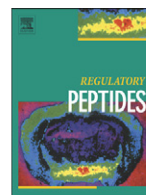
Regulatory Peptides xxx (2009) xxx–xxx

Contents lists available at ScienceDirect

Regulatory Peptides

journal homepage: www.elsevier.com/locate/regpep



Corticotropin-releasing hormone attenuates vascular endothelial growth factor release from human HaCaT keratinocytes

Chun-Lei Zhou [a,b], Xiao-Jing Yu [a], La-Mei Chen [a], Hong Jiang [b], Chun-Yang Li [a,*]

[a] Department of Dermatology, Qilu Hospital, Shandong University, Jinan 250012, PR China
[b] Key Laboratory of Cardiovascular Remodeling and Function Research, Chinese Ministry of Education and Chinese Ministry of Public Health, Qilu Hospital, Shandong University, Jinan 250012, PR China

ARTICLE INFO

Article history:
Received 11 August 2009
Received in revised form 15 September 2009
Accepted 10 October 2009
Available online xxxx

Keywords:
Corticotropin-releasing hormone
HaCaT keratinocyte
Mitogen-activated protein kinase
Vascular endothelial growth factor

ABSTRACT

Objectives: Corticotropin-releasing hormone (CRH) is a central component of the local hypothalamic-pituitary-adrenal (HPA) axis, which has a functional equivalent in the skin. To determine whether CRH and its receptor, CRH-R1, modulate the expression of vascular endothelial growth factor (VEGF), which is overexpressed in psoriatic epidermis and plays a causal role in the pathogenesis of psoriasis, we investigated the effect of CRH on the expression of VEGF in a human keratinocyte cell line (HaCaT) and whether this effect is via the mitogen-activated protein kinase (MAPK) signal transduction pathways.

Methods: Real-time RT-PCR, ELISA assay and western blot were used in the present study to investigate the expression of VEGF in CRH-treated HaCaT cells.

Results: The mRNA and protein levels of VEGF in CRH-treated HaCaT cells were significantly attenuated. However, this downregulation was abrogated by pretreatment with antalarmin, SB203580 and SP600125; pretreatment with PD98059 did not attenuate the effects of CRH on the expression of VEGF. In addition, CRH treatment induced rapid phosphorylation of p38 MAPK and JNK1/2, and antalarmin, SB203580 and SP600125 inhibited CRH-induced phosphorylation of p38 MAPK and JNK1/2.

Conclusions: CRH might downregulate the expression of VEGF through the CRH-R1 and MAPK (p38 MAPK and JNK1/2) signaling pathways in human HaCaT cells.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

Corticotropin-releasing hormone (CRH), a 41-amino acid neuropeptide, is produced mainly in the hypothalamus and regulates endocrine and behavioral responses to stress through the activation of the hypothalamic-pituitary-adrenal (HPA) axis [1]. CRH exerts its actions via interaction with specific CRH receptors (CRH-Rs) [2]. Three subtypes of CRH-Rs – CRH-R1, CRH-R2 and CRH-R3 – belong to the G protein-coupled seven-transmembrane receptors [2–5]. Recent research has indicated that CRH and CRH-Rs are expressed and have functions in the skin [6–9]. In human skin, CRH-R1 is the major receptor in epidermis and dermis [7].

The human skin is an independent peripheral endocrine organ [10]. It is a prominent target organ for numerous neurotransmitters and neuropeptide signals that have a profound impact on skin biology in health and disease [11]. Skin has its own functional peripheral equivalent of the HPA axis; CRH produced peripherally and other HPA axis components comprise the cutaneous HPA systems [2,6,12]. Cutaneous CRH is synthesized by cutaneous cells and immune cells in human skin [1,13,14] and regulates various functions of the skin, especially maintaining local homeostasis [2,15]. Compelling evidence has suggested that CRH inhibits the proliferation of both human primary keratinocytes and immortalized keratinocytes [15,16] and stimulates differentiation of keratinocytes [17,18]. Slominski et al. demonstrated a CRH-based homeostatic response system in the skin, and CRH acts as a pleiotropic cytokine [19]. The abnormal differentiation of keratinocytes resulting in a suboptimal barrier function of the skin may be evidence of a protective function for CRH and CRH-R1 in the skin [20].

Psoriasis is a chronic inflammatory disease characterized by erythematous plaques with silvery scales. Psoriatic lesions exhibit proliferation of epidermal keratinocytes, inflammatory cell infiltration, and increased angiogenesis of the superficial dermal vessels [21]. The prominence of dermal microvascular expansion in the psoriatic lesion demonstrates that psoriasis is an angiogenesis-dependent disease [22]. Vascular endothelial growth factor (VEGF) is a crucial regulator of angiogenesis and vascular permeability in both physiological and pathological conditions such as tumor growth and chronic inflammation [23–25]. It was originally identified as an endothelial cell-specific growth factor that can stimulate endothelial cells to undergo angiogenesis and induce vascular permeability, thus facilitating the development of some diseases [26]. VEGF is expressed and secreted by epidermal keratinocytes in normal human skin [27]. Keratinocytes overexpress VEGF in clinically involved and uninvolved skin of patients with chronic plaque psoriasis [28]. In transgenic mice with epidermis-specific overexpression of VEGF, enhanced skin vascularity and vascular

* Corresponding author. Tel.: +86 531 82169390; fax: +86 531 86927544.
E-mail address: lichunyangjinan@126.com (C.-Y. Li).

