ORIGINAL CONTRIBUTION

β-Endorphin Concentrations in Peripheral Blood Mononuclear Cells of Patients With Multiple Sclerosis

Effects of Treatment With Interferon Beta

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Context: It has been reported that the opioid peptide β-endorphin (BE) has immunosuppressive effects. Interferon beta (IFN-β) is a well-established therapy for multiple sclerosis (MS), but immunological mechanisms underlying its beneficial effects in MS are partially undefined.

Objectives: To determine BE levels in peripheral blood mononuclear cells (PBMCs) of patients with relapsing-remitting MS during different phases of disease activity and the possible modulating effects of IFN-β treatment on PBMC BE synthesis in patients with MS.

Design: We measured BE levels in blood samples collected from 6 patients with MS who had not experienced clinical changes during the previous 3 months (patients with stable MS) and from 7 patients with MS during a clinical relapse. We also surveyed BE levels in PBMC samples from 8 patients with MS before treatment and for 6 months after the beginning of IFN-β administration. The control group was 13 healthy subjects.

Results: Low PBMC BE levels were detected in patients with stable MS and in those entering IFN-β treatment compared with control subjects. Increased BE concentrations were observed in MS patients experiencing a clinical relapse compared with patients with stable MS. During IFN-β treatment, the levels of BE in PBMC samples from patients with MS increased significantly (after 1 month, $P = .02$; after 3 months, $P = .007$; and after 6 months, $P = .16$).

Conclusions: A reduction of BE levels was present in patients with clinically inactive MS. Treatment with IFN-β seems to induce an increase of this opioid in PBMCs of MS patients. The increase of BE concentration during a clinical relapse may represent a possible control mechanism aimed at counterbalancing the inflammatory phase of the disease.

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MULTIPLE sclerosis (MS) is the most common immune-mediated demyelinating disease of the central nervous system (CNS) affecting young adults. It is currently believed that the immune system may be involved primarily (ie, coordinated antigen-specific attack on myelin) or secondarily (ie, bystander, nonspecific immune activation) in mediating tissue injury in MS. Systemic recombinant interferon beta (IFN-β) administration was the first approved treatment for MS. The effectiveness provided by IFN-β in reducing the relapse rate in MS is likely because of its immunomodulatory effects. Its mechanism(s) of action remains to be completely elucidated, but the more convincing studies demonstrate an inhibitory effect of this cytokine on blood-brain barrier permeability to immune cells, possibly through decrease of metalloprotease activity and diminished expression of adhesion molecules.[1,2] Effects of IFN-β on immune cells in MS have been variably reported, the only undebated result being the increase of secretion of interleukin 10 (IL-10), a cytokine with an anti-inflammatory profile.[3]

There is growing evidence suggesting a tight cross-regulation between the neuroendocrine and immune systems. β-Endorphin (BE) is an opioid peptide known for its effect on modulation of pain, food assumption, endocrine secretion, and recently, for its immunomodulating effects.[4] The arcuate nucleus of the hypothalamus and the intermediate pituitary gland are the main sources of BE.[5,6] More recently, the synthesis of this opioid has been demonstrated even in the cells of the immune system, that is, by lymphocytes, thymocytes, monocytes, and splenocytes.[7,8] The production of BE in the CNS and immune cells has similar features: it is under inhibitory tonic control by dopamine and γ-aminobutyric acid and stimulatory tonic control by 5-hydroxytryptamine.[4,9]

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PATIENTS AND METHODS

PATIENTS

Twenty-one subjects (7 male and 14 female) with clinically definite relapsing-remitting MS, according to the criteria of Poser et al[16] were studied. At the time of study entry, patients were classified into 3 groups.

Clinically stable patients included 6 MS patients (3 male and 3 female) with a mean ± SD age of 29.5 ± 5.7 years; mean ± SD Expanded Disability Status Scale (EDSS) score of 2.4 ± 1.6; and mean ± SD duration of disease of 10.6 ± 5.5 years. We collected blood samples once, during a stable phase of the disease (no clinical change during the previous 3 months). During the period of study, 5 of the 6 patients underwent magnetic resonance imaging (MRI) analysis: no enhancing lesions were found in 4 patients and one small gadolinium-enhanced lesion was present in 1 patient.

