**Supplementary Files**

**Figure S1.**

UVB radiation enhances CORT plasma levels in C57Bl/6 mice in a dose (a) and time (b) dependent manner.

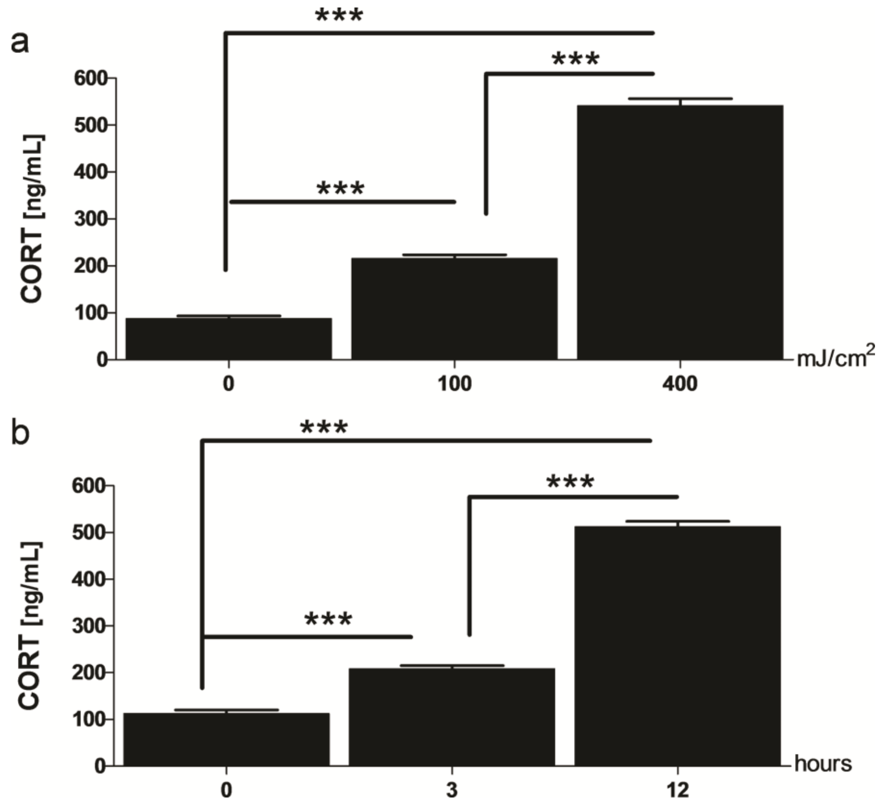

**Figure S2.**

Different routes of activation of the central HPA axis by exposure of the skin to UVB energy.

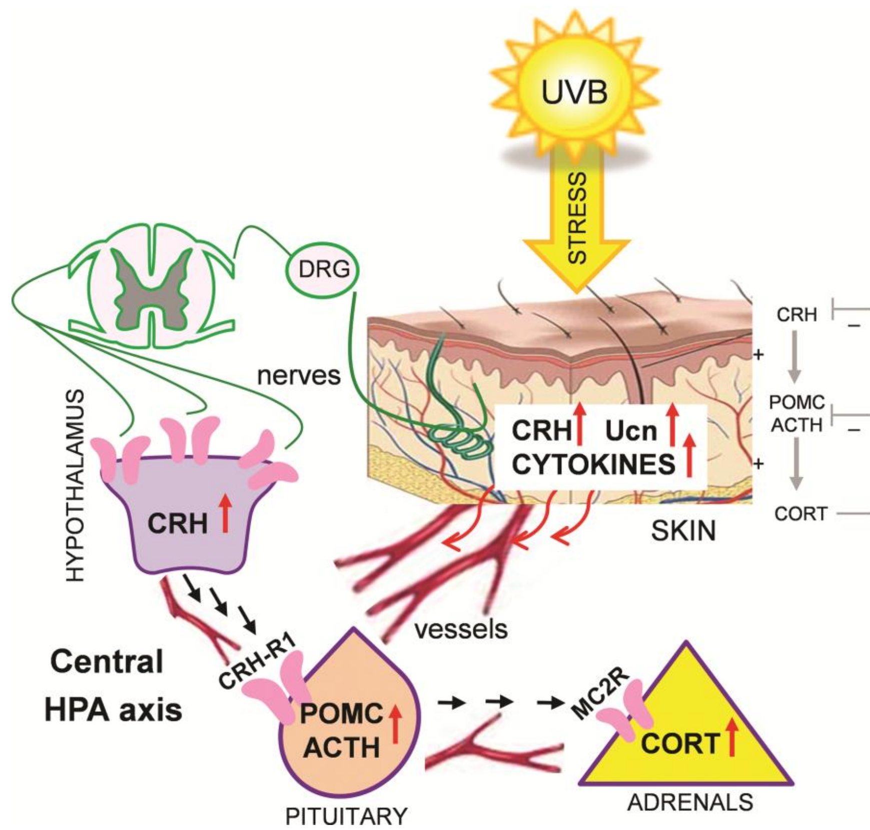

**Table S1.** Specification of the UVB bulb used in this study.

| Bulb name | Waveband (nm) | Dose* and Time of irradiation with SED and MED | |
|---|---|---|---|
| USHIO G15T8E | UVB (290-320) | Dose | 400 mJ/cm$^2$ |
| | | Time | 240 sec |
| | | SED | 4 |
| | | MED | 2.1 |

*Fraction delivered. Detailed specifications were described previously (Skobowiat *et al.*, 2013b).

