Clin. exp. Immunol. (1992) **87**, 178–182

Decreased immunoreactive beta-endorphin in mononuclear leucocytes from patients with rheumatic diseases

C. J. WIEDERMANN, P. SACERDOTE*, E. MUR, U. KINIGADNER, T. WICKER, A. E. PANERAI* & H. BRAUNSTEINER *Department of Internal Medicine, School of Medicine, University of Innsbruck, Innsbruck, Austria, and *Department of Pharmacology, School of Medicine, University of Milan, Milan, Italy*

(Accepted for publication 23 August 1991)

SUMMARY

The neuroendocrine polypeptide hormone beta-endorphin (β-END), which is released from various tissues including the anterior pituitary gland and cells of the immune system, has recently been implicated as having an immunoregulatory role. We used a radioimmunoassay to measure β-END levels in circulating mononuclear leucocytes from normal subjects and patients with various rheumatic diseases. Levels of β-END in leucocytes from patients were lower than in leucocytes from healthy subjects ($P < 0.001$). Whereas levels of β-END in leucocytes from patients with the various rheumatic disorders were not significantly different, an inverse correlation was found between β-END levels in leucocytes and expression of rheumatoid factor ($P < 0.025$) and erythrocyte sedimentation rate ($P < 0.025$). This study demonstrates decreased content of β-END in cells of the immune system related to parameters of inflammatory activity in rheumatic diseases.

Keywords Rheumatic diseases β-endorphin leucocytes

INTRODUCTION

Beta-endorphin (β-END), a polypeptide hormone involved in the regulation of pain experience, is derived from the precursor pro-opiomelanocortin (POMC), and is released primarily from the anterior pituitary gland in response to stress [1]. Furthermore, several studies have implied that POMC is produced in a number of other tissues, including immunological cells of blood, spleen, lymph nodes and mucosa-associated lymphoid tissues [2]. In lymphocytes, the augmentation of POMC (and β-END) by viral infection, or upon stimulation by endotoxin, corticotropin-releasing factor, IL-1 or catecholamines provides strong evidence that the hormone originates from cells of the immune system [2]. Endogenous opioids are probably involved not only in neuromodulation but also in immunomodulation via activation of both specific opioid and non-opioid receptors expressed on the surface of several types of leucocytes [2]. Opiatergic immunomodulation may not be exclusively ascribed to products of POMC released from the pituitary gland, since in some animal studies stimulation of pituitary opioid peptide release did not cause changes in immune functions [3]. It has been suggested that opiatergic immunomodulation *in vivo* is brought about locally by opioid peptides released from lymphocytes in a paracrine fashion, by a mechanism comparable to other lymphokines [4].

Human synovium is richly innervated by autonomic and sensory nerve fibres, and immunoregulatory neuropeptides have been discussed as being involved in the regulation of inflammatory and possibly protective reactions occurring in damaged joints when released locally [5,6]. In patients with various rheumatic diseases, or with rheumatoid arthritis, decreased levels of serum β-END have been demonstrated [7,8], and were related to chronic pain of arthritis, which in turn leads to depletion of central nervous system β-END [9]. It is not known whether β-END has an immunological role to play in patients with rheumatic diseases. In one study, elevation of β-END serum levels by physical exercise was observed to correlate with improved functional capacity of patients with rheumatoid arthritis, without affecting immunological parameters of disease activity, such as erythrocyte sedimentation rate (ESR) [10]. As seen in animal studies, such modulation of plasma levels of pituitary β-END (released by exercise) does not necessarily affect immunological parameters.

In vitro there is strong evidence for the involvement of β-END in the regulation of immune functions [2]. Therefore, the present study was undertaken to examine the levels of β-END occurring in peripheral blood mononuclear leucocytes (PBMC) from healthy subjects and patients with various rheumatic diseases and to see if any correlation can be found between these levels and immunological parameters in the patient group.

Correspondence: Christian J. Wiedermann, MD, Department of Internal Medicine, University of Innsbruck, Anichstraße 35, A-6020 Innsbruck, Austria.

