

Skin Corticotropin-Releasing Hormone Receptor Expression in Psoriasis

Journal of Investigative Dermatology advance online publication, 1 March 2007; doi:10.1038/sj.jid.5700757

TO THE EDITOR

Psoriasis is characterized by keratinocyte proliferation, inflammation, and mast cell activation (Schon and Boehncke, 2005). It is also triggered or exacerbated by acute stress (Katsarou-Katsari *et al.*, 1999; Saraceno *et al.*, 2006); however, this mechanism remains poorly understood. Stress typically results in release of corticotropin-releasing hormone (CRH) from the hypothalamus and regulates the hypothalamic-pituitary-adrenal (HPA) axis (Chrousos, 1995) through activation of CRH receptor-1 (CRH-R1), leading to immunosuppression. CRH is also found peripherally (Chrousos, 1995) and has pro-inflammatory effects through mast cell activation (Theoharides *et al.*, 1998). CRH and CRH-R gene expression has been documented in rodent and human skin (Slominski *et al.*, 2001). In fact, it has been proposed that skin has the equivalent of the HPA axis (Slominski *et al.*, 2000). In mice, CRH is released from nerve endings (Slominski *et al.*, 2001), whereas in humans it is synthesized by skin cells (Slominski *et al.*, 1998), immune cells (Karalis *et al.*, 1997), and human mast cells (Kempuraj *et al.*, 2004).

To study the effect of stress and the role of CRH in psoriasis, we investigated, by quantitative PCR, CRH-R expression in affected and unaffected skin of psoriasis patients ($n=13$) and skin from normal controls ($n=4$), as well as serum CRH levels from psoriasis patients ($n=8$) and controls ($n=4$). The characteristics of the subjects (Table S1) were as follows: male mean age 47.4 ± 7.0 years ($n=7$); female mean age 28.0 ± 5.2 years ($n=6$); normal

subjects (one male, three female subjects, mean age 40 ± 15.2 years). All skin biopsies requiring two stitches were collected for diagnostic purposes (Table S1). The Medical Ethics Commit-

tee of Attikon Hospital HIRB approved this protocol. All participants gave their written informed consent according to the Declaration of Helsinki Principles. Patients had moderate chronic plaque psoriasis with psoriasis area and severity index (PASI) scores 5–16 and had not received any therapy for psoriasis