0167-0115/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.regpep.2009.10.001

Please cite this article as: Zhou C-L, et al, Corticotropin-releasing hormone attenuates vascular endothelial growth factor release from human HaCaT keratinocytes, Regul Pept (2009), doi:10.1016/j.regpep.2009.10.001

ARTICLE IN PRESS

permeability [29], chronic transgenic delivery of VEGF to the skin induced inflammation and all characteristics of psoriasis spontaneously, and the VEGF antagonist reversed the phenotype. These findings suggested a causative role of VEGF in the pathogenesis of psoriasis [30].

However, little is known about the exact role of CRH in skin. We hypothesized that CRH may modulate VEGF expression and investigated the effect of CRH and its receptor CRH-R1 on the expression of VEGF in a human keratinocyte cell line, HaCaT. We examined whether this effect functioned via the mitogen-activated protein kinase (MAPK) signal transduction pathway, particularly p38 mitogen-activated protein kinase (p38 MAPK), extracellular signal-regulated protein kinase 1/2 (ERK1/2) and c-Jun N-terminal kinase (JNK) [31,32].

2. Materials and methods

2.1. Antibodies and reagents

Human CRH and the CRH-R1 antagonist antalarmin were from Sigma (St. Louis, MO, USA). SB203580 (the inhibitor of p38 MAPK), PD98059 (the inhibitor of ERK1/2) and SP600125 (the inhibitor of JNK1/2) were from Biosource (Camarillo, CA). Antibody against β-actin was from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies against phospho-p38 MAPK, p38 MAPK, phospho-JNK1/2 and JNK1/2 were from Cell Signaling Technology (Beverly, MA, USA); horseradish peroxidase-conjugated anti-β-actin and anti-rabbit IgG antibodies were from Santa Cruz Biotechnology. The human VEGF ELISA kit was from R&D Systems (Minneapolis, MN, USA).

2.2. Cell culture

Immortalized human HaCaT keratinocytes were maintained at 37 °C and 5% carbon dioxide (CO_2) in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin. HaCaT cells were digested for 5 min at 37 °C with phosphate-buffered saline (PBS) containing 0.25% trypsin and 0.02% EDTA. Then, the cells were collected by centrifugation at 800 rpm for 10 min at 4 °C, and cultured at a density of 1×10^6 cells/plate at 37 °C in a humid atmosphere of 5% CO_2 and 95% air. The culture medium was changed twice a week.

2.3. Cell pretreatment

HaCaT cells were seeded at a density of 1×10^6 cells/plate, grown for 48 h until 70% confluence, and then cells were washed with serum-free media and maintained with media without FBS at least 12 h prior to the experiments. HaCaT cells were pretreated with 10 μM antalarmin, SB203580, PD98059 or SP600125 respectively for 1 h, and then different concentrations of CRH (1, 10 and 100 nM) were added to the cells.

2.4. Real-time RT-PCR

After treatment, total RNA was extracted from HaCaT cells by use of TRIzol (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Reverse transcription was performed at 37 °C for 1 h by use of an MLV Kit (Promega, Madison, WI), and 1 μg total RNA was used. Real-time RT-PCR involved Light Cycler 2.0 (Roche Applied Science, USA). cDNA was amplified with the use of Light-Cycler-FastStart DNA Master SYBR Green I (Roche, Indianapolis, IN). The primers for VEGF (GenBank NM_001033756.1) were 5′-AAGTGGTCCCAGGCTGCA-3′ (forward), and 5′-ACTCCAGGCCCTCGTCA-3′ (reverse). The primers for human β-actin (GenBank NM_001101.2) were 5′-TGGACATCCGCAAAGAC-3′ (forward) and 5′-GAAAGGGTGTAACGCAACTA-3′ (reverse). The cycling conditions were denaturation for 10 s at 95 °C, amplification for 35 cycles, with denaturation for 5 s at 95 °C, annealing for 5 s at 55 °C for β-actin and 60 °C for VEGF, and extension for 15 s at 72 °C. At the end of each cycle, the fluorescence emitted by the SYBR Green I dye was measured. After amplification, a melting curve was generated by holding the reaction at 65 °C for 30 s and then heating slowly to 95 °C with a ramp rate of 0.1 °C/s. The data were analyzed by use of Light Cycler v4.0 (Roche Applied Science). VEGF mRNA expression was normalized to that of the housekeeping gene human β-actin. Relative VEGF mRNA levels were calculated using the 2(-Delta Delta C(T)) Method [33]. Three independent experiments were performed in triplicate.

2.5. VEGF ELISA

After stimulation for 24 h, culture supernatants of cells were collected, centrifuged (15,000 rpm, 5 min) and stored at −80 °C until analysis. The concentration of VEGF in the culture supernatant was measured by an immunoassay kit according to the manufacturer's instructions. The human VEGF ELISA kit was from R&D Systems (Minneapolis, MN, USA). Three independent experiments were performed in triplicate.

2.6. Western blot analysis

Stimulated HaCaT cells were lyzed in ice-cold RIPA buffer containing 1% phenyl methyl sulfonyl fluoride and centrifuged at 15,000 rpm for 10 min at 4 °C. Supernatants were collected, and the protein concentrations were measured with the use of a BCA protein assay kit (Beyotime, Jiangsu, China). Equal amounts of protein were boiled for 5 min, and 30 μg of total protein was separated on 12% SDS-PAGE and transferred to a 0.22-μm nitrocellulose membrane (Bio-Rad, Hercules, CA). After being blocked with 5% non-fat milk, the blots were washed with PBS containing 0.1% Tween 20 and incubated with an appropriate primary antibody at 4 °C overnight. Antibodies against phospho-p38 MAPK, p38 MAPK, phospho-JNK1/2 and JNK1/2 were used at 1:500 dilution; antibody against β-actin was used at 1:5000 dilution. After several washes, the membranes were incubated with horseradish peroxidase-conjugated secondary antibody (1:5000) for 1 h at 37 °C and then washed again. The blots were visualized with use of an enhanced chemiluminescence kit (Millipore, Billerica, MA, USA). The images were recorded by use of FluorChem9900 (Alpha Innotech, CA, USA) and analyzed with use of Quantity One software (Bio-Rad Laboratories, Hercules, CA). Three independent experiments were performed.