Table 1. Clinical data and β-endorphin (β-END) content of circulating blood mononuclear cells (PBMC) from patients with rheumatic diseases

Diagnosis	Number of patients M/F†	Age (years) (range)	ESR (mm after 1 h)	CRP (mg/dl)	RF (quant.*) (U/ml)	RF (qual.*) (% positive)	β-END (pg/10^6 MNC)
Osteoarthritis	22 10/12	59·1 ± 3·01‡ (25–85)	11·3 ± 1·82	0·06 ± 0·02	101 ± 47·1	13·6	11·9 ± 3·33
Rheumatoid arthritis	49 16/33	57·9 ± 1·73 (34–84)	34·4 ± 3·32	2·8 ± 0·45	260 ± 62·3	61·2	8·6 ± 1·34
Reactive arthritis	8 5/3	35·6 ± 5·07 (18–63)	37·1 ± 7·72	4·2 ± 1·00	30 ± 0·2	12·5	5·0 ± 4·01
Polymyalgia rheumatica	6 2/4	63·2 ± 5·36 (49–83)	37·7 ± 12·51	3·2 ± 0·98	79·7 ± 49·70	16·7	9·3 ± 3·99
Ankylosing spondylitis	5 4/1	39·4 ± 8·02 (20–66)	26·0 ± 8·81	3·0 ± 1·25	30·0 ± 0§	0	31·8 ± 13·66
Psoriatic arthritis	2 2/0	55·5 ± 16·50 (39–72)	36·0 ± 10·00	1·6 ± 0·96	30·0 ± 0§	0	11·0 ± 1·00
Fibrositis	2 1/1	46·0 ± 10·00 (36–46)	5·5 ± 0·50	0·6 ± 0	30·0 ± 0§	0	11·0 ± 1·00
Multiple group comparison¶	—	$P = 0·0051$	$P = 0·0001$	$P = 0·0001$	$P = 0·0008$	—	$P = 0·0754$

* Agglutination procedures that employ latex particles for detection of rheumatoid factors (RF) (see Patients and Methods).

† M, male; F, female.

‡ Mean ± s.e.m.

§ All patients had serum levels of RF (latex) of ≤ 30 U/ml (detection limit).

¶ Kruskal–Wallis analysis for non-parametric samples.

ESR, erythrocyte sedimentation rate; CRP, C-reactive protein.

PATIENTS AND METHODS

Subjects

Ninety-four patients seen in the rheumatology out-patient department and from whom verbal consent was obtained participated in the study. The arthritis patients were categorized into five clinical groups: osteoarthritis, rheumatoid arthritis, reactive arthritis, ankylosing spondylitis and psoriatic arthritis. Additionally, six patients with polymyalgia rheumatica, and two patients with non-inflammatory diffuse musculoskeletal pain were included. Clinical data of the patients are given in Table 1. Patients were treated with non-steroidal anti-inflammatory drugs. Physical and occupational therapy and assistive devices were provided when necessary. Some patients with mild-to-moderate rheumatoid arthritis received auranofin. Patients on treatment with anti-malarial drugs, penicillamine, methotrexate, systemic steroids or other immunosuppressive agents were not included. Routine laboratory tests included ESR (Westergren), qualitative rheumatoid factor (RF) latex test (Gamma Biologicals, Houston, TX), and nephelometric quantifications of RF and C-reactive protein (CRP) (Beckman, Brea, CA). Data on pain levels and duration of the disease were not available for analysis. A total of 55 healthy volunteers (20 women, 35 men) of comparable age (mean ± s.e.m. 40·8 ± 16·06 years; range 22–70 years) served as controls. Routine laboratory tests on the control subjects were not performed.

Cell preparation

From all subjects after an overnight fast, 15 ml of forearm venous blood were drawn into a heparinized syringe between 08.00 a.m. and 11.00 a.m. Samples were processed immediately. PBMC were isolated by Ficoll–Paque (Pharmacia, Uppsala, Sweden) density gradient centrifugation, as described previously [11]. Cells were washed twice in Hank's balanced salt solution (GIBCO, Eggenstein, Germany). Aprotinine (10 μl) at a concentration of 20 000 kallikrein-inactivating U/ml (Bayer, Leverkusen, Germany) was added to pellets of 10^7 MNC in order to inhibit proteolytic degradation of the peptide hormones during the further processing of samples. Cells were then stored at −70°C for 2 weeks to 4 months.