Clinically relapsing patients included 7 MS patients (1 male and 6 female) with a mean ± SD age of 33.4 ± 11.5 years; mean ± SD EDSS score of 3.2 ± 2.1; and mean duration of disease of 9.7 ± 6.5 years. We collected blood samples once during a *clinically active phase* of disease, which was defined as the development, within 10 days before blood sample collection, of new neurological symptoms and signs or as the worsening of previously existing neurological disturbances.

Patients treated with IFN-β included 8 patients with MS (3 male and 5 female) with a mean ± SD age of 33.1 ± 5.6 years; mean ± SD basal EDSS score of 1.8 ± 0.5; and mean ± SD duration of disease of 5.5 ± 4 years. Blood samples were collected at basal time and after 1, 3, and 6 months of IFN-β treatment. A subcutaneous injection of 8×10^6 IU of IFN-β (Betaferon; Shering AG, Berlin, Germany) was administered every other day to all patients but one, who was treated with a half dose after the start of treatment for a moderate lymphopenia. These patients experienced the last clinical relapse at least 1 month before the first dose of IFN-β was given.

No medication other than IFN-β was used. Patients who underwent relapses (7 clinically relapsing patients and 2 patients treated with IFN-β) were treated with methylprednisolone (1000 mg/d for 5 consecutive days). The treatment was started after blood sample collection.

β-Endorphin levels were also measured in 13 normal control subjects (5 male and 8 female) with a mean ± SD age of 31.0 ± 4.4 years.

METHODS

In all patients, peripheral blood samples were obtained and collected in a tube containing EDTA between 9 and 11 AM. The PBMC samples were separated by gradient sedimentation over Ficoll-Paque (Pharmacia, Uppsala, Sweden), and cells were washed and resuspended at 4×10^6/mL.

Aprotinin (Boehringer Ingelheim Pharmaceutical Inc, Ridgefield, Conn), 1000 kIU, was added to all samples before storage at –20°C until further processing to inhibit enzymatic degradation of the peptides. Pelleted cells were resuspended in 1 mL of 0.1N acetic acid, homogenized in a blade homogenizer, and then sonicated. Samples were centrifuged at 10000*g* for 10 minutes, and supernatants were frozen until the radioimmunoassay step.

β-Endorphin was measured by radioimmunoassay. The antiserum and the radioimmunoassay procedure were previously described and validated.[9] The antiserum used was directed to the C-terminal sequence of human BE. It showed 100% cross-reactivity with human β-lipotropin and low cross-reactivity with camel BE (5%).

Minor cross-reactivity was also observed with equimolar met-enkephalin (0.1%) but not with leu-enkephalin, dynorphin, α and β melanocyte-stimulating hormone, substance P, somatostatin, thyrotropin-releasing hormone, corticotropin-releasing hormone, neurotensin, arginine vasopressin, bombesin, cholecystokinin, vasoactive intestinal peptide, insulin, follicle-stimulating hormone, luteinizing hormone, prolactin, growth hormone, morphine, naloxone, or the cytokines IL-1α and IL-1β, and tumor necrosis factor α.

Sensitivity of the method was 10 pg per tube, and intra-assay and interassay variation coefficients were 8% and 11%, respectively.

STATISTICAL ANALYSIS

β-Endorphin data were analyzed by 1-way analysis of variance and by 1-way analysis of variance for repeated measures for the patients treated with IFN-β.