2.7. Statistical analysis

For each condition, data from at least three independent experiments were quantified and analyzed by one-way ANOVA with post-hoc LSD t test. A $P<0.05$ was considered statistically significant. Analysis involved use of SPSS v16.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Effect of CRH on VEGF production in HaCaT cells

To study the effect of CRH on VEGF mRNA expression in HaCaT cells, the cells were treated with different concentrations of CRH (1, 10 and 100 nM). Total RNA was extracted after 4 h and real-time RT-PCR for VEGF was performed. VEGF production was measured by ELISA kit from supernatants of CRH-treated cultured HaCaT cells collected 24 h after the exposure. Real-time RT-PCR and ELISA revealed VEGF mRNA expression and production, respectively, significantly decreased by CRH (1, 10 and 100 nM) in HaCaT cells in a dose-dependent manner, with the maximal effect at 100 nM (Fig. 1A, B).

3.2. CRH-R1 and MAPK signaling pathways are involved in VEGF production

Since CRH treatment led to decreased VEGF expression, we examined whether CRH-R1 and MAPK signaling pathways participated in the regulation of VEGF expression. HaCaT cells were pretreated with

UNCORRECTED PROOF

Please cite this article as: Zhou C-L, et al, Corticotropin-releasing hormone attenuates vascular endothelial growth factor release from human HaCaT keratinocytes, Regul Pept (2009), doi:10.1016/j.regpep.2009.10.001

ARTICLE IN PRESS

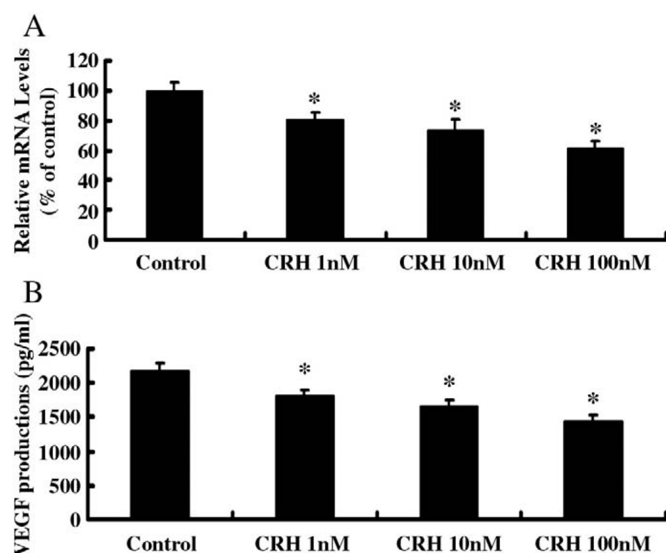


Fig. 1. Effect of CRH on the mRNA expression and production of vascular endothelial growth factor (VEGF) in HaCaT cells. (A) Real-time RT-PCR analysis of VEGF mRNA level in HaCaT cells treated with different concentrations of CRH (1, 10 and 100 nM) for 4 h. (B) ELISA of VEGF production in cells treated with the indicated concentrations of CRH for 24 h. Data are mean ± SD of experiments performed in triplicate. * $P<0.05$ compared with control cells.

antalarmin and SB203580, PD98059, or SP600125 at 10 μM for 1 h before CRH treatment (100 nM). 4 h later, the CRH-induced decrease in VEGF mRNA expression was significantly blocked by antalarmin, SB203580 and SP600125 pretreatment ($P<0.01$) but pretreatment with PD98059 did not attenuate the effects of CRH on the expression of VEGF ($P<0.05$) (Fig. 2A). Meanwhile, antalarmin, SB203580 and SP600125 blocked the CRH-induced decrease in VEGF production ($P<0.01$), but PD98059 did not ($P>0.05$) (Fig. 2B). Thus, CRH down-regulated VEGF expression in HaCaT cells by CRH-R1 through p38 MAPK and JNK1/2 but not ERK1/2 pathways.

3.3. CRH activates p38 MAPK and JNK1/2 phosphorylation in HaCaT cells

To give evidence of the activation of p38 MAPK and JNK1/2 pathways, we used western blot analysis to evaluate the phosphorylation of p38 MAPK and JNK1/2 in CRH-treated HaCaT cells. CRH induced a rapid phosphorylation of p38 MAPK and JNK1/2, with a peak at 5 min (Fig. 3A). Normalization to total p38 MAPK and JNK1/2 confirmed that CRH specifically altered p38 MAPK and JNK1/2 phosphorylation but not the expression levels. Pretreating HaCaT cells respectively with SB203580, SP600125 or antalarmin for 1 h significantly inhibited the CRH-induced phosphorylation of p38 MAPK and JNK1/2 (Fig. 3B). These data indicate that CRH activated p38 MAPK and JNK1/2 phosphorylation in HaCaT cells, and CRH-R1 was involved in the CRH-induced phosphorylation of p38 MAPK and JNK1/2.