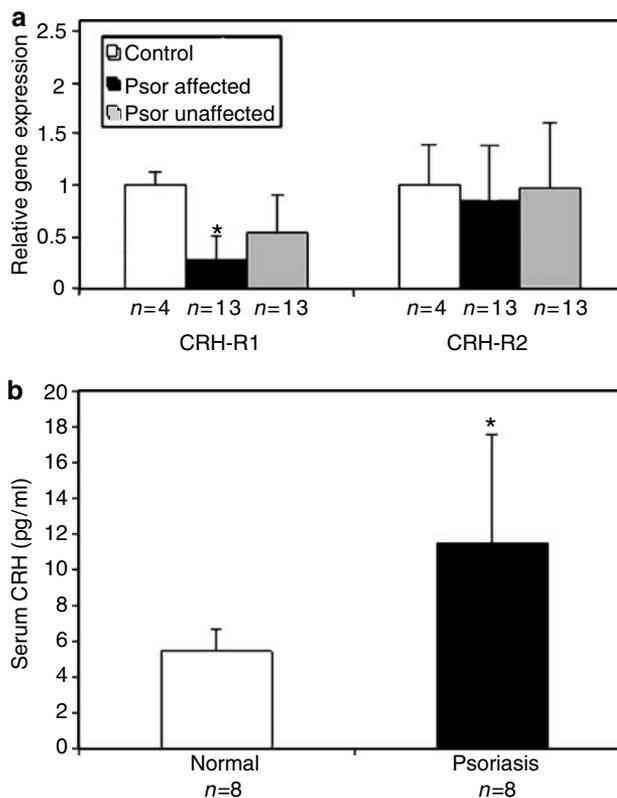


Figure 1. Skin CRH receptor expression and serum CRH levels in psoriasis. (a) CRH-R gene expression in skin samples from psoriasis patients ($n=13$) and controls ($n=4$) by quantitative real-time-PCR was obtained from non-exposed skin (back and gluteal). Samples of unaffected skin of psoriasis patients were obtained from sites at least 15 cm away from lesional areas. All biopsies were immediately placed in RNAlater solution (Ambion Inc., Austin, TX) and stored at -20°C . Relative quantities of mRNA expression were normalized using 18S as an internal control. TaqMan was performed with cDNA reverse transcribed from 100 ng RNA from each sample. (b) Serum CRH levels in psoriasis patients ($n=8$) and controls ($n=4$) measured using an ELISA kit (R&D Systems, Minneapolis, MN) ($*P<0.05$). Statistics: the efficiency of the quantitative PCR machine was shown to be 1.8 and the equation we use is $1/(1.8^{\Delta C_t})$. This value was calculated for each condition and results are expressed as a ratio of mRNA expression for CRH-R to 18S. Results are presented as mean \pm SD and were compared with controls (set at 1) using one-way analysis of variance on ranks followed by Dunn's correction for multiple comparisons. Significance is denoted by (psor = psoriasis) $*P<0.05$.

Abbreviations: CRH, corticotropin-releasing hormone; HPA, hypothalamic-pituitary-adrenal; CRH-R1, CRH receptor-1; PASI, psoriasis area and severity index

(topical or systemic) for the past month. The PASI score for males was 11.3 ± 13.5 and for females was 11.5 ± 3.7 .

Expression of CRH-R1 mRNA was lowest in affected samples from psoriasis patients (0.27 ± 0.23 , $n=13$, $P<0.05$), compared with control patients (Figure 1a). CRH-R1 expression in unaffected skin from psoriasis patients (0.53 ± 0.38) was not statistically different from that of affected samples or controls (Figure 1a). There was no statistically significant difference in CRH-R2 mRNA expression among the control samples, those obtained from affected (0.86 ± 0.51) and from unaffected (0.97 ± 0.65) psoriatic skin (Figure 1a).

The serum CRH level (11.52 ± 6.09 pg/ml) was higher ($n=8$, $P<0.05$) in psoriasis patients than controls (5.42 ± 1.2 pg/ml, $n=8$). There was no apparent correlation between the PASI scores and either CRH-R1 expression or serum CRH levels.

This study provides early evidence that affected psoriatic skin has decreased gene expression of CRH-R1 mRNA than normal controls. One possible explanation is that overstimulation by increased levels of local or systemic (serum) CRH in psoriasis patients, possibly in response to chronic stress, may lead to CRH-R1 downregulation. In fact, CRH protein expression was recently reported to be increased in the affected skin of three patients with active psoriasis than in one control; however, this effect was not quantitated (O'Kane *et al.*, 2006). As non-affected psoriatic skin apparently did not overexpress CRH-R, as shown by our quantitative real-time-PCR data, there was apparently no mechanism in place to lead to downregulation. Increased CRH-R expression in psoriatic skin was also mentioned as "unpublished observations" (O'Kane *et al.*, 2006) and it is, therefore, difficult to evaluate it. The reduction in CRH-R1 mRNA expression in affected skin of patients with psoriasis we observed could be due to the intense inflammation seen in plaques without any association with serum CRH levels; however, this possibility is not supported by the literature. In fact, we showed that the inflammation-related molecules IL-1, IL-4, and lipopolysaccharide had no

effect on CRH-R1 expression, but increased CRH-R2 expression in human mast cells (Papadopoulou *et al.*, 2005). CRH-R1 is expressed in keratinocytes (Slominski and Wortsman, 2000) and in a subpopulation of skin mast cells (Donelan *et al.*, 2006). Normal cultured human mast cells also express mRNA and protein for CRH-R1 and CRH-R2 (Cao *et al.*, 2005). Human skin, squamous cell carcinoma, and melanoma cells also express CRH and CRH-R1 (Slominski *et al.*, 1998, 2001). The lack of any significant difference between affected and unaffected or control skin may either indicate (a) that unaffected skin represents an early or intermediate stage, (b) that these areas have different number of cells expressing CRHR, or (c) the variability is too large, given the small number of patients.

Psoriasis is the most common chronic inflammatory skin disorder (Schon and Boehncke, 2005). It is worsened by stress (Katsarou-Katsari *et al.*, 1999) and is characterized by aberrant HPA function (Richards *et al.*, 2005); moreover, neuropeptides appear to induce skin neurogenic inflammation (Saraceno *et al.*, 2006). The skin may have its own equivalent of the HPA axis (Slominski *et al.*, 2000; Slominski and Wortsman, 2000) and the role of CRH in cutaneous inflammatory diseases was reviewed recently (O'Kane *et al.*, 2006). Chronic stress and CRH typically attenuate immune processes, whereas acute stress enhances antigen-specific, cell-mediated immunity (Dhabhar and McEwen, 1999). Stress also exacerbates contact dermatitis in rats (Kaneko *et al.*, 2003). Acute stress induces local release of CRH in the skin (Lytinas *et al.*, 2003) and increases skin vascular permeability (Singh *et al.*, 1999), an effect mimicked by intradermal CRH and absent in mast cell-deficient mice (Theoharides *et al.*, 1998). CRH also increased vascular permeability in human skin, an effect dependent on CRH-R1 and mast cells (Crompton *et al.*, 2003).

