalculated as pg/1 μ g of total proteins for brain tissue. Results are expressed as mean \pm SEM in pg/mL per treatment group and analyzed for statistical significance with ANOVA with exact P value provided (GraphPad Software, San Diego, CA).

Histological examination

Skin was fixed in buffered (pH=7.4) paraformaldehyde and processed into paraffin blocks. Ten μ m sections were deparaffinized and stained with hematoxylin-eosin (H&E). The slides

were scanned with Aperio ImageScope (Leica Biosystems Inc., Buffalo Grove, IL) analyzed, and pictures processed with ImageJ (NIH).

Statistical analyses

The data are presented as means \pm SD and are analyzed using Prism 4.00 (GraphPad Software, San Diego, CA) with Student's *t*-test or ANOVA (SigmaStat, San Jose, CA). Statistically significant differences are denoted by *, **, or ***, where *P* is considered statistically significant at <0.05, <0.005, or <0.001; respectively.

RESULTS

Previously we have documented the induction of the central HPA axis (27) or paraventricular POMC signaling (31) 12 or 24 h after single exposure to 400 mJ/cm² of UVB to shaved back murine skin. In the present study, we have evaluated systemic body responses to the same UVB protocol but after shorter, 30–90 min, time following irradiation.

Thus, exposure of the skin to 400mJ/cm² of UVB enhanced significantly hypothalamic CRH levels by 12% and 55%, after 30 and 90 min, respectively, compared to sham-irradiated animals (Fig. 2a). Significant increases in plasma CRH were noted only after 90 min following UVB challenge (Fig. 2b), while ACTH levels increased transiently after 30 min with a negligible decrease after 90 min of exposure (Fig. 2c). The plasma levels of CORT were significantly elevated by 155% at 30 min and 200% stimulation at 90 min after UVB radiation in comparison to control (Fig. 2d). Increases in plasma CORT levels were accompanied by enhanced expression of *Star* and *Cyp11b1* genes in adrenal cortex with stronger stimulation seen at 90 min vs. 30 min after irradiation (Fig. 2e, 2f).

To test a possible involvement of pituitary in plasma CORT responses, we used hypophysectomised (hypox) and control (sham-hypox) mice. Both hypox and control animals showed increased plasma CORT concentrations at 90 min after UVB exposure indicating extra-pituitary signal involvement in such stimulations (Fig. 2g).

UVB also enhanced β -END levels in the brain (hypothalamus) after 30 and 90 min (maximal stimulation) with plasma levels increasing significantly only after 90 min of exposure (Fig. 3a, 3b).

Next, we have tested the UVB effect on immune activity of lymphocytes isolated from spleens of mice, the skin of which was exposed to a single dose of UVB (400 mJ/cm²). In the first experiment, spleens were collected 30 min, 90 min, 12 h, and 24 h after UVB exposure, splenocytes isolated, and stimulated with T cell specific anti-CD3e MoAb for 48 h after which IFN γ levels were measured by ELISA. As shown in Table 1, a significant inhibition in IFN γ production by anti-CD3e-stimulated splenocytes after UVB treatment compared to control cells was seen 90 min following exposure. This inhibitory effect persisted in splenocytes isolated 12 h and 24 h after UVB treatment. Next, we tested production of IFN γ and IL-10 in anti-CD3e-stimulated splenocytes isolated from mice 90 min after UVB exposure and found significant reduction of levels of both cytokines in splenocyte cultures derived from UVB-treated vs. control group (Table 2). Next, we tested

whether supernatants derived from cultures of anti-CD3e stimulated splenocytes isolated from UVB-treated and control animals can affect IFN γ production. As shown in Table 3, supernatants from cultures of anti-CD3e-stimulated splenocytes obtained from UVB-treated mice had no significant effect on IFN γ production by normal cultured splenocytes, which was in contrast to supernatants from sham-irradiated animals, which activated IFN γ production in a dose-dependent manner. We also tested the effect of serum isolated from control and UVB-treated animals on IFN γ production. As shown in Table 4, normal mouse splenocytes treated with 0.1% serum derived from UVB-radiated animals exhibited lack of IFN γ stimulation after exposure to anti-CD3e MoAb T cell mitogen, which contrasted with significant increases of IFN γ levels from cultures of normal splenocytes to which were added 0.1% serum from sham-treated, non-irradiated animals (Table 4). Splenocytes not stimulated with anti-CD3e contained very low levels of IFN γ (ranging from 4–12 pg/ml) (data not shown).

