Acta Derm Venereol (Stockh) 1995; 75: 9–11

Increased Concentration of Beta-endorphin in the Sera of Patients with Severe Atopic Dermatitis

WIESLAW GLINSKI, HALINA BRODECKA, MARIA GLINSKA-FERENZ and DARIUSZ KOWALSKI

Department of Dermatology, Warsaw School of Medicine, Warsaw, Poland

Serum beta-endorphin was measured by radioimmunoassay in 25 patients with atopic dermatitis and 100 healthy subjects. The neuropeptide was found to be markedly ($p < 0.001$) increased in patients with atopic dermatitis (9.2 ± 3.4 pg/ml) as compared to normal controls (6.1 ± 1.5 pg/ml). A correlation between increased serum beta-endorphin concentration and some clinical parameters of the disease has been found. The statistically significant elevation of beta-endorphin was found in patients with widespread atopic dermatitis lesions involving more than 20% of the skin surface (11.1 ± 3.6 pg/ml), a high disease severity score (10.7 ± 3.7 pg/ml), and previous bronchial asthma symptoms (11.6 ± 3.1 pg/ml). A possible explanation of increased beta-endorphin is either its generation in atopic dermatitis lesions by inflammatory cells or activation of pituitary-adrenal axis by psychoneural factors in the mechanism of chronic stress. ***Key words: neuropeptide; opioid peptides; inflammation.***

(Accepted June 17, 1994.)

Acta Derm Venereol (Stockh) 1995; 75: 9–11.

Wieslaw Glinski, Department of Dermatology, Warsaw School of Medicine, 82A Koszykowa Str, 02008 Warsaw, Poland.

Neuropeptides play a role in the pathomechanism of several skin diseases, such as psoriasis, systemic scleroderma and delayed-type cutaneous hypersensitivity reactions (1–5).

In addition to their neurotransmitter (6) and neuroendocrine roles, neuropeptides have mitogenic activities in tissues (7) and can modulate the responses of the immune system (8). Some of them, i.e. substance P (SP), are potent releasers of histamine from mast cells and induce wheal-and-flare reactions, as well as itch, when injected into the skin (9).

Numerous neuropeptides have been identified by immunochemistry in human skin in both primary sensory dermal C fibers and small-size myelinated nerve A-delta endings, i.e. SP, neurokinin A, neurotensin, vasoactive intestinal peptide (VIP), neuropeptide Y and calcitonin gene-related peptide (CGRP) (10–13).

Neuropeptides may serve as a neuromodulatory factor underlying the pathogenesis of atopic dermatitis (AD), since stress exacerbates AD lesions and there is an altered erythemal response of AD skin. A reduction in the flare response to CGRP (14), but not to SP (15), has been demonstrated in patients with AD. Numbers of SP- and NPY-immunoreactive fibers were increased, whereas somatostatin immunoreactive nerves were depleted in lesional skin of atopic eczema (16). CGRP and VIP immunoreactivity did not differ between atopic eczema and normal skin.

There have been no studies on the opiate neuropeptide family in AD, however, they might be of importance as stress-related mediators (17–18).

The purpose of our paper was to study the concentration of beta-endorphin in the sera of patients with AD and healthy subjects. An additional aim was to correlate alterations in beta-endorphin concentrations to disease severity and occurrence of personal and familial atopy.

MATERIALS AND METHODS

Patients

Twenty-five patients suffering from AD (14 males, 11 females, mean age 34 years, range 15–58), and 100 age- and sex-matched healthy volunteers (55 males and 45 females, mean age 33 years, range 17–70, 55 M, 45 F) were studied. Only emollients were applied as topical treatment in a 2-week wash-out period in patients with AD, and they were not given any systemic drugs for at least 3 months.