The level of stress in these patients was not quantitated with any validated instrument and it is, therefore, premature to try to make any correlations between our findings and any level of stress in these patients.

Mast cells are involved not only in allergic reactions, but also in innate immunity (Galli *et al.*, 2005) and inflammation (Theoharides and Cochrane, 2004). Mast cells are juxtaposed to nerve endings during hair follicle formation (Roloff *et al.*, 1998) and are located close to CRH-positive nerve endings (Rozniecki *et al.*, 1999), suggesting that they are involved in a "brain-skin" connection (Paus *et al.*, 2006), as targets of CRH and related peptides (Theoharides *et al.*, 2004).

The present findings suggest that CRH and CRH-R1 may participate in the pathogenesis of psoriasis, especially when worsened by stress.

CONFLICT OF INTEREST

The authors state no conflict of interest. Use of CRH-R antagonists in stress-induced dermatoses is covered by US Patents no. 6020305 and 6689748 (TCT).

ACKNOWLEDGMENTS

Aspects of this work were supported by US NIH Grant no. AR47652 (TCT). We thank Dr Michael Court (Tufts University) for providing the 18S primers. We also thank Miss Jessica Christian for her word processing skills.

Michael Tagen¹, Linsey Stiles¹, Demetrios Kalogeromitros², Stamatios Gregoriou², Duraisamy Kempuraj¹, Michael Makris², Jill Donelan¹, Magdalini Vasiadi¹, Nikolaos G. Staurianees³ and Theoharis C. Theoharides^{1,4,5,6}

¹Departments of Pharmacology and Experimental Therapeutics, Tufts University School of Medicine, Tufts-New England Medical Center, Boston, Massachusetts, USA; ²Allergy Division, Attikon Hospital, University of Athens Medical School, Athens, Greece; ³Department of Dermatology, Attikon Hospital, University of Athens Medical School, Athens, Greece; ⁴Department of Biochemistry, Tufts University School of Medicine, Tufts-New England Medical Center, Boston, Massachusetts, USA; ⁵Department of Internal Medicine, Tufts University School of Medicine, Tufts-New England Medical Center, Boston, Massachusetts, USA and ⁶Department of Psychiatry, Tufts University School of Medicine, Tufts-New England Medical Center, Boston, Massachusetts, USA.
E-mail: theoharis.theoharides@tufts.edu

SUPPLEMENTARY MATERIAL

Methods.

Table S1. Characteristics of subjects providing the skin samples.