Studies in human and experimental animal models indicate an inhibitory effect of BE on the immune system.[10] The administration of naloxone hydrochloride, an opiate receptor antagonist, results in an increase of natural killer cell activity and proliferation of mitogen-induced peripheral blood mononuclear cells (PBMCs).[11-14] Moreover, BE concentrations in PBMCs are decreased in diseases in which the immune system is supposed to be overactivated, as in rheumatoid arthritis and Crohn disease, or during rejection of transplanted organs.[15] Decreased levels of BE have also been reported in cerebrospinal fluid (CSF) and PBMC samples from MS patients.[14]

We evaluated BE levels in PBMC samples from MS patients in a clinically stable phase of the disease, during a clinical relapse, and during treatment with IFN-β to investigate the hypothetical role of this opioid in the regulation of inflammation in MS and in mediating IFN-β therapeutic effects.

RESULTS

The values of BE levels in patients with stable MS and the basal values of patients selected for enrollment in IFN-β treatment were not significantly different ($P = .3$).

Mean ± SD BE levels in PBMC samples obtained from patients with stable MS (55 ± 25 pg/10^6 cells) and in basal samples obtained from MS patients who were going to enter IFN-β treatment (45 ± 8.9 pg/10^6 cells) were significantly lower compared with BE levels of matched normal control subjects (101 ± 47 pg/10^6 cells; $P<.001$) (**Figure 1**).

Patients with clinically relapsing MS showed a higher mean ± SD BE level (82 ± 4 pg/10^6 cells) compared with

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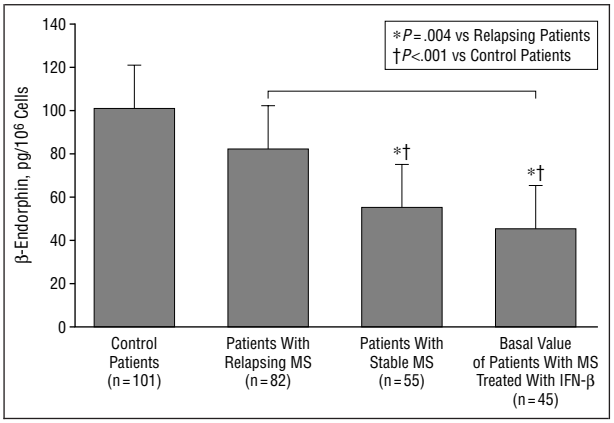

Figure 1. *Mean β-endorphin levels in peripheral blood mononuclear cells obtained from patients with multiple sclerosis (MS) and age-matched controls. Error bars indicate SDs. IFN-β indicates interferon beta.*

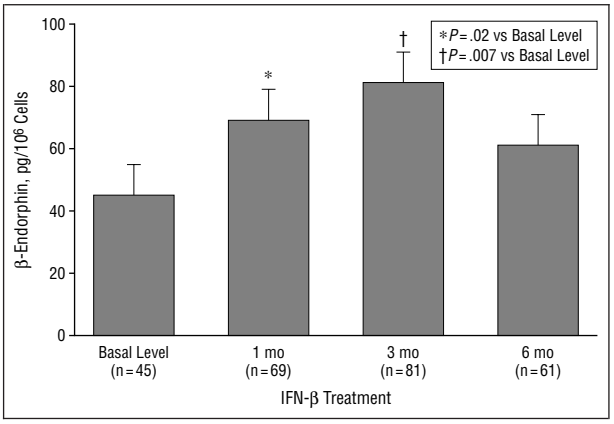

Figure 2. *Mean β-endorphin levels in lymphocytes obtained from patients with multiple sclerosis during treatment with interferon beta (IFN-β). Error bars indicate SDs.*

patients with stable MS and the basal value of patients treated with IFN-β (P = .004). The mean ± SD BE level of relapsing patients did not differ significantly from that of control subjects (P = .32).

Mean ± SD BE levels significantly increased in MS patients during IFN-β treatment (P = .004). Compared with the basal BE level (45 ± 9 pg/10^6 cells), the effect was already evident the first month after treatment began (70 ± 3 pg/10^6 cells), peaked at the third month (81 ± 7 pg/10^6 cells; P = .02), and was still present at the sixth month (61 ± 6 pg/10^6 cells; P = .007), although not statistically significant (P = .16) (**Figure 2**).