**Table S2.** Primers sequences used in this study.

| Target | Gene symbol | Forward sequence 5’-3’ | Reverse sequence 5’-3’ |
|---|---|---|---|
| Corticotrophin releasing hormone from exon 2 | *CRH Ex2* | agcccttgaatttcttgcag | acccatgcggatcagaac |
| Urocortin | *Ucn* | cgcgctcctcttgctgttag | atggacagtggagggtcgtc |
| Proopiomelanocortin | *POMC* | agtgccaggacctcacca | cagcgagaggtcgagtttg |
| Melanocortin receptor 2 | *MC2R* | aaatgattctgctgcttccaa | tggtgtttgccgttgactta |
| Steroid 11β-hydroxylase | *CYP11B1* | gccatccaggctaactcaat | cattaccaagggggttgatg |
| Steroidogenic acute regulatory protein | *STAR* | ttgggcatactcaacaacca | acttcgtccccgttctcc |
| β-actin | *β-ACTIN* | ctaaggccaaccgtgaaaag | accagaggcatacagggaca |

**Table S3.** List of immunoassay kits used in this study.

| Target Name | Symbol, Cat # | Vendor |
|---|---|---|
| Corticotrophin releasing hormone | CRH<br>FEK-019-06 | Phoenix Pharm.,<br>Burlingame, CA |
| Urocortin | Ucn<br>MBS941797 | MyBioSource,<br>San Diego, CA |
| β-Endorphin | β-END<br>S-1245 | Peninsula Lab.,<br>San Carlos, CA |
| Adrenocorticotropic hormone | ACTH<br>21-ACTHU-E01 | Alpco Diagnostic, Salem, NH |
| Corticosterone | CORT<br>ADI-900-097 | Enzo Life Sci.,<br>Plymouth Meeting, PA |

**Table S4.** List of antibodies and reagents used for IHC and WB.

| Primary antibody | Host | Cat # | Vendor |
|---|---|---|---|
| CRH | rabbit | PBL rC70 | Gift from Dr. W. Vale, Salk Institute, La Jolla, CA |
| Ucn | goat | sc-1825 | Santa Cruz Biotech., Santa Cruz, CA |
| ACTH* (POMC) | rabbit | – | Gift from Dr. T. Allen, Oregon Health Sciences University, Portland, OR |
| ACTH | rabbit | AFP-6328031 | Dr. A. F. Parlow, NIDDK, Torrance, CA |
| β-END | rabbit | AFP-6328031 | |
| 3β-HSD | rabbit | – | Gift from Dr. R. Parker, The University of Alabama at Birmingham, AL |
| P450scc | rabbit | – | Gift from Dr. R. Tuckey, University of Western Australia, Crawley, WA |
| **Secondary antibody and reagents** | | | |
| Biotinylated anti rabbit IgG | donkey | 711-065-152 | Jackson ImmunoResearch, West Grove, PA |
| Biotinylated anti goat IgG | donkey | 705-065-147 | |
| Streptavidin−CY3 | – | 016-160-084 | |
| Streptavidin−Alexa488 | – | 016-540-084 | |
| Anti rabbit IgG−HRP | goat | Sc-2030 | Santa Cruz Biotech., Santa Cruz, CA |
| β-actin IgG−HRP | mouse | A3854 | Sigma, St. Louis, MO |

*Antibody recognize the POMC precursor, used exclusively for WB method.

**Materials and Methods**

The methodology followed the protocols described previously (Skobowiat *et al.*, 2011; Skobowiat *et al.*, 2013a; Skobowiat *et al.*, 2013b).

**Quantitative real time RT-PCR (QRT-PCR)**

Total RNA was extracted with TRIZOL® (Invitrogen, Carlsbad, CA). One microgram of total RNA was reverse transcribed into cDNA with the high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA). Primers used for PCR amplification are listed in Table S2. The PCR reactions were performed in triplicate with KAPA SYBR® Fast Master Mix (Kapa Biosystems, Inc.,Woburn, MA). The data were collected on the Light Cycler 480 from Roche. The amount of amplified product for each gene was compared to that for reference gene (β-actin) using a comparative ΔΔCT method and presented as a fold change ± SD.

