Increased serum CRH levels with decreased skin CRHR-1 gene expression in psoriasis and atopic dermatitis

Magdalini Vasiadi, BSc[a,b,c], **Anastasia Therianou, MD**[d], **Kyriaki Sideri, MD**[c], **Marilena Smyrnioti, BSc, MA**[e], **Nikolaos Sismanopoulos, MD**[a,c], **Danae Anastasia Delivanis, MD**[a,#], **Shahrzad Asadi, PharmD**[a,f], **Alexandra Katsarou-Katsari, MD, PhD**[d], **Theodora Petrakopoulou, MD**[g], **Athanasios Theoharides, MD, PhD**[h], **Christina Antoniou, MD, PhD**[d], **Evaggelia Papadavid, MD, PhD**[i], **Nikolaos Stavrianeas, MD, PhD**[i], **Dimitrios Kalogeromitros, MD, PhD**[c,†], and **Theoharids Constantin Theoharides, MS, PhD, MD**[a,b,c,j,k]

[a]Molecular Immunopharmacology and Drug Discovery Laboratory, Department of Molecular Physiology and Pharmacology, Tufts University School of Medicine, Boston, MA, USA

[b]Sackler School of Graduate Biomedical Sciences, Tufts University, Boston, MA, USA

[c]Allergy Clinical Research Center, Attikon Hospital, Athens University Medical School, Athens, Greece

[d]First Department of Dermatology, A. Sygros Hospital, Athens University Medical School, Athens, Greece

[e]Pain Relief Clinic, Second Department of Anesthesiology, Attikon Hospital, Athens University Medical School, Athens, Greece

[f]Department of Pharmacy, Tufts Medical Center, Boston, MA, USA

[g]Oncology Hospital, IKA, Athens, Greece hAno Toumba Polyclinic, IKA, Thessaloniki, Greece

[h]Ano Toumba Polyclinic, IKA, Thessaloniki, Greece

[i]Second Department of Dermatology, Attikon Hospital, Athens University Medical School, Athens, Greece

[j]Department of Biochemistry, Tufts University School of Medicine, Boston, MA, USA

[k]Department of Internal Medicine, Tufts University School of Medicine and Tufts Medical Center, Boston, MA, USA

Abstract

There is increased serum CRH with decreased lesional skin CRHR-1 gene expression in psoriasis and atopic dermatitis, suggesting possible involvement in stress-induced worsening of symptoms.

Address for correspondence: T.C. Theoharides, M.S., Ph.D., M.D., Department of Molecular Physiology and Pharmacology, Tufts University School of Medicine, Harrison Avenue, Boston, MA 02111, Phone: 617-636-6866, Fax: 617-636-2456, theoharis.theoharides@tufts.edu.
[#]Department of Internal Medicine, University of Connecticut Medical Center, Hartford, CT
[†]Deceased

Keywords

Atopic dermatitis; corticotropin-releasing hormone; inflammation; mast cells; psoriasis; stress

---

To the Editor:

Autoimmune skin diseases, such as psoriasis (Ps) and atopic dermatitis (AD), are chronic inflammatory skin disorders mediated primarily by T cells [1], but mast cells are also implicated [2]. Symptoms of both Ps and AD worsen with stress [3]. Acute stress leads to increased skin vascular permeability and inflammation, through mast cell activation by corticotropin-releasing hormone (CRH) both in rodents [4] and humans [5]. CRH and CRHR-1 are both expressed in human skin [6], leading to the hypothesis that CRH may be involved in the pathophysiology of skin diseases [7].

Full length 3 mm^3 punch skin biopsies were collected for diagnostic purposes from subjects with Ps and AD who had not received any medication for 15 days prior to biopsy and were free from any systemic allergic or inflammatory disease. Serum was also collected from patients and controls. The Medical Ethics Committee approved this protocol. Psoriasis Area and Severity Index (PASI) score was recorded for some of the Ps patients; no severity index was obtained from the AD patients Also, some Ps and AD patients filled the State-Trait Anxiety Inventory (STAI), which has been validated for the Greek population [8], to investigate the extent of stress. The STAI measures separate constructs of psychosocial stress and clearly differentiates between the temporary condition of "state anxiety" (STATE now) and the more general and long-standing quality of "trait anxiety" (STATE trait).