Determination of immunoreactive β-END

After collection of samples had been completed, cells were suspended in 1 ml 0·1 mol/*l* acetic acid, homogenized in a blade homogenizer, sonicated, and centrifuged for 10 min at 10 000 ***g***. The supernatant was collected, and β-END was measured by radioimmunoassay and validated as previously described [12]. Specific antibodies were obtained against bovine serum albumin (BSA) conjugates of human β-END [12]. Briefly, 0·1 ml of PBMC supernatant was added to 0·2 ml of phosphosaline buffer containing BSA, the antiserum at an initial dilution of 1/12 000, and the tracer; tubes were incubated for 24 h, and free radioactive iodine counted after centrifugation. Assay sensitivity was 10 pg/tube and intra-assay and interassay variation coefficients were 8% and 11%, respectively. HPLC analysis of the molecular forms of immunoreactive (ir) β-END in human peripheral blood lymphocytes from healthy subjects revealed the presence of N-acetyl-β-END, with a ratio of native β-END to N-acetyl-β-END ranging from 1 to 2 [11].

RESULTS

Analysis of irβ-END levels in PBMC disclosed a range of concentrations between 0 and 78 pg/10^6 cells. In the healthy

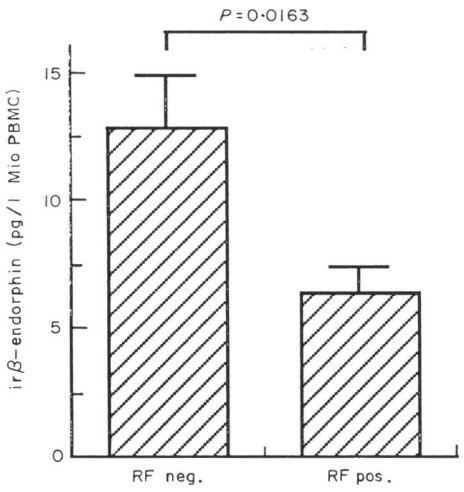

Fig. 1. Immunoreactive β-endorphin concentrations in peripheral blood mononuclear leucocytes from patients with rheumatic diseases who are negative ($n=59$) or positive ($n=35$) for rheumatoid factor as measured in the qualitative rheumatoid factor (RF) latex test. Mean ± s.e.m.; P = two-tail probability, unpaired Student's t-test.

Table 2. Correlation between β-endorphin (β-END) content of peripheral blood mononuclear cells (PBMC) from patients with rheumatic diseases and clinical data

β-END (ng/10^6 PBMC) *versus*	Number of patients	Correlation	Probability*
Age (years)	94	$r=-0{\cdot}164$	NS
ESR after 1 h (mm)	94	$r=-0{\cdot}178$	$P<0{\cdot}025$
CRP (mg/dl)	94	$r=-0{\cdot}104$	NS
RF quant. (U/ml)	94	$r=-0{\cdot}182$	$P<0{\cdot}025$
Leucocytes (G/l)	93	$r=-0{\cdot}094$	NS
Haemoglobin (g/dl)	91	$r=0{\cdot}075$	NS
Hematocrit (%)	90	$r=-0{\cdot}091$	NS
Platelets (G/l)	90	$r=-0{\cdot}088$	NS
Lymphocytes (G/l)	87	$r=0{\cdot}046$	NS
Monocytes (G/l)	87	$r=-0{\cdot}156$	NS

* Probability (two-tailed) calculation of significant correlations was performed according to Glass & Stanley [24].

NS, Not significant ($P>0{\cdot}05$).