The patients were divided into 2 subgroups of severe and mild disease by clinical criteria of disease activity described elsewhere (19). Shortly, six clinical features, i.e. erythema, purulence, excoriations or crusting, dryness or scaling, cracking or fissuring, and lichenification, were graded on a scale of 0 (none) to 3 (severe) for 6 defined body sites: hand and neck, anterior and posterior trunk, mid-upper to mid-lower arm (both sides), both hands, mid-thigh and mid-calf (both sides), and both feet. The maximal score was 108, severe cases had a score above 40, whereas mild cases had a score below 36.

The extent of the skin lesions was assessed according to the "rule of nines" (19). It was measured in each defined site by estimating whether none, a third, two-thirds, or all of the area was affected. For instance, with the head and neck, investigators calculated the area affected to give a score 0, 3, 6, and 9%, whereas for the upper extremities – 0, 6, 12, and 18%, etc. The extent of the disease in mild cases did not exceed 20% of the body surface. Further subgrouping of patients was by the co-existence of other atopic diseases and by history of familial atopy. However, they did not have any recent symptoms of asthma, conjunctivitis or rhinitis.

All 25 patients with AD had active skin lesions: severe lesions involved more than 20% of skin surface (10 cases), mild lesions, 10–20% (9 cases), and marginal lesions below 10% of skin area (6 cases).

Patients were asked about the presence of pruritis accompanying recent relapse of AD lesions and its intensity on the scale 1 to 10, and signs of itching, such as scratches and erosions, were noted during examination. Sixteen patients had intensive pruritus, whereas 9 patients suffered from less intensive itching although it was much more pronounced than in AD patients with itching.

Determination of serum beta-endorphin

The concentration of beta-endorphin in sera was measured by radioimmunoassay with the use of the NEN 125 I-RIA kit for plasma beta-endorphin. This test is based on the modification of Carr's method (20). Briefly, the procedure was performed at 4°C, serum was incubated with rabbit beta-endorphin antibodies for 20 h, and then 125 I-beta-endorphin was added for an additional period of 20 h. Fractions containing either bound or free 125 I-beta-endorphin were separated on activated carbon particles by centrifugation at 40°C for 15 min.

The results were read from a standard curve which was plotted on the basis of analysis of control sera containing known concentrations of beta-endorphin ranging from 5 to 1000 pg per 1 ml. The radioactivity of the samples was determined in the LKB – Wallac counter integrated with the MERA-CAMAC computer system.

© 1995 Scandinavian University Press. ISSN 0001-5555

Table I. *Serum beta-endorphin concentration in patients with atopic dermatitis and in healthy individuals*

Group (*n*)	Serum beta-endorphin (pg/ml)			*t*-test* *p*-value versus normal	% cases with increase**
	mean	SD	range		
Atopic dermatitis (25)	9.2	3.4	4.8–17.1	0.001	40%
Healthy subjects (100)	6.1	1.5	3.7–11.7	–	–

* statistical comparison by Student's t-test
** more than 2 SD above mean concentration in healthy subjects

Statistics was performed by Student's *t*-test, and determinations of the serum beta-endorphin concentration were performed on the same day for both the patients and the healthy volunteers of similar age and sex distribution.

RESULTS

The mean serum concentration of beta-endorphin in patients with AD (9.2 ± 3.4 pg/ml) was increased as compared to that of normal controls (6.1 ± 1.5 pg/ml). Forty percent of the cases had increased levels of beta-endorphin more than 2 SD above the mean serum neuropeptide concentration in healthy controls (Table I).

The values found in patients with more than 20% skin surface involved (11.1 ± 3.6 pg/ml) were much higher than those in patients with lower skin involvement, i.e. 10–20% (9.2 ± 2.8 pg/ml) and below 10% of body surface (6.0 ± 0.7 pg/ml). The increase in beta-endorphin was restricted also to the group of patients with severe disease (10.7 ± 3.7 pg/ml). In cases with a rather mild course of the disease the neuropeptide concentration (8.2 ± 2.5 pg/ml) was only slightly elevated as compared to controls. (Fig. 1).

There was no relationship of neuropeptide concentration to the grade of pruritus, grade 2–5 and grade 6–10, or to familial occurrence of atopy, nor to other symptoms of atopy besides bronchial asthma (11.6 ± 3.1 pg/ml).