Serum level and skin gene expression results from patients were compared to those of controls using the Mann-Whitney non parametric U-test. There was no statistical difference in the mean age between controls and patients. All subject characteristics and the summary of the results are presented in Table 1.

For CRH serum measurements in Ps patients (n=48, 27 of which had PASI=10-50), the mean age was 46±17 (23 women and 25 men), while the mean age for controls (n=19) was 47±16 (10 women and 9 men). Serum CRH levels were higher (p=0.0001) in Ps patients (22.5±13.7 pg/ml) as compared to controls (9.7±4.2 pg/ml) (Fig. 1A). When Ps patients were separated in those with PASI scores <10>, there was a statistically significant increase (p=0.0001) in Ps patients with PASI >10 (Fig. 1A), but not in those with <10 (data not shown). For CRH serum measurements in AD patients (n=15), the mean age was 36±18 (9 women and 6 men), while the mean age for control (n=19) was 47±16 (10 women and 9 men). Serum CRH levels were higher (p=0.0001) in AD patients (31.0±19.5 pg/ml) as compared to controls (9.7±4.2 pg/ml) (Fig. 1A).

For cytokine serum measurements, the mean age for Ps patients (n=56, 31 of which were Ps patients with PASI 10–50) was 40±14 (26 women and 30 men), while the mean age for controls (n=33) was 48±18 (14 women and 19 men). Serum cytokines and VEGF were evaluated using Milliplex microbead technology and the measurements were performed by Millipore (St. Charles, MI). Serum levels of IL-6, IL-9, IL-33, TNFβ, and TSLP in Ps patients were undetectable, except for VEGF and IL-8. Serum VEGF levels were not different in Ps patients (411.9±280 pg/ml) as compared to controls (257±123.8 pg/ml), (Fig. 1B). However, serum VEGF levels in Ps patients with PASI score >10 were significantly increased (p=0.0286) (Fig. 1B), while in Ps patients with PASI score <10 were not (data not shown). There was no difference between serum IL-8 levels in Ps patients (25.7±27.5 pg/ml) as compared to controls (31.3±31.2 pg/ml).

For serum measurements, the mean age for AD patients (n=18) was 38±20 (12 women and 6 men), while mean age for control (n=33) was 48±18 (14 women and 19 men). Serum levels of IL-6, IL-9, IL-33, TNFβ, and TSLP were undetectable, except for VEGF and IL-8. Serum VEGF levels were not different in AD patients (291.4±280.1 pg/ml) as compared to controls (257.2±123.8 pg/ml) (Fig. 1B). Serum IL-8 levels were lower (p=0.0171) in AD patients (19.7±17.2 pg/ml) as compared to controls (31.3±32.2 pg/ml) (data not shown). There was a reasonable correlation between serum CRH levels and STAI (STATE now) scores (Pearson r=0.55, p=0.04, n=14) for Ps patients with PASI score>10, but there was no correlation with STAI (STATE trait). There was no correlation between STAI scores and CRH serum measurements or VEGF gene expression for the AD patients (Fig. 1C).

For gene expression assays, the mean age for Ps patients (n=40) was 46±17 (19 women and 21 men), while mean age for controls (n=30) was 43±15 (22 women and 8 men). Skin CRHR-1 gene expression was lower (p<0.0001) in affected skin samples from Ps patients (0.1±0.1) as compared to controls (1±0.7) (Fig. 2A). CRHR-1 expression was documented in lesional Ps skin by immunohistochemistry and appeared to co-localize with mast cells (Fig. 2B) There was also statistically significant lower (p=0.0001) CRHR-1 gene expression in the samples obtained from affected versus unaffected Ps skin, while there was no statistically significant difference in CRHR-1 gene expression between the control and the non-lesion samples (data not shown). Lesional skin CRH gene expression was undetectable in Ps (data not shown). For CRHR-1 gene expression assays, the mean age for AD patients (n=16) was 35±21 (10 women and 6 men), while mean age for controls (n=30) was 43±15 (22 women and 8 men). Skin CRHR-1 gene expression was lower (p=0.0001) in affected samples from AD patients (0.2±0.1) as compared to controls (1±0.7) (Fig. 2A).