To test whether hypophysectomy can change immune responses secondary to UVB exposure, we isolated splenocytes from either hypox- or sham-hypox animals 90 min after UVB or sham exposure and tested their ability to produce IFN γ with and without stimulation of T cells with anti-CD3e MoAb (Fig. 4). Splenocytes isolated from both mouse groups showed similar and very low IFN γ production when incubated with PBS (vehicle) vs increased cytokine levels in cells treated with anti-CD3e MoAb. There was a significant decrease in IFN γ production in splenocytes isolated from UVB-treated animals with intact pituitary and sustained production of CORT, consistent with data presented in Tables 1–3. In contrast, UVB had an opposite effect in hypox mice (without pituitary, lacking the HPA axis-derived CORT production) e.g., mitogen significantly ($P<0.041$) increased production of IFN γ by splenocytes (Fig. 4). There was no statistical difference in IFN γ production by anti-CD3e MoAb stimulated splenocytes isolated from unirradiated hypox and intact (sham-hypox) animals.

DISCUSSION

In this study we show for the first time, that a single dose of UVB applied exclusively to the skin rapidly (30–90 min after exposure) enhances brain and/or plasma concentrations of CRH, β -END, ACTH, and CORT. The stimulation of CORT was independent of the pituitary (seen also in hypox mice) and was associated with an enhanced expression of *Cyp11b1* and *Star* mRNA in adrenals. This single dose UVB exposure also led to rapid (already at 90 min after exposure) and significant suppression of T-cell mitogen-stimulated immune activity of lymphocytes isolated from spleen of exposed animals. This effect was reversed by hypophysectomy where UVB exposure enhances IFN γ production in mitogen-activated splenocytes compared to control.

Our previous study demonstrated that plasma CORT was elevated 12 h and 24 h after UVB treatment which was the result of the activation of the central HPA axis, and required an intact pituitary since in hypophysectomized animals the effect was abolished (27). Similarly, significant activation of all elements of the cutaneous (local) HPA axis by UVB was also seen 12 h and 24 h in human skin (20) and 6 h, 12 h, and 24 h in mouse skin (23) after exposure. In addition, activation of the POMC signaling system in the Arc of the

hypothalamus was also observed 12 h and 24 h following irradiation (31). Therefore, the stimulation of CORT 30 and 90 min after UVB that was accompanied by increased levels of CRH, β -END, and ACTH was an unexpected finding and requires explanation. Similar response to UVB by hypox animals and mice with intact pituitary, both show similar increases of plasma CORT levels and demonstrate that this stimulation follows a pathway independent of the HPA axis. Thus, the described in brain (hypothalamus) and/or plasma increases in CRH, β -END, and ACTH are dissociated from the classical HPA axis and represent separate activities specific for hypothalamus (CRH and β -END) and serum (CRH, β -END, and ACTH) organized along HP axis (hypothalamic CRH \rightarrow pituitary ACTH). Clarification of these signaling pathways operating either in the brain or endocrine levels will require extensive *in vivo* studies. However, the rapid process seen in this study suggests neuronal signaling in conveying afferent information to the brain (hypothalamus) with descending transmission to peripheral organs including activation of the adrenal cortex to synthesize and release CORT and probably other mediators. Observed stimulatory action triggered by UVB was manifested by the increased production of CRH in the hypothalamus, as early as 30–90 min after radiation. Likewise, an rapid production of POMC-derived ACTH and β -END with rapid increases in CORT plasma levels indicates that the rapid activation of paraventricular nucleus of hypothalamus takes place via neuronal activation rather than via endocrine/immune mediators released by the skin. This proposition is supported by bidirectional communication between the skin and brain in regulation of body homeostasis in reaction to external noxious environmental factors (52, 32, 1, 8). Although detailed routing of such activation requires extensive *in vivo* studies, we speculate that activation of adrenocortical CORT production is secondary to neural stimulation. Since the hypothalamus governs both HPA axis (11, 10, 13) and sympathetic nervous system activity (53, 13), there is a high probability for mutual activation of the above mentioned centers by UVB light, with CRH perhaps playing a crucial role. Adrenals, unlike other organs, receive direct “preganglionic” cholinergic inputs from hypothalamus, mediolateral spinal cord (54) and are the so-called “modified sympathetic ganglia”. Based on this present study and previous studies (27, 31), we propose that UVB light leads to activation of several brain areas, including preganglionic sympathetic neurons localized in the hypothalamus (55) with subsequent activation of corticosteroidogenic activity of adrenal cortex as an alternative to the HPA-led activation.