4. Discussion

In the present study, we investigated the regulatory effect of CRH on the expression of VEGF in HaCaT cells and the MAPK pathway involved. CRH attenuated VEGF expression in human HaCaT cells through CRH-R1. Furthermore, the effect of CRH on VEGF expression was associated with activation of p38 MAPK and JNK1/2 signal transduction pathways through CRH-R1.

VEGF is a major epidermis-derived vessel-specific growth factor and regarded as a potent angiogenic factor in many cutaneous diseases [34,35]. The level of VEGF is an important parameter in maintaining balanced skin angiogenesis, and it has been identified as a major keratinocyte-derived vessel-specific growth factor [36]. Keratinocytes in the lesional skin are a major source of pro-angiogenic cytokines in psoriasis; studies have identified several angiogenic factors from psoriatic epidermis, including tumor necrosis factor-α and VEGF [37,38]. VEGF was reported to be strongly up-regulated in psoriatic skin lesions

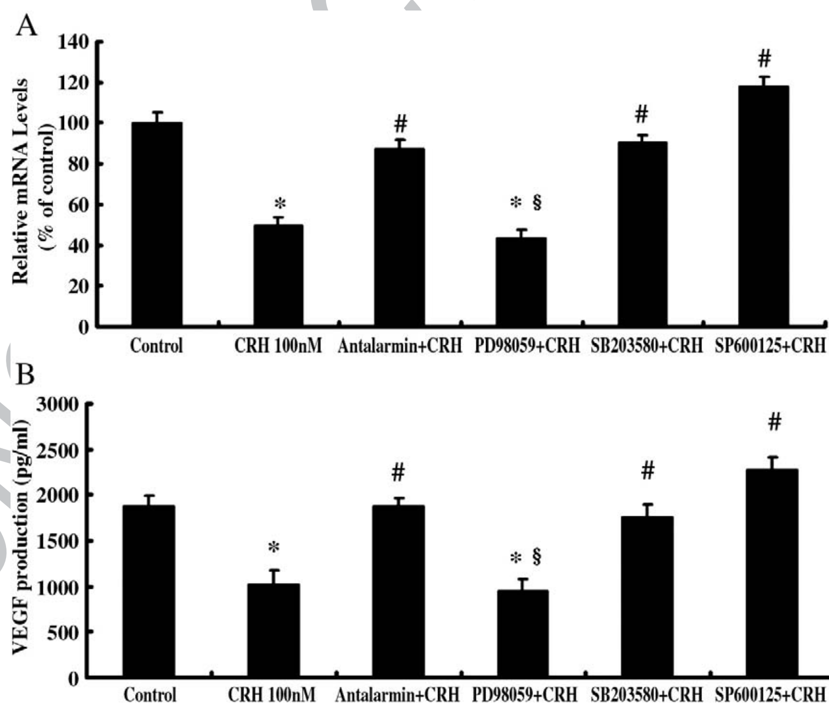


Fig. 2. Effect of the antagonist of CRH-R1 and MAPK inhibitors on VEGF expression induced by CRH in HaCaT cells. HaCaT cells were treated respectively with antalarmin (the CRH-R1 antagonist) and SB203580 (the inhibitor of p38 MAPK), PD98059 (the inhibitor of ERK1/2), or SP600125 (the inhibitor of JNK1/2) at 10 μM for 1 h before CRH (100 nM). (A) Real-time RT-PCR analysis of VEGF mRNA expression after 100 nM CRH treatment for 4 h. (B) ELISA of VEGF production in cells treated with 100 nM CRH for 24 h. Data are mean ± SD of three independent experiments performed in triplicate. * $P<0.01$ vs control; # $P<0.01$, § $P>0.05$ vs CRH-treated group.

Please cite this article as: Zhou C-L, et al, Corticotropin-releasing hormone attenuates vascular endothelial growth factor release from human HaCaT keratinocytes, Regul Pept (2009), doi:10.1016/j.regpep.2009.10.001