Skin VEGF gene expression was lower (p=0.0121) in affected samples from Ps patients (0.6±0.4) as compared to controls (1±0.7) (Fig 2C). There was statistically significant lower VEGF gene expression (p=0.0009) among the samples obtained from affected and from unaffected psoriatic skin (data not shown). Skin VEGF gene expression was also lower (p=0.0001) in affected samples from AD patients (0.2±0.06) as compared to controls (1.0±0.7) (Fig. 2C).

There was a positive correlation between CRH serum levels and VEGF gene expression in Ps patients with PASI score >10 (Pearson r=0.44, n=22, p=0.04, Fig. 2D), which is known to be association with increased skin vascularization. (This finding imply that high serum CRH correlates best only in severe Ps patients.

Our findings suggest that overstimulation by the increased serum levels of CRH and VEGF, possibly in response to stress, leads to decreased gene expression of skin CRH and CRHR-1, as well as skin VEGF gene expression, respectively. The positive correlation between serum CRH levels and the STAI-now scores in severe Ps patients supports this possibility. The lack of similar correlation in AD patients may be because we did not have access of severity index scores for these patients, unlike in Ps patients, in order to carry out a subgroup analysis.

The present findings suggest that high serum CRH, possibly in response to stress, stimulates skin mast cells to release VEGF and contribute to skin inflammatory evident in severe Ps patients. Continuous or repeated stimulation may lead to decreased expression of skin CRHR-1 as we showed recently with cultured mast cells [9], and hence decreased VEGF as was evident in patients with mild disease. CRH and CRHR-1 may therefore participate in the pathogenesis of Ps and AD, especially when worsened with stress, through mast cell activation to release VEGF. Mast cell blockers may provide novel treatment approaches.

Acknowledgments

Abbreviations used:

CRH	corticotropin-releasing hormone
CRHR-1	CRH receptor 1
Ps	psoriasis
STAI	State-Trait Anxiety Inventory
VEGF	Vascular endothelial growth factor

Biographies

Magdalini Vasiadi, BSc

Graduate Student, Sackler School of Graduate Biomedical Sciences, Department of Molecular Physiology and Pharmacology, Tufts University, Boston, MA, USA

Graduate student, Allergy Clinical Research Center, Attikon Hospital, Athens University Medical School, Athens, Greece

Anastasia Therianou, MD

Graduate Student, First Department of Dermatology, A. Sygros Hospital, Athens University Medical School, Athens, Greece

Kyriaki Sideri, MD

Graduate Student, Allergy Clinical Research Center, Attikon Hospital, Athens University Medical School, Athens, Greece

Marilena Smyrnioti, BSc, MA

Clinical Psychologist, Pain Relief Clinic, Second Department of Anesthesiology, Attikon Hospital, Athens University Medical School, Athens, Greece

Nikolaos Sismanopoulos, MD

Graduate Student, Allergy Clinical Research Center, Attikon Hospital, Athens University Medical School, Athens, Greece

Research Fellow, Molecular Immunopharmacology and Drug Discovery Laboratory, Department of Molecular Physiology and Pharmacology, Tufts University School of Medicine, Boston, MA, USA

Danae A Delivani, MD

Research Fellow, Molecular Immunopharmacology and Drug Discovery Laboratory, Department of Molecular Physiology and Pharmacology, Tufts University School of Medicine, Boston, MA, USA

References

1. Guttman-Yassky E, Nograles KE, Krueger JG. Contrasting pathogenesis of atopic dermatitis and psoriasis-Part I: Clinical and pathologic concepts. J Allergy Clin Immunol. 2011; 127(5):1110–1118. [PubMed: 21388665]

2. Metz M, Grimbaldeston MA, Nakae S, Piliponsky AM, Tsai M, Galli SJ. Mast cells in the promotion and limitation of chronic inflammation. Immunol Rev. 2007; 217:304–328. [PubMed: 17498068]