Although systemic immunosuppressive effects of UVB are well documented (41, 43, 42, 44), the mechanisms of such UVB activity are not fully understood. They include production of immunosuppressive cytokines and neuropeptides, reprogramming of immune competent cells, and vitamin D dependent and independent suppression of adaptive immunity (43, 42, 44, 40, 1). Thus, our finding on UVB immunosuppression was expected. However, the rapidity (90 min) with which it occurred was unexpected. The immunosuppressive effect in the internal immune organ (spleen) affected both Th1 (IFN γ) and Th2 (IL-10) cytokines, which persisted after 12 h or 24 h after irradiation. While the latter effects can be explained either by activation of the central HPA axis (27) or mechanisms extensively discussed by others (43, 42, 44, 40, 1), the rapid induction of immune suppression cannot be explained by activation of HPA, because an opposite effect (immunostimulation, Figure 4) was seen in hypox animals exposed to UVB. An alternative explanation could be triggering by UVB of

neural stimuli transmitted from the skin up to spinal cord and brain with direct neurotransmission to the spleen through the sympathetic system (56). This hypothesis appears to be supported by sympathetic innervation of spleen in rodents (57, 58), aggravation of immune responses by sympathectomy with 6-OHDA (59), neuronal origin of immunosuppression in chronic (60) or acute (61, 62) stress and expression of receptors for neurotransmitters on lymphocytes (63, 64). However, a supplemental role of serum factors in the rapid UVB-induced immunosuppression is indicated by the inhibition of splenocyte immune activity by serum collected from UVB-treated animals. This effect could again be secondary to CORT production/release induced by neural impulse sent to the adrenal cortex or activation of the adrenal medulla to produce catecholamines (55). Neuroanatomical study with the use of retrograde tracers provided the evidence that spleen in mice and rat receives exclusively sympathetic innervation with no sensory inputs either from vagal nerve or dorsal root ganglia (DRG) (57, 58), which would be consistent with the hypothesis that UVB triggers central coordination of sympathetic inhibition of immune activity in the spleen. Other serum-derived factors would include β -END and α -MSH, of which serum levels are stimulated by UVB, and which show immunosuppressive properties (65–68). Clarification of these possible routes of UVB-induced immunosuppression will require challenging and extensive experimental effort. The nature of the triggering stressor (UVB in this case) determines the body stress-response manifested as a rapid action mediated through what is called “HPA cross-sensitization” with immune and behavioral consequences (69).

In summary, we provided evidence that UVB light can rapidly activate neuroendocrine effects leading to increases in plasma levels of neuropeptides and CORT that is pituitary independent, leading to dramatic immunosuppression.

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Possible neuro-endocrine pathways involved in splenic immunosuppressive action evoked by exposure of murine skin to ultraviolet B (UVB) radiation.

UVB-induced afferent neural signals activate the central HPA axis resulting in pituitary POMC-derived ACTH and adrenal corticosterone release to plasma. Slower immunosuppressive action, takes 12 – 24 h (upper part).

UVB-induced afferent neuronal signals affect CNS and directly activate adrenal gland (CORT, neurotransmitters) and spleen (neurotransmitters, neuropeptides). Rapid immunosuppression, 30 – 90 min (lower part).