ESR, erythrocyte sedimentation rate; CRP, C-reactiveprotein; RF, rheumatoid factor.

subjects ($n=55$) a mean ± s.e.m. concentration of irβ-END of $34{\cdot}9 \pm 2{\cdot}33$ pg/10^6 PBMC was found, whereas in the patients with rheumatic disorders ($n=94$) a mean ± s.e.m. concentration of irβ-END of $10{\cdot}4 \pm 1{\cdot}32$ pg/10^6 PBMC was found (the difference is statistically significant: two-tailed, unpaired t-test; $P<0{\cdot}001$). Table 1 shows the mean ± s.e.m. concentrations in the different clinical groups of patients with rheumatic disorders. Only in the five patients with ankylosing spondylitis did irβ-END levels appear to be in the range found in healthy subjects. However, differences among the clinical groups were not statistically significant. Comparable concentrations of irβ-END were found in the osteoarthritis and rheumatoid arthritis

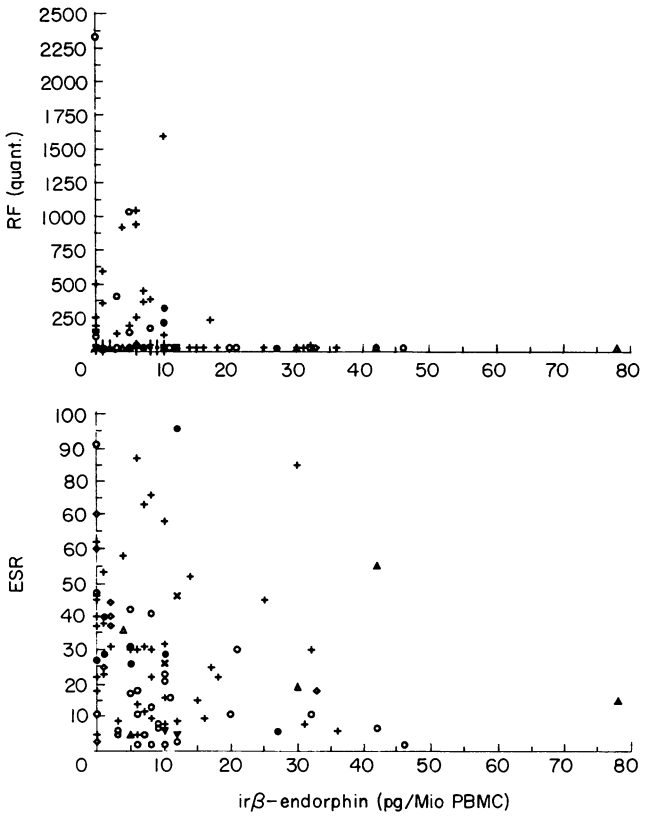

Fig. 2. Scattergrams of immunoreactive β-endorphin concentrations in peripheral blood mononuclear leucocytes from patients with various rheumatic diseases against erythrocyte sedimentation rates (ESR, mm after 1 h) and rheumatoid factor levels (RF, U/ml); $n=94$. ○, Osteoarthritis; △, ankylosing spondylitis; +, rheumatoid arthritis; ◇, reactive arthritis; ●, polymyalgia rheumatica; ×, psoriatic arthritis; ▼, fibrositis.

groups. Statistical evaluation of the data revealed significantly lower levels of irβ-END in patients positive for RF as measured in the qualitative test for RF (Fig. 1). Inverse correlations were also found between irβ-END in PBMC and quantitative measures of RF and ESR; a lack of correlation of irβ-END to other clinical parameters was, however, observed, including CRP, haemoglobin, platelets and peripheral blood lymphocytes (Table 2, Fig. 2).

DISCUSSION

The majority of patients studied were found to have detectable levels of irβ-END in PBMC, in a range significantly lower than the levels of irβ-END in PBMC from age-matched healthy humans. The measurement of irβ-END in circulating leucocytes is of considerable interest, since most of the evidence for irβ-END in leucocytes so far stems from experiments on *in vitro* stimulated lymphocytes [2]. In the present study, similar decreased levels of irβ-END have been found in PBMC from patients with different rheumatic diseases, in particular osteoarthritis and rheumatoid arthritis. As the pathogenesis of osteoarthritis and rheumatoid arthritis is characterized by different mechanisms, the data suggest that decreased irβ-END levels in PBMC are not directly related to the immunological mechanisms of rheumatoid arthritis. Whether PBMC from

patients with ankylosing spondylitis contain more irβ-END than PBMC from patients with other rheumatic diseases, will require a larger number of cases for adequate study.