ARTICLE IN PRESS

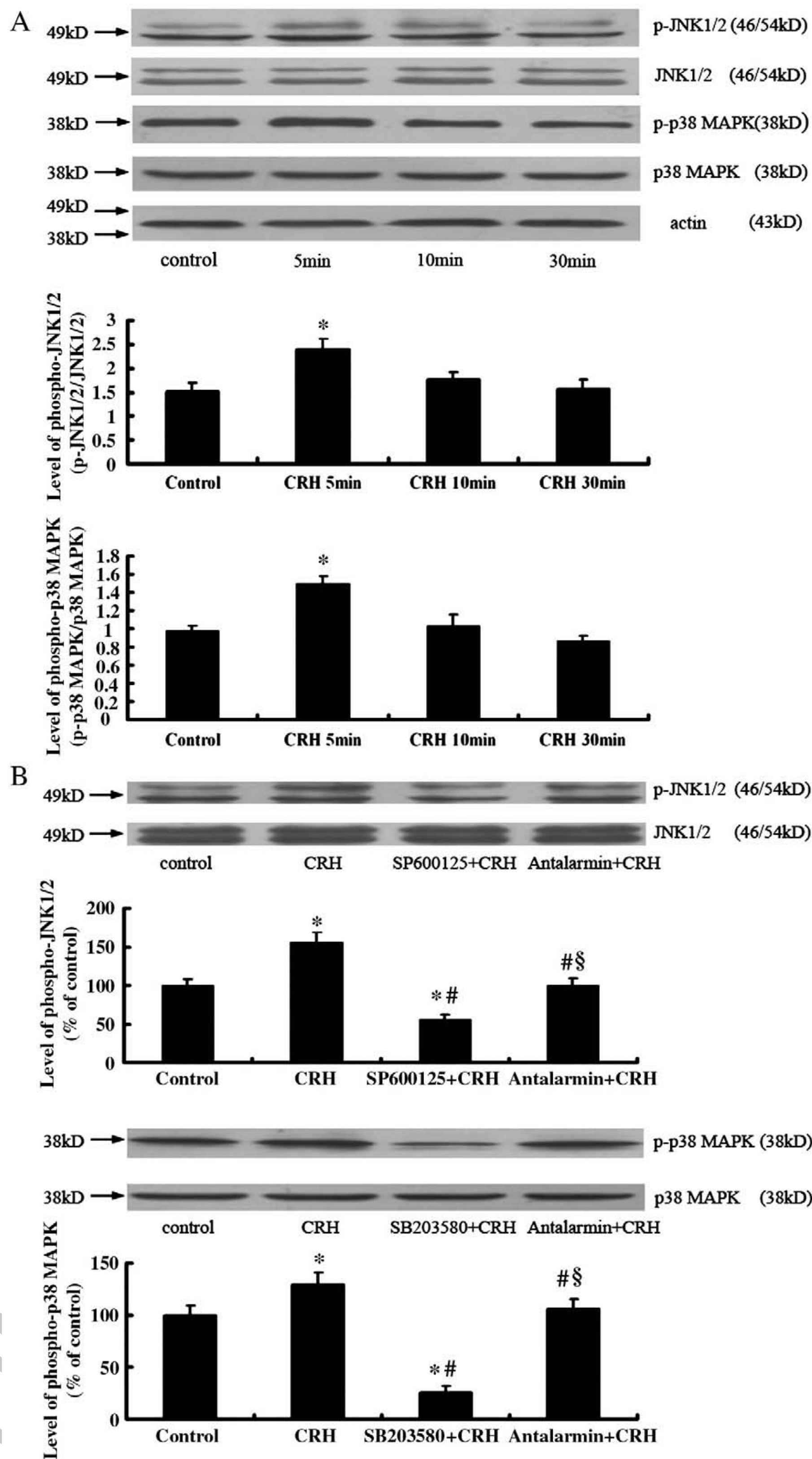


Fig. 3. CRH activates p38 MAPK and JNK1/2 phosphorylation in HaCaT cells. (A) Western blot analysis of the activation of p38 MAPK and JNK1/2 pathways and phosphorylation of p38 MAPK and JNK1/2 in HaCaT cells treated with CRH. CRH induced a rapid activation of p38 MAPK and JNK1/2 as determined by phosphorylation levels. Phosphorylation of p38 MAPK and JNK1/2 peaked at 5 min. Data are mean ± SD of three independent experiments. * $P<0.05$ vs control. (B) Effect of pretreating HaCaT cells with SB203580 (the p38 MAPK inhibitor), SP600125 (the JNK1/2 inhibitor) or antalarmin (the CRH-R1 antagonist) for 1 h on the CRH-induced phosphorylation of p38 MAPK and JNK1/2. Data are mean ± SD of three independent experiments. * $P<0.05$, § $P>0.05$ vs control; # $P<0.05$ vs CRH-treated group.

Please cite this article as: Zhou C-L, et al, Corticotropin-releasing hormone attenuates vascular endothelial growth factor release from human HaCaT keratinocytes, Regul Pept (2009), doi:10.1016/j.regpep.2009.10.001

ARTICLE IN PRESS

and may play a key role in mechanisms underlying the development of psoriasis [28,30].

Recently, CRH has been shown to modulate cytokine production and induce changes in growth and differentiation [15,18]; it may induce a shift away from proliferation activity and towards immunoreactivity [16]. Previously, the pro-inflammation role of CRH was reported with CRH-induced activation of mast cells in stress-related exacerbation of cutaneous inflammatory diseases, such as psoriasis [39,40]. Meanwhile, exogenously added CRH was found to stimulate production of interleukin (IL)-6 and IL-11 and inhibit production of IL-1β in HaCaT cells, and CRH inhibited lipopolysaccharide (LPS)-stimulated IL-6 production [41]. CRH also could inhibit VEGF mRNA expression in cultured extravillous trophoblasts, suggesting that CRH may inhibit angiogenesis during early placentation [42]. In another study, CRH downregulated IL-18 expression, a pro-inflammatory factor [43]. Thus, CRH also has an anti-inflammatory role.

HaCaT cells are a unique spontaneously immortalized keratinocyte cell line derived from normal adult human skin [44]. HaCaT cells exhibit most of the characteristics of basal keratinocytes, especially hyperproliferation, which is typical of epidermal keratinocytes in psoriatic lesions [45]. Therefore, the line has been widely used for *in vitro* testing of anti-psoriatic compounds [46,47]. We used HaCaT cells to examine the effect of CRH on VEGF production. VEGF mRNA expression and production were significantly downregulated by CRH in a dose-dependent manner, with the maximal effect at 100 nM CRH. CRH at 100 nM was used in comparable models, such as regulation of IL-1β production in monocytes [48], suppression of the stress-related NF-κB pathway in human HaCaT keratinocytes [49], and in human trophoblast cells, in which CRH augmented LPS induced cytokine secretion [50]. Although CRH produced locally plays an active and pivotal role in peripheral organs, especially in regulating local homeostasis [2,15], the biological actions of CRH are mediated through interaction with CRH-Rs, which may be a central element [2]. We found the effect of CRH on VEGF expression antagonized by the CRH-R1 antagonist, antalarmin, which indicates that CRH-R1 was involved in the role of CRH in HaCaT cells.

