Proopiomelanocortin, corticotropin releasing hormone and corticotropin releasing hormone receptor genes are expressed in human skin

A. Slominski[a,b,*], G. Ermak[a], J. Hwang[a], A. Chakraborty[c], J.E. Mazurkiewicz[b], M. Mihm[a]

[a]*Department of Pathology and Laboratory Medicine, Albany Medical College, Albany, NY 12208, USA*
[b]*Department of Microbiology, Immunology and Molecular Genetics, Albany Medical College, Albany, NY 12208, USA*
[c]*Department of Dermatology, Yale University School of Medicine, New Haven, CT 06520, USA*

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Abstract Evidence is provided that human skin, the largest body organ exposed to multiple stressors, expresses proopiomelanocortin (POMC), corticotropin releasing hormone (CRH) and CRH-receptor (CRHR) genes in vivo. In vitro studies show that POMC and CRHR mRNAs are transcribed in melanocytes, cells derived from the neural crest, and in keratinocytes, cells derived from the ectoderm. CRH mRNA is transcribed in cultured melanocytes but not in keratinocytes. It is proposed that an equivalent of the 'hypothalamus-pituitary axis' composed of the CRH-CRHR-POMC loop is conserved in mammalian skin.

Key words: POM; Corticotropin releasing hormone (CRH); Corticotropin releasing hormone receptor; Hypothalamus-pituitary axis; Melanocyte; Keratinocyte

1. Introduction

Skin is the largest body organ that is directly and permanently exposed to multiple physical, chemical and biological influences. Therefore, the skin should be able to respond immediately by generating appropriate signals for defense against these stressors and to reestablish tissue homeostasis. The first and at present best understood step in response to stress by the organism is the activation of the hypothalamus-pituitary-axis [1–3]. It starts with an induction of CRH production in the hypothalamus, which after delivery to the pituitary activates CRH receptors [2]. This cascade culminates with an increased expression of POMC and secretion of POMC peptides [3].

Recent reports documented that the skin is not only a target for POMC peptide (MSH, ACTH and β-endorphin) bioregulation but also a site of POMC gene expression itself [4]. Cutaneous POMC expression was demonstrated in the skin of C57 BL/6 mice [5] and in rodent melanomas [6]. Furthermore, it was shown that the human epidermal carcinoma cell line A431 expresses POMC, and that this expression was enhanced by exposure of cells to UVB or phorbol mirystate acetate (PMA) [7]. In vivo, localized and cell restricted accumulation of POMC-derived ACTH, β-endorphin and β-MSH was demonstrated in human skin biopsies both under pathologic and physiologic conditions [8]. Although these data suggested that accumulation of POMC products may arise from local production due to normal skin reactivity or disease-related phenomena, experiments demonstrating POMC mRNA expression in human skin remained to be performed [8]. Moreover we decided to test whether CRH and CRHR genes are expressed in human skin.

2. Materials and methods

2.1. Skin biopsies and cell culture

Skin biopsy samples were obtained from patients seen at the Albany Medical College. Routine histopathologic analyses were performed in sections stained with hematoxylin-eosin. For molecular analyses the tissues were frozen in liquid nitrogen. Human pituitaries were obtained from the National Hormone and Pituitary Program, National Institute of Diabetes, Digestive and Kidney Diseases (NIDDKD).

Semiconfluent cultures of foreskin human melanocytes and keratinocytes were grown in Ham's F10 medium supplemented with 5% fetal calf serum, 85 nM 12-O-tetradecanoylphorbol-13 acetate (TPA), 0.1 mM isobutylmethylxanthine (IBMX), 1 mM insulin and 40 μg bovine pituitary extract (GIBCO, NY) and in keratinocyte-SFM supplemented with growth factors (GIBCO, NY), respectively [9]. Human melanocytes and keratinocytes were cultured in 75 cm^2 flasks without any treatment or were treated either with the UVB at 25 mJ/cm^2 or 100 nM TPA [9].

2.2. Reverse transcription-polymerase chain reaction (RT-PCR) assays

Total RNA from frozen tissue and cells was extracted using the RNAzol B isolation kit following the manufacturer's protocol (CINNA/BIOTECX Laboratories, Houston, TX). For the RT-PCR assays 2 μg of total RNAs were reverse transcribed using oligo(dT) as primers and the Superscript preamplification system (GIBCO-BRL, Gaithersburg, MD). Only samples that were proven to be free from DNA contamination by running PCR amplification without prior RT were used for the experiments. Fragments derived from the coding regions of human POMC [10], CRH [11] and CRH-R [12,13] cDNAs were amplified using primers designed and synthetized commercially (National Bioscience, Plymouth, MN). The reaction mixtures contained 25 mM $(NH_4)_2SO_4$ buffer, pH 9.0 (POMC cDNA) or pH 9.5 (CRH-R and CRH cDNAs), 2 mM $MgCl_2$, 0.4 mM dNTP and 4 μM of primers. The cDNAs were heated at 94°C for 3 min and then amplified. The PCR products were separated on 1.5% agarose, stained with ethidium bromide and photographed under UV light.