3. Theoharides TC, Cochrane DE. Critical role of mast cells in inflammatory diseases and the effect of acute stress. J Neuroimmunol. 2004; 146:1–12. [PubMed: 14698841]

4. Theoharides TC, Singh LK, Boucher W, Pang X, Letourneau R, Webster E, et al. Corticotropin-releasing hormone induces skin mast cell degranulation and increased vascular permeability, a possible explanation for its pro-inflammatory effects. Endocrinology. 1998; 139:403–413. [PubMed: 9421440]

5. Crompton R, Clifton VL, Bisits AT, Read MA, Smith R, Wright IM. Corticotropin-releasing hormone causes vasodilation in human skin via mast celldependent pathways. J Clin Endocrinol Metab. 2003; 88:5427–5432. [PubMed: 14602784]

6. Slominski A, Wortsman J. Neuroendocrinology of the skin. Endocr Rev. 2000; 21:457–487. [PubMed: 11041445]

7. Slominski A. On the role of the corticotropin-releasing hormone signalling system in the aetiology of inflammatory skin disorders. Br J Dermatol. 2009; 160(2):229–232. [PubMed: 19187344]

8. Fountoulakis KN, Papadopoulou M, Kleanthous S, Papadopoulou A, Bizeli V, Nimatoudis I, et al. Reliability and psychometric properties of the Greek translation of the State-Trait Anxiety Inventory form Y: Preliminary data. Ann Gen Psychiatry. 2006; 5:2. [PubMed: 16448554]

