Ultraviolet B stimulates production of corticotropin releasing factor (CRF) by human melanocytes

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Abstract Here we show that human melanocytes express the corticotropin releasing factor (CRF) gene and produce CRF peptide. The CRF production and secretion is markedly stimulated by ultraviolet B (UVB) radiation. This is the first demonstration that cutaneous melanocytes respond to environmental stress (UVB) through the production of CRF.

Key words: Corticotropin releasing factor; Ultraviolet B; Melanocyte; Skin; Environmental stress

1. Introduction

A classical pathway for response to stress in vertebrates is via induction of corticotropin releasing factor (CRF) in the hypothalamus with subsequent production and processing of proopiomelanocortin (POMC) by the pituitary [1,2]. Recently, it was documented that skin, the largest body organ, is not only a target for POMC peptide regulation but also a site of POMC gene expression itself [3–10].

Ultraviolet radiation is a major environmental stressor of the skin. Following damage by UVB radiation, both keratinocytes and melanocytes are activated through cascade reactions resulting in production and processing of POMC, up-regulation of MSH receptors, and increased melanin content [8,9,11,12]. Because CRF is the main regulator of POMC expression we decided to investigate whether CRF is produced by skin cells and whether this production is stimulated by UVB.

2. Materials and methods

2.1. Cell culture

Culture conditions of foreskin human melanocytes and UVB treatment protocols were detailed previously [8,9,11]. Briefly, during UVB treatment the culture medium was replaced with phosphate-buffered physiologic saline (PBS). Doses of 10 and 20 mJ/cm^2 were used (energy range of 260–380 nm with peak at 302 nm; UVM-57 LAMP, UVP Inc., CA). After treatment the PBS was replaced by regular media and cells were incubated at 37°C for 24 h. The media and cells were collected separately and used for RNA or peptide extractions.

2.2. Reverse transcription-polymerase chain reaction and Southern blot hybridization

Reverse transcription-polymerase chain reaction (RT-PCR) assays were performed as described previously [7]. Briefly, total RNA after extraction with the TRIzol kit (Gibco-BRL, Gaithersburg, MD) was reverse-transcribed using the Superscript preamplification system (Gibco-BRL). The fragment of 413 bp derived from the CRH exon 2 transcript [13] was amplified using program and primers with sequences described previously [7]. As a negative control RNA samples without prior reverse transcription were run in parallel. The GAPDH control gene was amplified as described [7]. The PCR products after separation on 1.5% agarose were Southern blotted to nylon membranes (Oncor, Gaithersburg, MD) [7,14]. The amplified cDNA was hybridized with [α-^{32}P]dCTP-labelled human CRF cDNA and after washing membranes were exposed on Kodak XAR film [7].

2.3. CRF immunoassays

CRF peptides were extracted from melanocytes and conditioned media as described [8,9] using SEPCOL-1 containing 200 mg of C18 and followed the manufacturer's protocol (Cat. No. RIK-SEPCOL1, Peninsula, Belmont, CA). The eluted peptides after lyophilization were suspended in RIA buffer and CRF immunoreactivity was measured using CRF RIA kit (Advanced ChemTech, Kentucky) or were suspended in 0.1% trifluoroacetic acid (TFA) and separated by reverse-phase high pressure liquid chromatography (RP-HPLC) as follows. The reconstituted samples in 0.1% TFA (100 μl) were injected onto the RP-HPLC Beckman Ultrasphere C18 IP column (4.6 mm × 15.0 cm, 5 μm pore size). Peptides were eluted with an increasing linear gradient of acetonitrile in 0.1% TFA (flow rate = 1 ml/min) using an ISCO Dual Pump Model 2350 HPLC system with an ISCO V_4 variable wavelength UV detector. Starting at 11 min, 1 ml fractions were collected, lyophilized and assayed using CRF RIA kit. The corrected elution time of the CRF peptide was calibrated prior the sample separation with human CRF peptide synthesized commercially (Molecular Research Lab., North Carolina).