**ELISA/EIA, Peptide extraction**

Plasma was diluted 1:100 for CORT, 1:15 for ACTH, 1:13 for β-END with 0.9 % NaCl and processed with commercially available kits, listed in Table S3, according to the manufacturer's directions. Tissues (skin, brain) samples were homogenized with T-PER® buffer (Thermo Sci., Rockford, IL) supplemented with protease inhibitor cocktail (10µl/1ml; Sigma, St. Louis, MO), with the use of homogenizer (Polytron PT-MR2100, Swiss) and stand for 30 min, 4 °C and centrifuged at 12,000 g, 25 min, 4 °C. Supernatants containing extracted proteins were normalized, i.e. adjusted to the same concentration (4 µg/µl for brain and 3 µg/µl for skin) after calculation with BCA assay (Thermo Sci., Rockford, IL). Samples were run in triplicate, the OD's or fluorescence was read with spectrometer (SpectraMax M2, Molecular Devices, Sunnyvale, CA) and the concentrations were calculated from the 4 parametric standard curve with the software (SoftMax Pro, Molecular Devices, Sunnyvale, CA) and presented in pg/mL or in ng/mL.

Before CRH assay peptide extraction was performed (required by the manufacturer). 100 µl of plasma or 100 µl of adjusted protein extract from tissues were acidified, centrifuged and loaded onto equilibrated

SEP-COLUMN containing 200 mg of C18 (RK-SEPCOL-1, Phoenix Pharm., Burlingame, CA). After washing, peptide fraction was eluted by acetonitrile containing 1% trifluoroacetic acid and dried with speed vacuum (SpeedVac® Plus, Savant, West Palm Beach, FL) and diluted with sample buffer provided by the manufacturer.

**Immunohistochemistry**

Skin (3 samples per animal) and brain samples were fixed in 4% buffered (pH=7.4) paraformaldehyde for 12 h. After extensive rinsing in PBS (pH=7.4), tissues were submerged in 18% sucrose, frozen in OTC media and cut on a cryostat (Leica 3050, Bannockburn, IL) using the technique for mouse skin described by Paus *et al.,* 1999 (Paus *et al.*, 1999). The hypothalamus was isolated at the level of anterior Bregma +1 mm up to posterior Bregma -2.70 mm, by the use of the Brain Slicer Matrix (Zivic Instrument, Pittsburgh, PA). Region resembling the whole hypothalamus (Bregma ~ -0.34 to -2.70) were characterized under light microscope based on the The Allen Reference Atlas (http://mouse.brain-map.org/static/atlas) and 10 µm coronal sections were mounted onto silanized slides (Dako, Carpinteria, CA), rinsed several times with PBS and subjected to a single immunofluorescence. Blocking was performed with 5% donkey serum, 0.1% BSA, 0.3% Triton X-100 diluted in PBS for 1 h at RT. Following extensive rinsing in PBS, the primary antibody diluted in the same blocking solution was applied overnight, at 4 °C. The next day, tissues were rinsed and biotinylated species-specific IgG, were applied for 1 h. Following rinsing in PBS, the streptavidine-CY3 as red fluorophore or streptavidine-Alexa488 as green fluorophore was applied for 50 min. Next, sections were submerged in fluorescent mounting medium (Dako) and topped with a cover glass. Negative controls were performed the same way except primary antibodies (omission) or serum from non-immunized goats or rabbits was applied (replacement). Antibodies and reagents used in this study are listed in Table S4. At least 3 sections per skin sample, i.e., 9 per animal of each primary antibody were studied under a fluorescent microscope (Leica, Digital DM4000B, Buffalo Grove, IL) equipped with the filter capable of visualization of λ excitation/emission 550/570 nm (red), and 490/525 nm (green) and conjugated to a digital camera. Pictures were further analyzed for quantitative intensity of

immunocomplexes (6 measurements per assessment) with the use of ImageJ software (National Health Institute, Bethesda, MD) and presented as mean ± SD.

**References:**

Paus R, Muller-Rover S, Van Der Veen C, *et al.* (1999) A comprehensive guide for the recognition and classification of distinct stages of hair follicle morphogenesis. *J Invest Dermatol* 113:523-32.

Skobowiat C, Dowdy JC, Sayre RM, *et al.* (2011) Cutaneous hypothalamic-pituitary-adrenal axis homolog: regulation by ultraviolet radiation. *Am J Physiol Endocrinol Metab* 301:E484-93.

Skobowiat C, Nejati R, Lu L, *et al.* (2013a) Genetic variation of the cutaneous HPA axis: an analysis of UVB-induced differential responses. *Gene* 530:1-7.

Skobowiat C, Sayre RM, Dowdy JC, *et al.* (2013b) Ultraviolet radiation regulates cortisol activity in a waveband-dependent manner in human skin ex vivo. *Br J Dermatol* 168:595-601.