REFERENCES

- Cao J, Papadopoulou N, Kempuraj D, Boucher WS, Sugimoto K, Cetrulo CL *et al.* (2005) Human mast cells express corticotropin-releasing hormone (CRH) receptors and CRH leads to selective secretion of vascular endothelial growth factor. *J Immunol* 174:7665-75
- Chrousos GP (1995) The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. *N Engl J Med* 332:1351-62
- Crompton R, Clifton VL, Bisits AT, Read MA, Smith R, Wright IM (2003) Corticotropin-releasing hormone causes vasodilation in human skin via mast cell-dependent pathways. *J Clin Endocrinol Metab* 88:5427-32
- Dhabhar FS, McEwen BS (1999) Enhancing *versus* suppressive effects of stress hormones on skin immune function. *Proc Natl Acad Sci USA* 96:1059-64
- Donelan J, Marchand J, Kempuraj D, Papadopoulou N, Theoharides TC (2006) Perifollicular and perivascular mouse skin mast cells express corticotropin-releasing hormone receptor. *J Inv Dermatol* 126:929-32
- Galli SJ, Nakae S, Tsai M (2005) Mast cells in the development of adaptive immune responses. *Nat Immunol* 6:135-42
- Kaneko K, Kawana S, Arai K, Shibasaki T (2003) Corticotropin-releasing factor receptor type 1 is involved in the stress-induced exacerbation of chronic contact dermatitis in rats. *Exp Dermatol* 12:47-52
- Karalis K, Louis JM, Bae D, Hilderbrand H, Majzoub JA (1997) CRH and the immune system. *J Neuroimmunol* 72:131-6
- Katsarou-Katsari A, Filippou A, Theoharides TC (1999) Effect of stress and other psychological factors on the pathophysiology and treatment of dermatoses. *Int J Immunopathol Pharmacol* 12:7-11
- Kempuraj D, Papadopoulou NG, Lytinas M, Huang M, Kandere-Grzybowska K, Madhapan B *et al.* (2004) Corticotropin-releasing hormone and its structurally related urocortin are synthesized and secreted by human mast cells. *Endocrinology* 145:43-8
- Lytinas M, Kempuraj D, Huang M, Boucher W, Esposito P, Theoharides TC (2003) Acute stress results in skin corticotropin-releasing hormone secretion, mast cell activation and vascular permeability, an effect mimicked by intradermal corticotropin-releasing hormone and inhibited by histamine-1 receptor antagonists. *Int Arch Allergy Immunol* 130:224-31
- O'Kane M, Murphy EP, Kirby B (2006) The role of corticotropin-releasing hormone in immune-mediated cutaneous inflammatory disease. *Exp Dermatol* 15:143-53
- Papadopoulou NG, Oleson L, Kempuraj D, Donelan J, Cetrulo CL, Theoharides TC (2005) Regulation of corticotropin-releasing hormone receptor-2 expression in human cord blood-derived cultured mast cells. *J Mol Endocrinol* 35:R1-8
- Paus R, Theoharides TC, Arck PC (2006) Neuro-immunoendocrine circuitry of the "brain-skin connection". *Trends Immunol* 27:32-9
- Richards HL, Ray DW, Kirby B, Mason D, Plant D, Main CJ *et al.* (2005) Response of the hypothalamic-pituitary-adrenal axis to psychological stress in patients with psoriasis. *Br J Dermatol* 153:1114-20
- Roloff B, Fechner K, Slominski A, Furkert J, Botchkarev VA, Bulfone-Paus S *et al.* (1998) Hair cycle-dependent expression of corticotropin-releasing factor (CRF) and CRF receptors in murine skin. *FASEB J* 12:287-97
- Rozniecki JJ, Dimitriadou V, Lambracht-Hall M, Pang X, Theoharides TC (1999) Morphological and functional demonstration of rat dura mast cell-neuron interactions *in vitro* and *in vivo*. *Brain Res* 849:1-15
- Saraceno R, Kleyn CE, Terenghi G, Griffiths CE (2006) The role of neuropeptides in psoriasis. *Br J Dermatol* 155:876-82
- Schon MP, Boehncke WH (2005) Psoriasis. *N Engl J Med* 352:1899-912
- Singh LK, Pang X, Alexacos N, Letourneau R, Theoharides TC (1999) Acute immobilization stress triggers skin mast cell degranulation via corticotropin releasing hormone, neurotensin and substance P: a link to neurogenic skin disorders. *Brain Behav Immunity* 13:225-39
- Slominski A, Ermak G, Mazurkiewicz JE, Baker J, Wortsman J (1998) Characterization of corticotropin-releasing hormone (CRH) in human skin. *J Clin Endocrinol Metab* 83:1020-4
- Slominski A, Wortsman J (2000) Neuroendocrinology of the skin. *Endocr Rev* 21:457-487
- Slominski A, Wortsman J, Luger T, Paus R, Solomon S (2000) Corticotropin releasing hormone and proopiomelanocortin involvement in the cutaneous response to stress. *Physiol Rev* 80:979-1020
- Slominski A, Wortsman J, Pisarchik A, Zbytek B, Linton EA, Mazurkiewicz JE *et al.* (2001) Cutaneous expression of corticotropin-releasing hormone (CRH), urocortin, and CRH receptors. *FASEB J* 15:1678-93
- Theoharides TC, Cochrane DE (2004) Critical role of mast cells in inflammatory diseases and the effect of acute stress. *J Neuroimmunol* 146:1-12
- Theoharides TC, Donelan JM, Papadopoulou N, Cao J, Kempuraj D, Conti P (2004) Mast cells as targets of corticotropin-releasing factor and related peptides. *Trends Pharmacol Sci* 25:563-8
- Theoharides TC, Singh LK, Boucher W, Pang X, Letourneau R, Webster E *et al.* (1998) Corticotropin-releasing hormone induces skin mast cell degranulation and increased vascular permeability, a possible explanation for its pro-inflammatory effects. *Endocrinology* 139:403-13