3. Results and discussion

Human melanocytes expressed CRF mRNA (Fig. 1). This expression was accompanied by production of CRF peptide

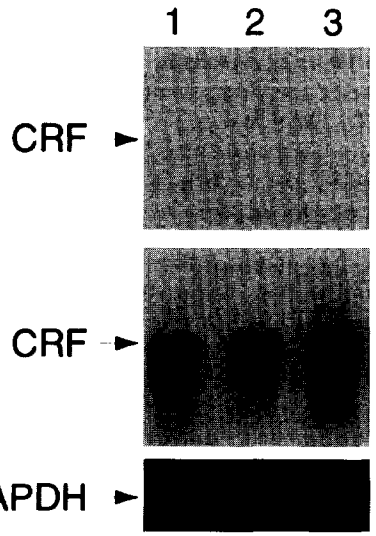

Fig. 1. RT-PCR Southern blot analysis of CRF mRNA expression in human melanocytes. Upper panel: PCR amplified RNA without reverse trascription (negative control); middle panel: RT-PCR amplified CRF fragment; lower panel: RT-PCR amplified GAPDH (semiquantitative control). UVB doses (mJ/cm^2): 0, control (lane 1); 10 (lane 2); 20 (lane 3).

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Table 1
Production of CRF by human melanocytes exposed to UVB radiation

Treatment	CRF production (pg/0.5 mg cellular protein)			
	Cells	*P*-value	Media	*P*-value
Control	31.3 ± 15.0	–	23.0 ± 2.7	–
UVB 10 mJ/cm^2	93.7 ± 5.1	0.005	38.9 ± 1.1	0.001
UVB 20 mJ/cm^2	270.0 ± 10.1	0.000	49.3 ± 9.3	0.021

Data represent means ± S.E. from 5 (cells) or 6 (media) assays combined from two independent experiments. *P*-values of the difference between control and UV treated cells were calculated using independent Student *t*-tests.

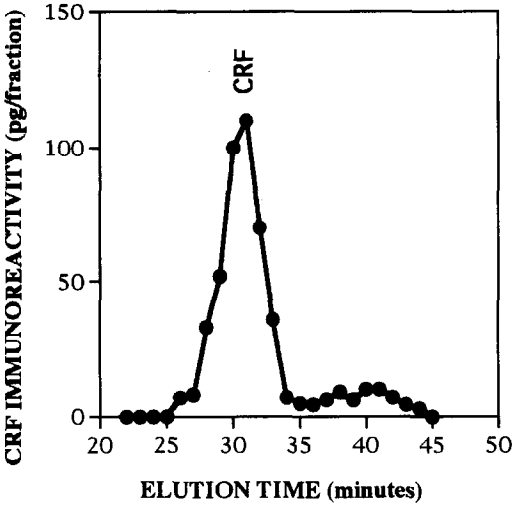

Fig. 2. RP-HPLC identification of CRF immunoreactivity from UVB treated melanocytes. CRF: CRF peptide standard eluting at the same time (30–32 min) as the radioimmunoreactivity peak detected by specific anti-CRF RIA kit.

(Fig. 2). UVB stimulated both production and secretion of CRF by human melanocytes in culture (Table 1). The stimulation occurred at doses of 10 and 20 mJ/cm^2, which in our previous experiments were optimal in stimulation of POMC peptide production, MSH receptor expression and stimulation of melanin synthesis [8,9,11,12].

CRF is a central regulator of POMC expression throughout the vertebrates [1,2]. Mammalian skin and melanocytes can produce POMC peptides including αMSH and ACTH [3–10], a process enhanced by UV radiation [4,8–10]. The skin also expresses CRF receptor 1 (CRF-R1) gene [14] and production of CRF-R1 mRNA can be stimulated by UVB [7]. Previously, we speculated that an equivalent to the 'hypothalamic-pituitary-adrenal axis' may operate in mammalian skin as a main coordinator and executor of the cutaneous response to stress [15]. Here we show for the first time that human melanocytes produce CRF peptide and that this process is markedly stimulated by UVB. Therefore, we propose that part of the skin response to UVB involves CRF production that in turn could induce POMC expression followed by an activation of the melanogenic system.

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