2.3. Southern blot hybridization

Specificity of the amplified products in all cases was proven by hybridization of the RT-PCR products with cDNAs corresponding to the analysed gene, i.e. POMC, CRH or CRHR as described below. After denaturation in 0.5 M NaOH and 1.5 M NaCl, products of RT-PCR were transferred to nylon membranes (Oncor, Gaithersburg, MD) and UV crosslinked to the membranes. Membranes were prehybridized in Hybridizol I (Oncor, Gaithersburg, MD) at 42°C for 4 h and hybridized with [α-^{32}P]dCTP-labelled human cDNAs for an additional 15 h at 42°C. After washing with 2 × SSC plus 0.1% SDS at room temperature and with 0.1 × SSC plus 0.1% SDS at 60°C membranes were exposed on Kodak XAR film at −70°C.

*Corresponding author. Department of Pathology and Laboratory Medicine, Albany Medical College, Albany, NY 12208, USA.
Fax: (1) (518) 2625927; E-mail: EBrisson@ccgateway.amc.edu

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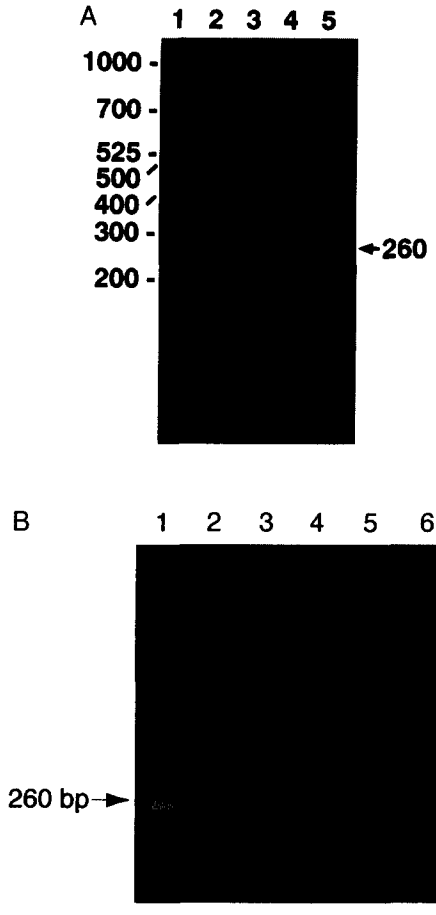

Fig. 1. POMC gene expression in human skin. (A) RT-PCR detection of 260 bp product representative of POMC exon 3 mRNA (arrow) in skin biopsies. Size markers (bp) (1), compound nevus (2), basal cell carcinoma (3), human scalp skin (4), pollution control (reaction mixture without DNA template) (5). (B) RT-PCR detection of 260 bp product representative of POMC exon 3 mRNA (arrow) in cultured melanocytes and keratinocytes. Pituitary (1), pollution control (reaction mixture without DNA template) (2), DNA size markers of 700, 525, 500, 400 and 300 bp (3), melanocytes (4), amplified RNA from melanocytes without prior RT (control) (5), keratinocytes (6), amplified RNA keratinocytes without prior RT (control) (7). RT-PCR detection was done using sense primer, 5′-GAG GGC AAG CGC TCC TAC TCC-3′ (nucleotides (nt) 605 to 625) and antisense primer, 5′-GGG GCC CTC GTC CTT CTT CTC-3′ (nt 845 to 865) for the human POMC cDNA [10]. The PCR program was 30 cycles of 45 s at 95°C, 1 min at 58°C and 2 min at 72°C with final extension of 7 min at 72°C.

3. Results and discussion

In the present study, using reverse transcription polymerase chain reaction (RT-PCR) (30 cycles), we demonstrated that the POMC gene is expressed in human skin in vivo, e.g. in normal scalp, compound nevus and basal cell carcinoma (Fig. 1A). The predicted 260 bp product is specific for exon 3 of the POMC gene. It is absent in control RNA samples that have been amplified without previous reverse transcription, and it hybridized to the POMC cDNA (not shown). Using cultured in vitro normal human melanocytes and keratinocytes, we detected by RT-PCR the 260 bp specific product representative of exon 3 of the POMC mRNA (Fig. 1B). The presence of POMC mRNA correlates with previous detection of ACTH, β-MSH and β-endorphin immunoreactivity in human scalp, basal cell carcinoma and in pigmented skin lesions [8]. Thus, human skin has the capability to transcribe and translate the POMC gene, and the keratinocytes and melanocytes are the potential source of POMC peptides in vivo.