Significant changes of irβ-END have been found in PBMC from patients with elevated ESR and RF levels. Elevation of these two parameters indicates increased inflammatory disease activity, including increased levels of local and circulating cytokines/lymphokines [13] and activation of T and B lymphocytes [14]. Therefore, decreased content of irβ-END in PBMC from patients with elevated ESR and RF levels may reflect release of β-END by a secretagogue action of IL-1 [15] or activation of lymphocytes [16]. Last but not least, lymphocytes from patients with rheumatic diseases may synthesize decreased amounts of opioid peptides. If β-END is released from PBMC by immunocyte activation *in vivo*, it may play an immunoregulatory role in patients with rheumatic diseases. This view is supported by *in vitro* studies where β-END caused activation of T lymphocytes and inhibition of antibody production by β-lymphocytes [17,18]. Furthermore, decreased opioids from immunocytes may be related to increased pain perception in these patients via mechanisms involving peripheral sensory nerves [19].

Decreased levels of β-END detected in the cerebrospinal fluid of patients suffering from chronic arthritis or chronic low back pain [20] led to the findings in chronic pain models that peripheral lesions can induce important changes in brain concentrations of opioid peptides involved in the modulation of pain [9]. Similar changes of opioid peptide levels in the central nervous system may be induced by neuropharmacological treatment of experimental animals [21,22]. Recent investigations revealed that changes of β-END levels produced by neuropharmacological compounds such as the serotonin receptor antagonist metergoline, the tricyclic anti-depressant chlorimipramine, or the GABA agonist sodium valproate, are similar in both the central nervous system and PBMC, which suggests that similar control mechanisms may exist for nervous system and immune system derived β-END [11]. Hence, decreased levels of irβ-END found in PBMC from rheumatic patients, including those with disorders where elevated indicators of inflammatory disease activity are present, may reflect an increase in pain experience without having a causal relation to immunological disease activity [23]. The two fibrositis patients, who do not have an activation of the immune system, also had low levels of irβ-END in PBMC, which may be related to the pain present in these patients [19,23]. Thus, degree of inflammation and/or pain level might be more important than RF. This view is supported by the findings of similar levels of irβ-END in PBMC from both patients with osteoarthritis and rheumatoid arthritis. Therefore, the role of pain in regulating opioid peptide levels in immunocytes will be studied in patients with non-rheumatic pain and in patients with immunological diseases presenting without pain. Results of such future studies will also clarify why low levels of irβ-END in PBMC did not correlate with other indicators of inflammation in rheumatic diseases, including white blood cell count and level of CRP.

The demonstration of differential levels of the neuroendocrine hormone β-END in PBMC from patients with inflammatory diseases may lend further support to the suggestion that the nervous system and the immune system are linked in the regulation of inflammatory processes in rheumatic diseases [5,6]. New experimental protocols for exploring the role of β-END and other opioid agonists and antagonists in rheumatic diseases with immune system dysfunction need to be designed. A better understanding of the mechanisms by which pain, opioid peptides and other neuropeptides may interact with the immune system can be expected to contribute significantly towards effective therapeutic strategies for the treatment of various rheumatic diseases.

ACKNOWLEDGMENTS

The study was supported by the Austrian Science Funds, grant 7476-Med to C.J.W.