To investigate the precise mechanism underlying the role of the CRH/CRH-R1 system in HaCaT cells, we studied the activation of MAPKs, the most extensively analyzed cytoplasmic signal transduction pathways. Three main MAPKs have been well characterized: ERK1/2, p38 MAPK and JNK [31,32]. In our study, pretreating HaCaT cells with SB203580 (the inhibitor of p38 MAPK) or SP600125 (the inhibitor of JNK1/2) completely abrogated the effects of CRH on the downregulation of VEGF, whereas pretreatment with PD98059 (the inhibitor of ERK1/2) did not attenuate the effects of CRH on the expression of VEGF. These data indicate that p38 MAPK and JNK1/2 but not ERK1/2 play an important role in the downregulation of VEGF under the CRH/CRH-R1 signaling pathway.

The roles of MAPKs have previously been demonstrated in psoriasis, with contradictory results. The activation of p38 MAPK and JNK1/2 is mainly related to stress and inflammatory cytokines, and the kinases therefore modulate various inflammatory responses [51]. The p38 MAPK is activated in keratinocytes in psoriasis, in wound healing and in response to different stimuli such as inflammatory cytokines, UV radiation and oxidative stress [52,53]. JNK1/2 is activated in skin in response to UV light and other stress signals [53]. Psoriatic epidermis showed selective activation of ERK and JNK but not p38 MAPK, which might be related to hyperproliferation and abnormal differentiation of psoriatic epidermis [54]. In our previous study, we found the levels of phosphorylated ERK1/2 and p38 MAPK enhanced in lesional psoriatic skin [55]. In another study, the activity of p38 MAPK and ERK1/2 was increased in lesional psoriatic skin compared with nonlesional psoriatic skin, and clearance of psoriasis normalized the p38 MAPK and ERK1/2 activity [56]. MAPK signal pathways have multiple roles in the pathogenesis of psoriasis. In our present study, the activation of p38 MAPK and JNK1/2 was involved in the downregulation of VEGF by CRH in HaCaT cells *in vitro*, which suggests an anti-angiogenic role of CRH/CRH-R1. Although the effects of ERK activation in skin to a large extent parallel those of p38 MAPK [57], ERK was not involved in the downregulation of VEGF expression in HaCaT cells by CRH/CRH-R1.

5. Conclusion

In summary, we demonstrate that CRH attenuates the expression of VEGF through CRH-R1 and p38 MAPK–JNK1/2 signaling pathways in human HaCaT cells. Overall, our study provides further evidence for the active and pivotal role of CRH and CRH-R1 in the skin, and may provide an insight into the pathophysiology of neuroinflammatory skin diseases such as psoriasis.

6. Uncited reference Q1

[58]

Acknowledgements

This work was supported by a Natural Science Grant (Y2005C67) from the Shandong Ministry of Science and Technology. We thank Rong Wang, Xuping Wang and Jinbo Feng for technical assistance.

References

[1] Karalis K, Muglia LJ, Bae D, Hilderbrand H, Majzoub JA. CRH and the immune system. J Neuroimmunol 1997;72:131–6.
[2] Slominski A, Wortsman J, Pisarchik A, Zbytek B, Linton EA, Mazurkiewicz JE, Wei ET. Cutaneous expression of corticotropin-releasing hormone (CRH), urocortin, and CRH receptors. Faseb J 2001;15:1678–93.
[3] Grammatopoulos DK, Chrousos GP. Functional characteristics of CRH receptors and potential clinical applications of CRH-receptor antagonists. Trends Endocrinol Metab 2002;13:436–44.
[4] Wiesner B, Roloff B, Fechner K, Slominski A. Intracellular calcium measurements of single human skin cells after stimulation with corticotropin-releasing factor and urocortin using confocal laser scanning microscopy. J Cell Sci 2003;116:1261–8.
[5] Slominski A, Pisarchik A, Tobin DJ, Mazurkiewicz JE, Wortsman J. Differential expression of a cutaneous corticotropin-releasing hormone system. Endocrinology 2004;145:941–50.
[6] Slominski A, Wortsman J, Luger T, Paus R, Solomon S. Corticotropin releasing hormone and proopiomelanocortin involvement in the cutaneous response to stress. Physiol Rev 2000;80:979–1020.
[7] Pisarchik A, Slominski A. Molecular and functional characterization of novel CRFR1 isoforms from the skin. Eur J Biochem 2004;271:2821–30.
[8] Zouboulis CC. The human skin as a hormone target and an endocrine gland. Hormones (Athens) 2004;3:9–26.
[9] Kono M, Nagata H, Umemura S, Kawana S, Osamura RY. In situ expression of corticotropin-releasing hormone (CRH) and proopiomelanocortin (POMC) genes in human skin. Faseb J 2001;15:2297–9.
[10] Slominski A, Mihm MC. Potential mechanism of skin response to stress. Int J Dermatol 1996;35:849–51.
[11] Arck PC, Slominski A, Theoharides TC, Peters EM, Paus R. Neuroimmunology of stress: skin takes center stage. J Invest Dermatol 2006;126:1697–704.
[12] Slominski AT, Botchkarev V, Choudhry M, Fazal N, Fechner K, Furkert J, Krause E, Roloff B, Sayeed M, Wei E, Zbytek B, Zipper J, Wortsman J, Paus R. Cutaneous expression of CRH and CRH-R. Is there a “skin stress response system”? Ann N Y Acad Sci 1999;885:287–311.
[13] Kempuraj D, Papadopoulou NG, Lytinas M, Huang M, Kandere-Grzybowska K, Madhappan B, Boucher W, Christodoulou S, Athanassiou A, Theoharides TC. Corticotropin-releasing hormone and its structurally related urocortin are synthesized and secreted by human mast cells. Endocrinology 2004;145:43–8.
[14] Slominski A, Ermak G, Mazurkiewicz JE, Baker J, Wortsman J. Characterization of corticotropin-releasing hormone (CRH) in human skin. J Clin Endocrinol Metab 1998;83:1020–4.
[15] Slominski AT, Roloff B, Zbytek B, Wei ET, Fechner K, Curry J, Wortsman J. Corticotropin releasing hormone and related peptides can act as bioregulatory factors in human keratinocytes. In Vitro Cell Dev Biol Anim 2000;36:211–6.
[16] Quevedo ME, Slominski A, Pinto W, Wei E, Wortsman J. Pleiotropic effects of corticotropin releasing hormone on normal human skin keratinocytes. In Vitro Cell Dev Biol 2001;37:50–4.
[17] Zbytek B, Slominski AT. Corticotropin-releasing hormone induces keratinocyte differentiation in the adult human epidermis. J Cell Physiol 2005;203:118–26.
[18] Zbytek B, Pikula M, Slominski RM, Mysliwski A, Wei E, Wortsman J, Slominski AT. Corticotropin-releasing hormone triggers differentiation in HaCaT keratinocytes. Br J Dermatol 2005;152:474–80.
[19] Slominski A, Zbytek B, Pisarchik A, Slominski RM, Zmijewski MA, Wortsman J. CRH functions as a growth factor/cytokine in the skin. J Cell Physiol 2006;206:780–91.