9. Asadi S, Alysandratos KD, Angelidou A, Miniati A, Sismanopoulos N, Vasiadi M, et al. Substance P (SP) induces expression of functional corticotropin-releasing hormone receptor-1 (CRHR-1) in human mast cells. J Invest Dermatol. 2011
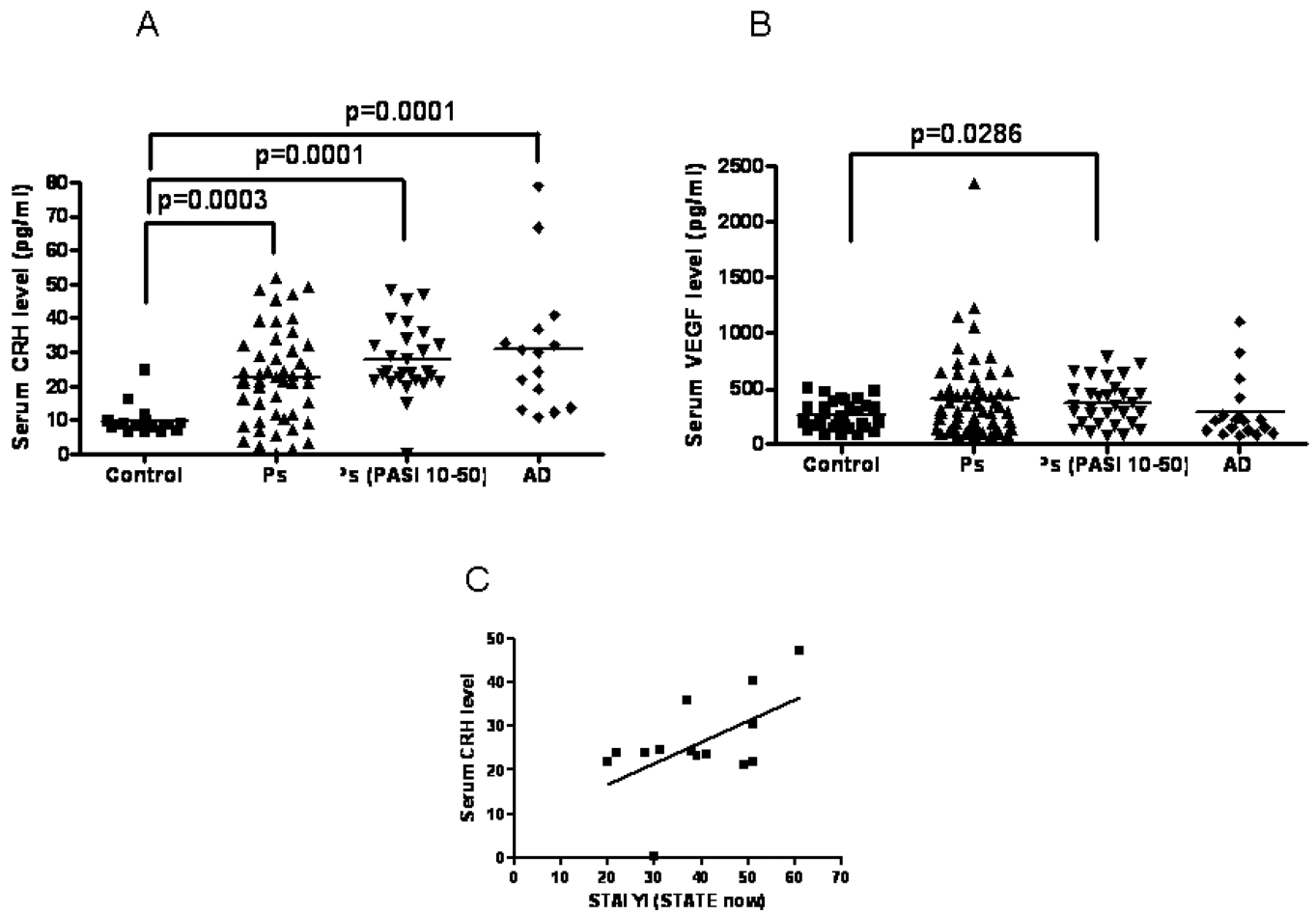
Figure 1.
CRH and VEGF serum levels in Ps and AD patients and controls (A–B), as well as correlation of serum CRH levels in severe Ps patients with STAI (STATE now), (C).
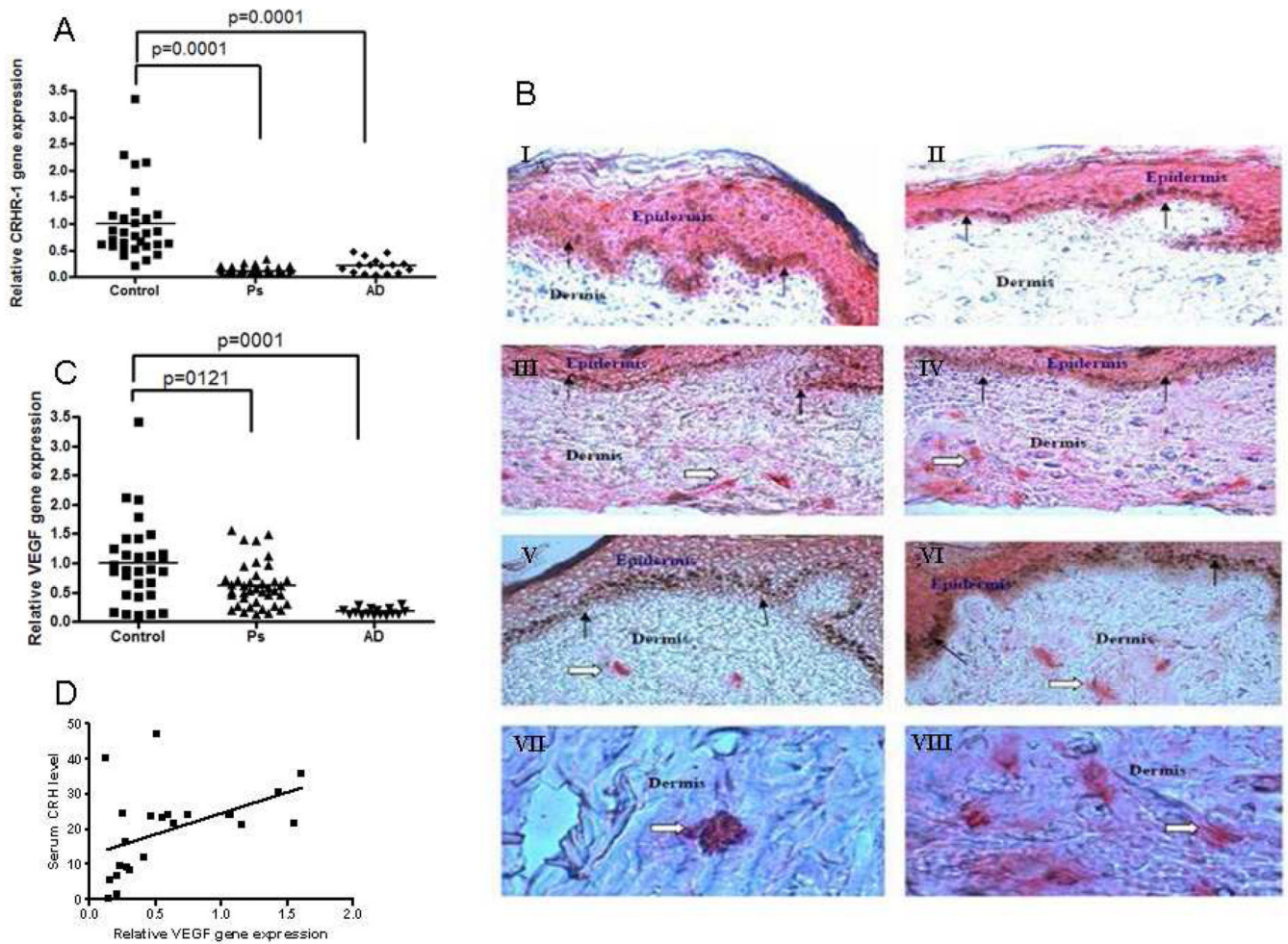
Figure 2.
CRHR-1 and VEGF skin gene expression in Ps and AD patients and controls (A,C), and
CRHR-1 immunostaining in Ps skin (B). Cryostat sections were prepared and fixed with
acetone for 2–3 min and incubated with normal blocking serum for 20 min. Sections were
then incubated with primary antibodies (Goat polyclonal antibody to CRHR-1; Abcam Inc,
Cambridge, MA, Cat # ab59023) diluted to 1:100 for 30 min and then immunostained with
Vectastain ABC AP kit (Vectastain Lab, Burlingame, CA) and Vector Red Alkaline
Phosphatase Substrate kit (Vector Lab) as per the kit's directions. Presence of red color
indicate the positive reaction for CRHR-1. (A&B). epidermis and mast cells in dermis
(C,D,E,F) and only mast cells (G&H). Solid arrow show epidermollow arrow show mast
cells. Correlation of serum CRH levels with VEGF gene expression in severe Ps patients
(D).