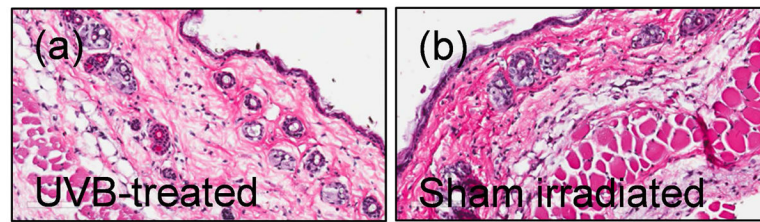


Figure 1. Single 400 mJ/cm² dose of UVB light does not lead to visible damage in murine skin. No detectable epidermal necrosis, keratinocyte apoptosis, or acute inflammatory responses were noticed in UVB- treated (a) or sham-irradiated (b) skin of C57Bl/6. H&E staining, magnification 100x.

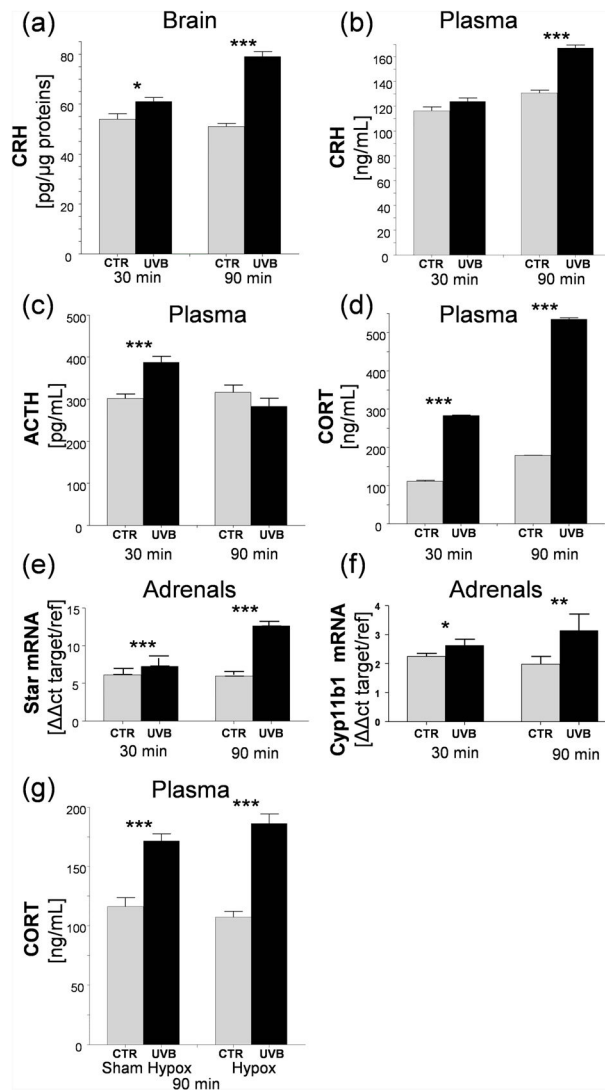


Figure 2.

UVB induces rapid increases in gene expression, neuropeptide and CORT production in murine brain, adrenals and plasma. CRH levels in brain (a) and plasma (b); levels of ACTH (c) and CORT (d) in plasma, evaluated with ELISA. The *Star* (e) and *Cyp11b1* (f) gene expression in murine adrenals; qRT-PCR data is presented as fold change based on method with β -actin used as housekeeping gene. Plasma CORT levels increases after UVB radiation in hypox and shame-hypox mice (g). Data presented as means \pm SD were analyzed using Student's *t*-test: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