DISCUSSION

The mean concentration of beta-endorphin in AD sera was about 50% increased as compared to normal controls. The highest concentration of beta-endorphin was found in the sera of patients with severe exacerbated active lesions (10.7 +3.7 pg/ml), and those with more than 20% of body surface (11.1 +3.6 pg/ml). It is likely that increase in beta-endorphin is related to phenomena occurring in AD lesional skin, i.e. it is generated by a lymphocytic infiltrate within the skin and/or by circulating lymphocytes when they recirculate. This peptide might be produced also in peripheral tissues, i.e. skin, since it is released during the inflammation processes from cells such as lymphocytes, macrophages, monocytes and plasmocytes (21).

$CD4^+$ T lymphocytes, in addition to eosinophils, are characteristic constituents of the late phase reactions to intracutaneous allergen challenge, and abnormalities in non-specific peripheral blood T cell responsiveness and lymphocyte subpopulations have been demonstrated in patients with AD (22, 23). Furthermore, the chronic skin lesions of AD contain perivascular dermal cell infiltrates comprising activated $CD4^+$ T lymphocytes and potential antigen-presenting cells with $CD1^+$ (dendritic cells) and $CD14^+$ (macrophages) surface markers (24, 25).

The role of T cells in the pathomechanism of AD has become clearer since recent findings pointed out that $CD4^+$ T cells

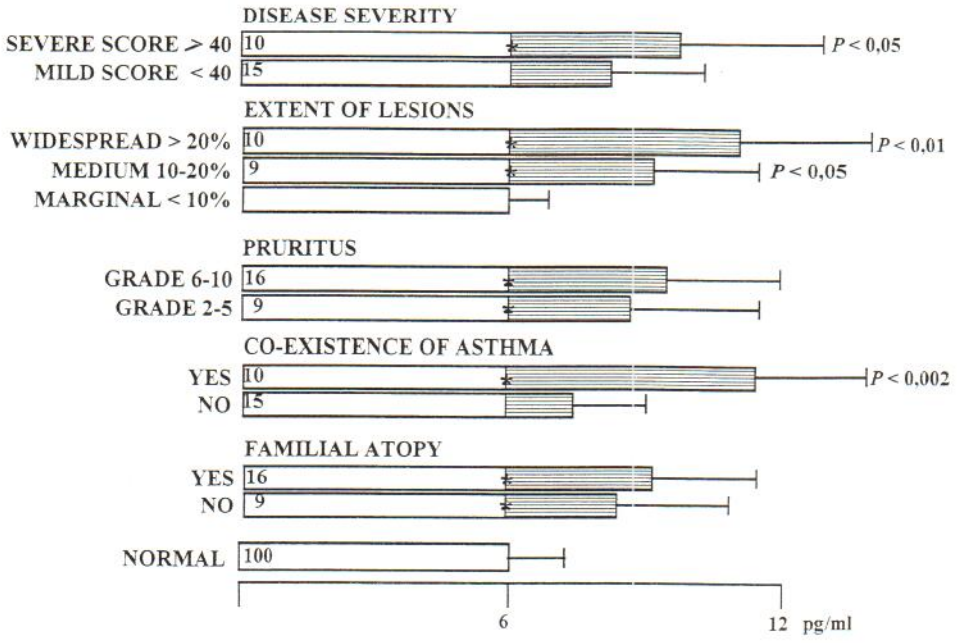

Fig. 1. The relationship between serum concentration of beta-endorphin (mean + SD) and clinical manifestations of the disease. Statistical comparison (*t*-test) of the difference between the group with indicated *p*-value and the group with no symptom or the lowest clinical parameter. Star indicates the difference compared to normal controls; *p*-value of at least $p < 0.05$.

cloned from AD dermis are allergen-specific (26) and that these T cell clones have the cytokine production profile of Th2 cells (27). These cells recognize inhalatory and food allergens in the dermis, become activated and mediate cytotoxic reactions in response to these antigens (26, 27), and in addition promote IgE production (28).