Table 1

Summary of subject characteristics and results

	Sex; No; Age	Serum CRH	Serum VEGF	Skin CRHR-1	Skin VEGF
AD	F; n=13; 41±23 M; n=7; age: 28±9	F; n=8; 42±23 M; n=5; 28±11	F; n=12; 43±22 M; n=6; 27±9	F; n=10; 40±24 M; n=6; 27±9	F; n=10; 40±24 M; n=6; 27±9
Results		31±19.5 pg/ml (p<0.0001)	291.4±280.1 pg/ml	Lower than control (p<0.0001))	Lower than control (p<0.0001)
Ps	F; n=30; 40±15 M;n=36;49±18	F; n=23; 41±14 M;n=25;42±15	F; n= 26; 40±14 M;n=30;48±19	F; n=19; 42±16 M;n=21;48±18	F; n=19; 42±16 M;n=21;48±18
Results		22.5±13.7 pg/ml (p<0.0001) †	411.9±280 pg/ml ‡	Lower than control (p<0.0001)	Lower than control (p=0.121)
Control	F; n=22; 49±16 M; n=26; 54±14	F; n=10; 10±5 M; n=9; 9±3	F; n=14; 53±16 M; n=19; 49±19	F; n=22; 52±18 M; n=8; 40±16	F; n=22; 52±18 M; n=8; 40±16
Results		9.7±4.2 pg/ml	257.2±123.8 pg/ml		

*
mean±SD

† For Ps patients with PASI>10 serum CRH levels were statistically significant increased (27.8±10.4, p=0.0001)

‡ For Ps patients with PASI>10 serum VEGF levels were significantly increased (369.8±197.5, p=0.0286)