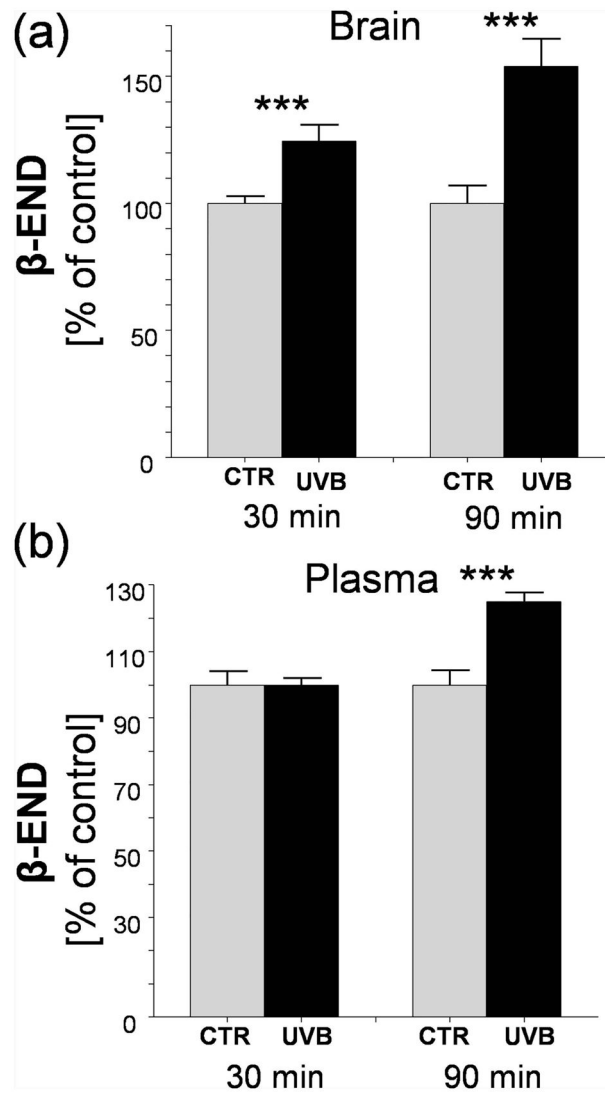


Figure 3. Cutaneous UVB enhances brain (a) and plasma (b) β -endorphin levels. Data presented as means \pm SD were analyzed using Student's *t*-test; *** $P < 0.001$.

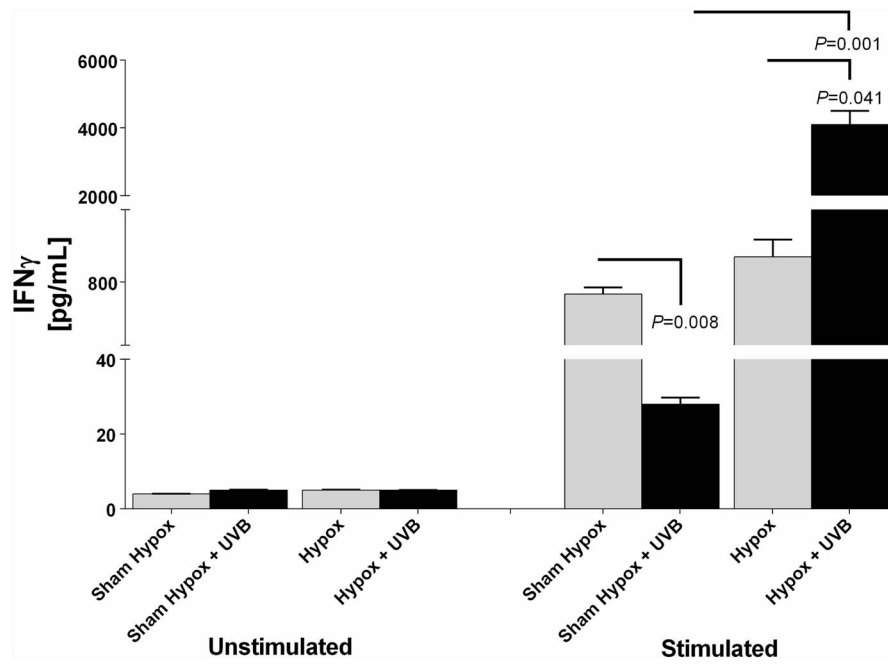


Figure 4. Hypophysectomy reverses UVB-induced suppression of splenocyte mitogen-induced IFN γ production. Results are expressed as means \pm SEM in pg/mL per treatment group and statistically analyzed with Anova with exact *P* values provided (GraphPad Prism 4).