REFERENCES

1 Terenius L. Endogenous peptides and analgesia. Ann Rev Pharmacol Toxicol 1978; **18**: 189–204.
2 Heijnen C, Kavelaars A, Ballieux RE. β-Endorphin: cytokine and neuropeptide. Immunol Rev 1991; **119**:41–63.
3 Flores CM, Hernandez MC, Hargreaves KM *et al.* Restraint stress-induced elevations in plasma corticosterone and β-endorphin are not accompanied by alterations in immune function. J Neuroimmunol 1990; **28**:219–25.
4 Teschemacher H, Koch G, Scheffler H *et al.* Opioid peptides. Immunological significance? Ann NY Acad Sci 1990; **594**:66–77.
5 Levine JD, Clark R, Devor M *et al.* Intraneuronal substance P contributes to the severity of experimental arthritis. Science 1984; **226**:547–9.
6 Kidd BL, Gibson SJ, O'Higgins F *et al.* A neurogenic mechanism for symmetrical arthritis. Lancet 1989; **ii**:1128–30.
7 Denko CW, Aponte J, Gabriel P *et al.* Serum beta-endorphin in rheumatic disorders. J Rheumatol 1982; **9**:827–33.
8 Editorial. Beta-endorphin levels lower in arthritis patients. JAMA 1981; **246**:203.
9 Panerai AE, Sacerdote P, Bianchi M *et al.* Brain and spinal cord neuropeptides in adjuvant induced arthritis in rats. Life Sci 1987; **41**:1297–303.
10 Ekdahl C, Ekman R, Andersson SI *et al.* Dynamic training and circulating levels of corticotropin-releasing factor, beta-lipotropin and beta-endorphin in rheumatoid arthritis. Pain 1990; **40**:35–42.
11 Sacerdote P, Rubboli F, Locatelli L *et al.* Pharmacological modulation of neuropeptides in peripheral mononuclear cells. J Neuroimmunol 1991; **32**:35–41.
12 Panerai AE, Martini A, Di Giulio AM *et al.* Plasma β-endorphin, β-lipotropin and met-enkephalin concentrations during pregnancy in normal and drug addicted women and their newborn. J Clin Endocrinol Metab 1983; **57**:537–42.
13 Duff G. Peptide regulatory factors in non-malignant disease. Lancet 1989; **i**:432–5.
14 Cush JJ, Lipsky PE, Postlethwaite AE *et al.* Correlation of serologic indicators of inflammation with effectiveness of nonsteroidal antiinflammatory drug therapy in rheumatoid arthritis. Arthritis Rheum 1990; **33**:19–28.
15 Kavelaars A, Ballieux RE, Heijnen CJ. Beta-endorphin secretion by human peripheral blood mononuclear cells: regulation by glucocorticoids. Life Sci 1990; **46**:1233–40.
16 Kavelaars A, Ballieux RE, Heijnen CJ. *In vitro* beta-adrenergic stimulation of lymphocytes induces the release of immunoreactive beta-endorphin. Endocrinology 1990; **126**:3028–32.
17 Hemmick LM, Bidlack JM. β-Endorphin stimulates rat T lymphocyte proliferation. J Neuroimmunol 1990; **29**:239–48.
18 Morgan EL, McClurg MR, Janda JA. Suppression of human B lymphocyte activation by β-endorphin. J Neuroimmunol 1990; **28**:209–17.
19 Stein C, Hassan AHS, Przewlocki R *et al.* Opioids from immunocytes interact with receptors on sensory nerves to inhibit nociception in inflammation. Proc Natl Acad Sci USA 1990; **87**:5935–9.

20 Almay BGL, Johansson F, von Knorring L *et al.* Endorphins in chronic pain. I. Differences in CSF endorphin levels between organic and psychogenic pain syndromes. Pain 1978; **5**:153–62.

21 Locatelli V, Petraglia F, Penalva A *et al.* Effect of dopaminergic drugs on hypothalamic and pituitary immunoreactive beta-endorphin concentrations in the rat. Life Sci 1983; **33**:1711–7.

22 Martini A, Sacerdote P, Mantegazza P *et al.* Antiepileptic drugs affect hypothalamic β-endorphin concentrations. J Neurochem 1984; **43**:871–3.

23 Kazis LE, Meenan RF, Anderson JJ. Pain in the rheumatic diseases: investigation of a key health status component. Arthritis Rheum 1983; **26**:1017–22.

24 Glass GV, Stanley JC. *Statistical Methods in Education and Psychology*. Englewood Cliffs, Prentice Hall, 1970:521.