UNCORRECTED PROOF

Please cite this article as: Zhou C-L, et al, Corticotropin-releasing hormone attenuates vascular endothelial growth factor release from human HaCaT keratinocytes, Regul Pept (2009), doi:10.1016/j.regpep.2009.10.001

ARTICLE IN PRESS

[20] O'Kane M, Murphy EP, Kirby B. The role of corticotropin-releasing hormone in immune-mediated cutaneous inflammatory disease. Exp Dermatol 2006;15:143–53.
[21] Gaspari AA. Innate and adaptive immunity and the pathophysiology of psoriasis. J Am Acad Dermatol 2006;54:S67–80.
[22] Barker JN. The pathophysiology of psoriasis. Lancet 1991;338:227–30.
[23] Ferrara N. Role of vascular endothelial growth factor in physiologic and pathologic angiogenesis: therapeutic implications. Sem Oncol 2002;29:10–4.
[24] Dvorak HF. Angiogenesis: update 2005. J Thromb Haemost 2005;3:1835–42.
[25] Ferrara N, Gerber HP. The role of vascular endothelial growth factor in angiogenesis. Acta Haematol 2001;106:148–56.
[26] Byrne AM, Bouchier-Hayes DJ, Harmey JH. Angiogenic and cell survival functions of vascular endothelial growth factor (VEGF). J Cell Mol Med 2005;9:777–94.
[27] Brown LF, Yeo KT, Berse B, Yeo TK, Senger DR, Dvorak HF, van de Water L. Expression of vascular permeability factor (vascular endothelial growth factor) by epidermal keratinocytes during wound healing. J Exp Med 1992;176:1375–9.
[28] Detmar M, Brown LF, Claffey KP, Yeo KT, Kocher O, Jackman RW, Berse B, Dvorak HF. Overexpression of vascular permeability factor/vascular endothelial growth factor and its receptors in psoriasis. J Exp Med 1994;180:1141–6.
[29] Detmar M, Brown LF, Schon MP, Elicker BM, Velasco P, Richard L, Fukumura D, Monsky W, Claffey KP, Jain RK. Increased microvascular density and enhanced leukocyte rolling and adhesion in the skin of VEGF transgenic mice. J Invest Dermatol 1998;111:1–6.
[30] Xia YP, Li B, Hylton D, Detmar M, Yancopoulos GD, Rudge JS. Transgenic delivery of VEGF to mouse skin leads to an inflammatory condition resembling human psoriasis. Blood 2003;102:161–8.
[31] Robinson MJ, Cobb MH. Mitogen-activated protein kinase pathways. Curr Opin Cell Biol 1997;9:180–6.
[32] Raman M, Cobb MH. MAP kinase modules: many roads home. Curr Biol 2003;13:R886–8.
[33] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods (San Diego, Calif) 2001;25:402–8.
[34] Bowden J, Brennan PA, Umar T, Cronin A. Expression of vascular endothelial growth factor in basal cell carcinoma and cutaneous squamous cell carcinoma of the head and neck. J Cutan Pathol 2002;29:585–9.
[35] Gira AK, Brown LF, Washington CV, Cohen C, Arbiser JL. Keloids demonstrate high-level epidermal expression of vascular endothelial growth factor. J Am Acad Dermatol 2004;50:850–3.
[36] Detmar M, Yeo KT, Nagy JA, Van de Water L, Brown LF, Berse B, Elicker BM, Ledbetter S, Dvorak HF. Keratinocyte-derived vascular permeability factor (vascular endothelial growth factor) is a potent mitogen for dermal microvascular endothelial cells. J Invest Dermatol 1995;105:44–50.
[37] Ettehadi P, Greaves MW, Wallach D, Aderka D, Camp RD. Elevated tumour necrosis factor-alpha (TNF-alpha) biological activity in psoriatic skin lesions. Clin Exp Immunol 1994;96:146–51.
[38] Bhushan M, McLaughlin B, Weiss JB, Griffiths CE. Levels of endothelial cell stimulating angiogenesis factor and vascular endothelial growth factor are elevated in psoriasis. Br J Dermatol 1999;141:1054–60.
[39] Crompton R, Clifton VL, Bisits AT, Read MA, Smith R, Wright IM. Corticotropin-releasing hormone causes vasodilation in human skin via mast cell-dependent pathways. J Clin Endocrinol Metab 2003;88:5427–32.
[40] Wakahashi S, Nakabayashi K, Maruo N, Yata A, Ohara N, Maruo T. Effects of corticotropin-releasing hormone and stresscopin on vascular endothelial growth factor mRNA expression in cultured early human extravillous trophoblasts. Endocrine 2008;33:144–51.
[41] Zbytek B, Mysliwski A, Slominski A, Wortsman J, Wei ET, Mysliwska J. Corticotropin-releasing hormone affects cytokine production in human HaCaT keratinocytes. Life Sci 2002;70:1013–21.