COMMENT

β-Endorphin has been shown to exert several effects on the immune system, including suppression of peripheral lymphocyte proliferation, inhibition of natural killer cell activity, and IL-2 and IFN-γ production.[12] The abovementioned responses can be up-regulated by the administration of opioid receptor antagonists.[12] Straub et al[17] have shown that norepinephrine and endogenous opioids like BE can inhibit macrophage IL-6 secretion. The disease-promoting activity of this cytokine in MS is suggested by its role in inducing B-cell differentiation into immunoglobulin-secreting cells, in T-cell activation, and in the production of perforin.[18] β-Endorphin may therefore down-regulate proinflammatory cytokines, which have been shown to be elevated in MS patients.[19] Moreover, a study on experimental autoimmune encephalomyelitis (EAE) in Lewis rats, the animal model for MS, reported that the administration of the opioid antagonist naloxone dramatically increases EAE severity.[19] Not only can BE regulate cytokine pattern secretion, it can also inhibit antigen-induced T-cell proliferation.[20] This mechanism likely occurs by nitric oxide–induced apoptosis[20]; inducible nitric oxide synthetase is in fact expressed in human PBMCs after incubation with BE.[21]

In our study, we found that MS patients during a stable phase of the disease had low mean ± SD BE levels compared with age-matched control subjects.

The intracellular concentration of a peptide reflects the balance between synthesis and release. However, in previous work,[22] it has been shown that high intracellular levels of BE correspond to a sustained secretion and low BE concentrations correspond to a reduced amount of secretion. Moreover, in vitro stimulation with corticotropin-releasing hormone or IL-1, potent activators of the *POMC* gene, leads to both synthesis and release.[4,23]

A steep rise of BE concentrations in PBMCs has been reported in young healthy subjects in the age range of the highest incidence of autoimmune diseases.[4] In the MS patients we studied, this increase of BE concentrations in PBMCs was not present, possibly as a consequence of the ongoing inflammatory process, as in other chronic inflammatory diseases.[15] We have no data to support the hypothesis that the decrease in BE concentration precedes the onset of MS, acting as a putative cofactor for disease development.

Our results also showed a significantly higher mean BE level during worsening in clinically relapsing patients compared with patients with stable MS and compared with the mean basal value of patients treated with IFN-β. The relatively high levels of BE soon after a relapse may be interpreted as a control mechanism aimed at down-regulating the inflammatory process. We have shown previously in a rat EAE model that an increase in hypothalamic and immunocyte BE concentrations is present at the peak of the disease, immediately before the recovery period.[19]

Our study is the first to show an increase of BE levels during IFN-β treatment: already after 1 month of therapy, BE concentrations were comparable with those measured in healthy control subjects. The mechanisms related to the BE increase during IFN-β treatment are unknown. Interferon beta may reset a pattern of cytokines (IL-1, IL-6, and tumor necrosis factor α) known to be able to increase BE levels in PBMCs.[4,19,24] Moreover, it is well known that cytokines, including IFN-α and IFN-β, can stimulate the hypothalamic-pituitary-adrenal axis.[25-27] Therefore, the effect of IFN-β on corticotropin-releasing hormone, one of the main inducers of BE synthesis, may also explain the BE increase.[24]

The possible beneficial activity of BE in MS, directly or indirectly triggered by IFN-β treatment, needs

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to be evaluated by studies with a larger number of MS patients who are followed up for a longer time.

We are aware that we are dealing with a mixed cell population. In healthy individuals and rodents, macrophages, B cells, and T cells can all produce and release BE.[4] We cannot say whether the altered BE levels that we observed are due to a specific augmentation in a certain cell type, an altered percentage of cells expressing BE, or uniformly increased production by all PBMCs. Since BE can affect the helper T cell balance (T_H1/T_H2), skewing it toward T_H2,[28] it would be worthwhile to evaluate BE levels in $CD4^+$ cells. Moreover, comparing the clinical evolution of MS patients with BE levels during IFN-β treatment would allow further investigation of the possible protective role of this opioid.

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