The inflammatory cells in AD dermal infiltrates are a possible source of beta-endorphin (21). This skin-derived beta-endorphin might diffuse to the plasma, and/or lymphocytes upon recirculation from the skin lesions to peripheral blood might still secrete this peptide to the plasma.

It is likely that in patients with AD, beta-endorphin is released in response to some stress-generating factor. Beta-endorphin, that belongs to the endogenous opiate family, is a neuropeptide known to be generated upon stimulation of the pituitary – adrenal axis after stress (29, 30). It exerts an anti-nociceptive effect upon binding to specific receptors on the peripheral nerve endings (21, 30) and induces an immunomodulation of the lymphocyte response (10, 11). Of special interest is the relationship between beta-endorphin concentration and the existence of bronchial asthma in anamnesis even though the patients have neither shown any recent symptoms of asthma nor received any anti-asthmatic drugs.

The presence of high amounts of beta-endorphin in AD lesions might presumably affect peripheral sensory nerve function. Beta-endorphin is bound probably to its specific receptors on the peripheral nerves, inducing an anti-nociceptive effect (21, 31, 32). The transmission of sensory stimuli is inhibited, which may account for the impaired induction in AD of neurogenic erythemal response by CGRP (14).

REFERENCES

1. Wallengren J. Substance P antagonist inhibits immediate and delayed type cutaneous hypersensitivity reactions. Br J Dermatol 1991; 124: 324–328.
2. Eedy DJ, Johnston CF, Shaw C, Buchanan KD. Neuropeptides in psoriasis: an immunocytochemical and radioimmunoassay study. J Invest Dermatol 1991; 96: 434–438.
3. Molina FA, Burrows NP, Russel Jones R, Terenghi G, Polak JM. Increased sensory neuropeptides in nodular prurigo nodularis: a quantitative immunohistochemical analysis. Br J Dermatol 1992; 127: 344–351.
4. Terenghi G, Bunker CB, Liu YF, Springall DR, Cowen T, Dowd PM, et al. Image analysis quantification of peptide-immunoreactive nerves in the skin of patients with Raynaud's phenomenon and systemic sclerosis. J Pathol 1991; 164: 245–252.
5. Karanth SS, Springall DR, Lucas S, Levy D, Ashby Levene MM. Changes in nerves and neuropeptides in skin from 100 leprosy patients investigated by immunocytochemistry. J Pathol 1989; 157: 15–26.
6. Barker JL. Peptides: roles in neuronal excitability. Physiol Rev 1976; 56: 435–452.
7. Nillson J, von Euler AM, Dalsgaard C-J. Stimulation of connective tissue cell growth by substance P and substance K. Nature 1985; 315: 61–63.
8. Wybran J. Enkephalins and endorphins as modifiers of the immune system: present and future. Fed Proc 1985; 44: 92–96.
9. Brain SD, Tippins JR, Morris HR, McIntyre I, Williams TJ. Potent vasodilator activity of calcitonin gene-related peptide in human skin. J Invest Dermatol 1986; 87: 533–536.
10. Bjorklund H, Dahlsgaard CJ, Jonasson CE, Herma H, Dahlsgaard CJ, Jonasson CE, et al. Sensory and autonomic innervation of non-hairy skin. An immunohistochemical study. Cell Tissue Res 1986; 43: 51–57.
11. Gibbins IL, Wattehow D, Coventry B. Two immunohistochemical identified populations of calcitonin gene-related peptide (CGRP)-immunorective axons in human skin. Brain Res 1987; 4: 143–148.
12. Tainio H, Vaalasti A, Rechardt L. The distribution of substance P-, CGRP-, galanin-, and ANP-like immunoreactivity nerves in human sweat gland. Histochem J 1987; 87: 375–380.