POMC expression and processing are under stimulatory control, mediated mainly by CRH through the activation of CRH receptors [1–3]. With the use of coding sequences of the CRH gene cloned from the human brain [11] and CRHR cloned from the human pituitary and brain [12, 13], primers for RT-PCR assays were designed (cf. Figures legend). The expression of CRH mRNA in human skin biopsies from the scalp, compound nevus and basal call carcinoma is shown in Fig 2A. The RT-PCR assay demonstrated the predicted 413 bp product representative of the CRH exon 2 transcript (Fig. 2A) that hybridized to human CRH cDNA and was absent in RNA amplified

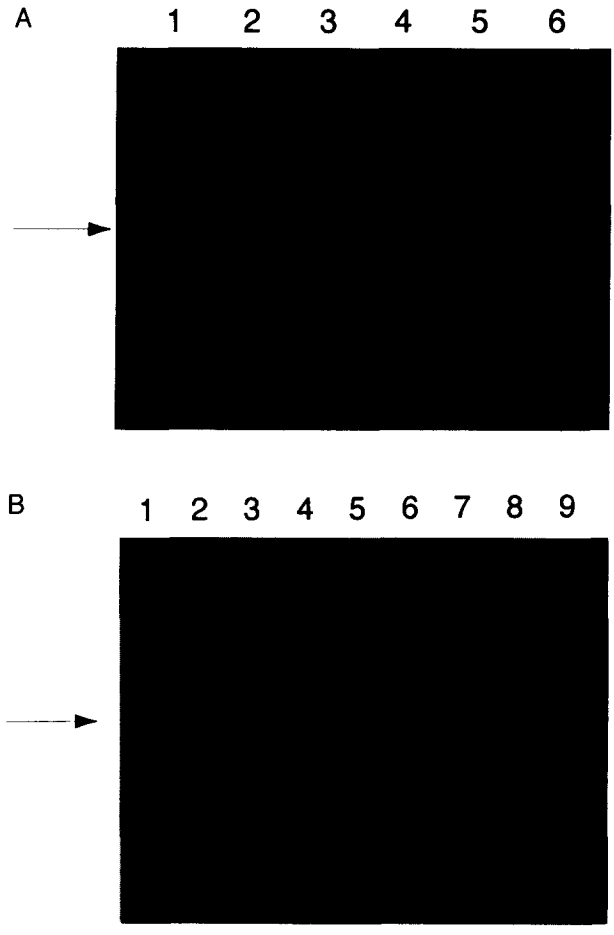

Fig. 2. Expression of CRH gene in human skin. Arrow shows a 413 bp product representative of mRNA from the CRH coding region of exon 2 [11]. (A) Pituitary control (1), DNA size markers of 700, 525, 500, 400, 300 and 200 bp (2), pollution control (reaction mixture without DNA template) (3), compound nevus (4), basal cell carcinoma (5), human scalp skin (6). (B) Pituitary control (1), pollution control (reaction mixture without DNA template) (2), DNA size markers (3); untreated melanocytes (4), melanocytes treated with UVB 25 mJ/cm^2 (5), melanocytes treated with 100 nM of TPA (6); untreated keratinocytes (7), keratinocytes treated with UVB 25 mJ/cm^2 (8), keratinocytes treated with 100 nM of TPA (9). RT-PCR detection was done using sense primer, 5′-CAC CCT CAG CCC TTG GAT TTC-3′ (nt 1436 to 1456) and antisense primer, 5′-GCC CTG GCC ATT TCC AAG AC-3′ (nt 1828 to 1848) for the CRH cDNA [11]. The PCR program was 35 cycles of 30 s at 96°C, 30 s at 55°C and 2 min at 72°C with final extension of 7 min at 72°C.