[42] Cao J, Cetrulo CL, Theoharides TC. Corticotropin-releasing hormone induces vascular endothelial growth factor release from human mast cells via the cAMP/protein kinase A/p38 mitogen-activated protein kinase pathway. Mol Pharmacol 2006;69:998–1006.
[43] Park HJ, Kim HJ, Lee JH, Lee JY, Cho BK, Kang JS, Kang H, Yang Y, Cho DH. Corticotropin-releasing hormone (CRH) downregulates interleukin-18 expression in human HaCaT keratinocytes by activation of p38 mitogen-activated protein kinase (MAPK) pathway. J Invest Dermatol 2005;124:751–5.
[44] Fusenig NE, Boukamp P. Multiple stages and genetic alterations in immortalization, malignant transformation, and tumor progression of human skin keratinocytes. Mol Carcinogen 1998;23:144–58.
[45] Bonnekoh B, Wevers A, Geisel J, Rasokat H, Mahrle G. Antiproliferative potential of zidovudine in human keratinocyte cultures. J Am Acad Dermatol 1991;25:483–90.
[46] Stein M, Bernd A, Ramirez-Bosca A, Kippenberger S, Holzmann H. Measurement of anti-inflammatory effects of glucocorticoids on human keratinocytes in vitro. Comparison of normal human keratinocytes with the keratinocyte cell line HaCaT. Arzneimittel-Forschung 1997;47:1266–70.
[47] Muller K, Prinz H. Antipsoriatic anthrones with modulated redox properties. 4. Synthesis and biological activity of novel 9, 10-dihydro-1, 8-dihydroxy-9-oxo-2-anthracenecarboxylic and -hydroxamic acids. J Med Chem 1997;40:2780–7.
[48] Paez Pereda M, Sauer J, Perez Castro C, Finkielman S, Stalla GK, Holsboer F, Arzt E. Corticotropin-releasing hormone differentially modulates the interleukin-1 system according to the level of monocyte activation by endotoxin. Endocrinology 1995;136:5504–10.
[49] Zbytek B, Pfeffer LM, Slominski AT. Corticotropin-releasing hormone inhibits nuclear factor-kappaB pathway in human HaCaT keratinocytes. J Invest Dermatol 2003;121:1496–9.
[50] Wang W, Nan X, Ji P, Dow KE. Corticotropin releasing hormone modulates endotoxin-induced inflammatory cytokine expression in human trophoblast cells. Placenta 2007;28:1032–8.
[51] Pearson G, Robinson F, Beers Gibson T, Xu BE, Karandikar M, Berman K, Cobb MH. Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. Endocr Rev 2001;22:153–83.
[52] Chen W, Bowden GT. Role of p38 mitogen-activated protein kinases in ultraviolet-B irradiation-induced activator protein 1 activation in human keratinocytes. Mol Carcinogen 2000;28:196–202.
[53] Kobayashi H, Aiba S, Yoshino Y, Tagami H. Acute cutaneous barrier disruption activates epidermal p44/42 and p38 mitogen-activated protein kinases in human and hairless guinea pig skin. Exp Dermatol 2003;12:734–46.
[54] Adachi M, Gazel A, Pintucci G, Shuck A, Shifteh S, Ginsburg D, Rao LS, Kaneko T, Freedberg IM, Tamaki K, Blumenberg M. Specificity in stress response: epidermal keratinocytes exhibit specialized UV-responsive signal transduction pathways. DNA Cell Biol 2003;22:665–77.
[55] Takahashi H, Ibe M, Nakamura S, Ishida-Yamamoto A, Hashimoto Y, Iizuka H. Extracellular regulated kinase and c-Jun N-terminal kinase are activated in psoriatic involved epidermis. J Dermatol Sci 2002;30:94–9.
[56] Yu XJ, Li CY, Dai HY, Cai DX, Wang KY, Xu YH, Chen LM, Zhou CL. Expression and localization of the activated mitogen-activated protein kinase in lesional psoriatic skin. Exp Mol Pathol 2007;83:413–8.
[57] Johansen C, Kragballe K, Westergaard M, Henningsen J, Kristiansen K, Iversen L. The mitogen-activated protein kinases p38 and ERK1/2 are increased in lesional psoriatic skin. Br J Dermatol 2005;152:37–42.
[58] Yano S, Komine M, Fujimoto M, Okochi H, Tamaki K. Mechanical stretching in vitro regulates signal transduction pathways and cellular proliferation in human epidermal keratinocytes. J Invest Dermatol 2004;122:783–90.

UNCORRECTED PROOF

Please cite this article as: Zhou C-L, et al, Corticotropin-releasing hormone attenuates vascular endothelial growth factor release from human HaCaT keratinocytes, Regul Pept (2009), doi:10.1016/j.regpep.2009.10.001