13. Wallengren J, Ekman R, Sundler F. Occurrence and distribution of neuropeptides in the human skin. Acta Derm Venereol (Stockh) 1987; 67: 185–192.
14. Giannetti A, Girolomoni G. Skin reactivity to neuropeptides in atopic dermatitis. Br J Dermatol 1989; 121: 681–688.
15. Coulson IH, Holden CA. Cutaneous reactions to substance P and histamine in atopic dermatitis. Br J Dermatol 1990; 122: 343–349.
16. Pincelli C, Fantini F, Massini P Girolomoni G, Seidenari S, Gianetti A. Neuropeptides in skin from patients with atopic dermatitis: an immunological study. Br J Dermatol 1990; 122: 745–750.
17. Madden J. Stress induced parallel changes in central opioid levels and pain responsiveness in the rat. Nature 1977; 265: 358.
18. Levis IW, Tordof MG, Shernan IE, Liebesking IC. Adrenal medullary enkefalin-like peptides may mediate stress analgesia. Science 1982; 217: 557–559.
19. Sowden JM, Berth-Jones J, Ross JS, Motley RJ, Marks R, Finlay AJ, et al. Double-blind, controlled cross over study of cyclosporin in adults with severe refractory atopic dermatitis. Lancet 1991; 338: 137–140.
20. Carr DR, Bullen BA, Skrinar GS, Arnold MA, Rosenblatt M, Beitens IZ, et al. Physical conditioning facilitates the exercise induced secretion of beta-endorphin and beta-lipotropin in women. N Engl J Med 1983; 305: 560–563.
21. Stein C, Ahmed HS, Hassan F, Przewlocki R, Gramsch C, Peter K, et al. Opioids from immunocytes interact with receptors on sensory nerves to inhibit nociception in inflammation. Proc Natl Acad Sci USA 1990; 87: 5935–5939.
22. Zachary CB, MacDonald DM. Quantitative analysis of lymphocyte subsets in atopic eczema using monoclonal antibodies and flow cytofluorimetry. J Invest Dermatol 1983; 108: 411–422.
23. Horsmanheimo M, Horsmanheimo A, Banov CH, Ainsworth SK, Fudenberg HH. Cell-mediated immunity in vitro in atopic dermatitis. Arch Dermatol 1979; 115: 161–164.
24. Zachery CB, Allen MH, MacDonald DM. In situ quantification of T lymphocyte subsets and Langerhans cells in the inflammatory infiltrate of atopic eczema. Br J Dermatol 1985; 112: 149–156.
25. Sillevis Smitt JH, Bos JD, Hulsebosh HJ, Krieg SR. In situ immunophenotyping of antigen-presenting cells and T cell subsets in atopic dermatitis. Clin Exp Dermatol 1986; 11: 159–168.
26. Rawle FC, Mitchell EB, Platts-Mills TAE. T cell responses to the major allergen from house dust mite Dermatophagoides pteronyssinus antigen P1: comparison of patients with asthma, atopic dermatitis and perennial rhinitis. J Immunol 1984; 133: 195–201.
27. Kimura JY, Ohta N, Ishii A, Nagano T, Usui M. Functional characterization of lymphocyte responses to fractioned house dust mite antigens (Dermatophagoides pteronyssinus) in atopic and non-atopic individuals. Immunology 1990; 70: 385–390.
28. Kapsenberg ML,Wierenga EA, van der Heijen FL, Bos JD. Atopic dermatitis and $CD4^+$ allergen-specific Th2 lymphocytes. Eur J Dermatol 1992; 2: 601–607.
29. Morley JE. The endocrinology of the opiates and opioid peptides. Metabolism 1981; 30: 195.
30. Olson GA, Olson RD, Kastin AJ. Endogenous opiates: 1985. Peptides 1986; 7: 907–933.
31. Monroe EW. Efficacy and safety of nalmefane in patients with severe pruritus caused by chronic urticaria and atopic dermatitis. J Acad Dermatol 1989; 2: 135–136.
32. Harrison PV. Nalmefen and pruritus (letter). J Am Acad Dermatol 1990; 23: 530.