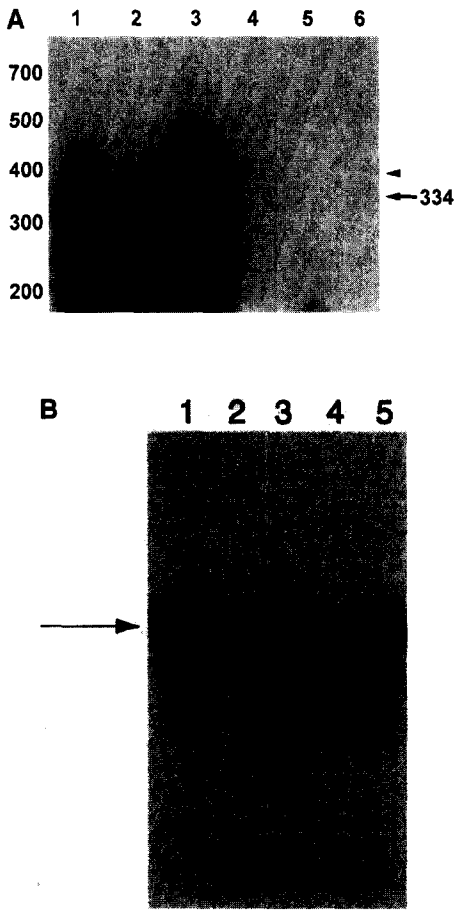

Fig. 3. Expression of CRHR gene in human skin. (A) RT-PCR detection of 334 bp (arrow) and of 400 bp (arrowhead) products representative of CRHR mRNA. From the left: DNA size markers (bp), RT-PCR products from compound nevus (1), basal cell carcinoma (2) and human scalp skin (3), PCR amplified RNA without prior RT from compound nevus (4), basal cell carcinoma (5) and human scalp skin (6). (B) RT-PCR detection of 334 bp (arrow) product representative of CRHR mRNA. Pituitary control (1), untreated melanocytes (2), melanocytes treated with UVB at 25 mJ/cm^2 (3), untreated keratinocytes (4), keratinocytes treated with UVB at 25 mJ/cm^2 (5). RT-PCR was done using sense primer, 5′-GCC CTG CCC TGC CTT TTT CTA-3′ (nt 424 to 444) and antisense primer, 5′-GCT CAT GGT TAG CTG GAC CA-3′ (nt 738 to 757) for the coding region of the CRHR cDNA [12,13]. The PCR program was 35 cycles of 45 s at 95°C, 1 min at 58°C and 2 min at 72°C with final extension of 7 min at 72°C. The RT-PCR products were separated on a 1.5% agarose gel, blotted to nylon membranes and hybridized to ^{32}P-labelled CRHR cDNA.

without RT (not shown). The RT-PCR of RNA isolated from human cultured cells showed the CRH gene expression in melanocytes but not in keratinocytes (Fig. 2B). Thus, the human skin should be added to the growing list of peripherial organs that express differentially CRH gene in vivo [2].

Using the RT-PCR and primers for the coding region of the CRHR cDNA, we detected two products in skin biopsies (Fig. 3A). One had a size of 334 bp (arrow) corresponding to predicted size of the pituitary CRHR cDNA fragment and was expressed in a compound nevus, a basal cell carcinoma and normal human scalp skin (Fig. 3A). The second one was larger (400 bp product marked by arrowhead) and was expressed in the scalp only (Fig. 3A). We suggest that both fragments are representative of the CRHR transcript(s) because they were absent in corresponding RNA amplified without RT and they hybridized to the CRHR cDNA (Fig. 3A). The smaller fragment represents the CRHR mRNA of the gene cloned from the pituitary [12,13]. The larger fragment (arrowhead, Fig. 3A) may represent either alternatively spliced CRHR mRNA or an additional member of the CRHR family. Additional members of the CRHR family that are expressed by peripherial tissues have been identified [14,15], and some of the cloned genes produce alternatively spliced CRHR mRNAs [13–15]. Furthermore, the anagen hair follicle of the scalp is a miniorgan with the highest mitotic activity in the body generated by a coordinated interaction of epithelial, mesenchymal cells and melanocytes as well as by a local skin immune system [16]. Therefore, it is likely that more than one CRHR gene can be expressed by different cell populations depending on the developmental status of the skin.

To identify cells expressing the CRHR gene we tested melanocytes and keratinocytes cultured in vitro that had been exposed to UVB. The predicted 343 bp message characteristic for the human pituitary (positive control, lane 1) that hybridized to the CRHR cDNA was detected in UVB treated melanocytes (lane 3) and keratinocytes (lane 5) but was absent in untreated melanocytes (lane 2) and keratinocytes (lanes 4) (Fig. 3B). This inducible gene expression suggests that expression of the CRHR can be an additional control point of the organized skin response to UVB, which already involves an increased production of POMC mRNA, ACTH and α-MSH peptides as well as α-MSH receptors by malignant melanocytes and keratinocytes cultured in vitro [9].

The presented data show for the first time that the control points of the 'hypothalamus-pituitary axis', POMC, CRH and CRHR genes are expressed in human skin. The cell-type restricted CRH mRNA expression in melanocytes and the inducible expression of CRHR mRNA in melanocytes and keratinocytes suggest a tight regulation of the CRH-CRHR-POMC loop through an autocrine (melanocyte) and paracrine (melanocyte versus keratinocyte) mechanism. These findings are not surprising taking into consideration the size of the skin, its anatomical distribution and functional diversity. The skin, because of its continous exposure to variable stressors must have a rapid, local and efficient mechanism of defense and of stabilization of tissue homeostasis. In this context, a local CRH-CRHR-POMC loop would represent a more economical and optimal defense program than reliance on the centrally driven hypothalamus-pituitary-axis.

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