Table 1

Cutaneous exposure to UVB light suppresses splenocyte T cell mitogen (Anti-CD3e MoAb)-induced IFN γ production in C57BL/6 in time dependent manner.[#]

Time after treatment	IFN γ concentration	
	Sham-treated	UVB-treated
	pg/mL	
30 min	119 \pm 45	285 \pm 98
90 min	2,470 \pm 362	518 \pm 97*
12 h	357 \pm 130	102 \pm 30**
24 h	360 \pm 51	191 \pm 37**

[#]Splenocytes from UVB-treated or sham-treated mice were harvested 30 min, 90 min, 12 h, or 24 h later and cultured with or without anti-CD3e MoAb for 48 h after which splenocyte culture supernatants were analyzed for levels of IFN γ as described in the Materials and Methods section. Data presented as Mean \pm SEM (n=5-6 mice per time point after UVB treatment per sham- or UVB-treated groups;

* $P < 0.05$,

** $P < 0.01$ by Student's *t*-test.

Table 2

UVB radiation suppresses generation of Th1 (IFN γ) and Th2 (IL-10) cytokine production by cultured splenocytes stimulated *in vitro* by mitogen (Anti-CD3e MoAb) 90 min after radiation.[#]

Treatment of mice	IFN γ concentration	
	IFN γ	IL-10
	pg/mL	
Sham (control)	2,470 \pm 362	70 \pm 11
UVB (400 mJ/cm ²)	300 \pm 94 ^{**}	30 \pm 12 [*]

[#]Splenocytes from UVB- or Sham-treated mice (90 min later) were stimulated for 48 h with anti-CD3e MoAb and harvested supernatants were analyzed for levels of IFN γ and IL-10 as described in the Materials and Methods section. Data presented as mean \pm SEM (n=6–7),

^{**} $P < 0.01$, or

^{*} $P < 0.05$ by Student's *t*-test.

Table 3

Supernatants from splenocytes isolated from UVB-treated animals have no effect, while those from Sham-irradiated animals stimulate IFN γ production in normal mouse splenocytes.[#]

Final Supernatant Concentration	Splenocyte Supernatant (Sham-treated)	Splenocyte Supernatant (UVB-treated)	Statistical Analysis
%	IFN γ , pg/mL		<i>P</i> value
0	669 \pm 20	570 \pm 47	0.221
3.125	863 \pm 72	636 \pm 48	0.026
6.25	852 \pm 39	574 \pm 28	0.046
12.5	1,091 \pm 49	684 \pm 39	0.011
25	1,466 \pm 13	789 \pm 36	0.004

[#]Supernatants from splenocytes stimulated by anti-CD3e MoAb for 48 h from 5 sham- and 5 UVB-treated mice (90 min survival time) were pooled together and added at the % indicated to the cultures of normal mouse spleens stimulated for 48 h with anti-CD3e MoAb for 48 h. Levels of IFN γ quantitated as described in detail in the Materials and Methods section. Data presented as Mean \pm SEM of triplicate cultures; exact *P* values evaluated with ANOVA (SigmaStat, San Jose, CA).

Table 4

Serum-derived factors from UVB-treated animals inhibit IFN γ production by mouse splenocytes cultured with (Anti-CD3e MoAb).[#]

Splenocytes incubated with 0.1% serum	IFN γ , pg/mL
Sham-treated mice	64 \pm 0.9
UVB-treated mice	8.1 \pm 0.9 *

[#]Sera from 6 UVB-treated and 5 Sham-treated mice were harvested 90 min after irradiation and pooled together (condition-dependent) and added for 2 h at 0.1% by volume to cultures of normal mouse splenocytes treated either with PBS or anti-CD3e MoAb in triplicates and incubated for 48 additional hours. IFN γ levels in harvested supernatants were analyzed ELISA as described in the Materials and Methods section. Statistical differences were evaluated with Student's *t*-test, where

* *P*<0.05.

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