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Lower serum activity of prolyl endopeptidase in fibromyalgia is related to severity of depressive symptoms and pressure hyperalgesia

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ABSTRACT

Background. The aims of the present study were to examine serum activities of peptidases, i.e. prolyl endopeptidase (PEP) and dipeptidyl peptidase IV (DPP IV), in patients with fibromyalgia and to examine the effects of subchronic treatment with sertraline on these variables.

Method. Serum PEP and DPP IV activity were measured in 28 normal volunteers and 21 fibromyalgia patients, classified according to the American College of Rheumatology criteria. Tenderness at tender points was evaluated by means of dolorimetry. Fibromyalgia patients had repeated measurements of serum PEP and DPP IV both before and after repeated administration of sertraline or placebo for 12 weeks.

Results. Patients with fibromyalgia had significantly lower serum PEP activity than normal volunteers. There were significantly negative correlations between serum PEP activity and severity of pressure hyperalgesia and the non-somatic, cognitive symptoms of the Hamilton Depression Rating Scale. Fibromyalgia patients with severe pressure hyperalgesia had significantly lower PEP activity than normal controls and fibromyalgia patients with less severe hyperalgesia. Fibromyalgia patients with severe non-somatic depressive symptoms had significantly lower serum PEP activity than normal volunteers. There were no significant changes in serum DPP IV activity in fibromyalgia. There were no significant effects of repeated administration of sertraline on serum PEP and DPP IV activity in patients with fibromyalgia.

Conclusions. The results show that fibromyalgia, and aberrant pain perception and depressive symptoms in fibromyalgia are related to lower serum PEP activity. It is hypothesized that lower serum PEP activity may play a role in the biophysiology of fibromyalgia through diminished inactivation of algesic and depression-related peptides.

INTRODUCTION

Dipeptidyl peptidase IV (DPP IV, EC 3.4.14.5) and prolyl endopeptidase (PEP, EC 3.4.21.26, post-proline cleaving enzyme, prolyl oligopeptidase) are two peptidases that are widely distributed among human tissues and body fluids (Hopsu-Havu & Glenner, 1966; Kato *et al.* 1980*a*; Vanhoof *et al.* 1992; Goossens *et al.* 1996). PEP is a cytosolic endopeptidase that cleaves peptide bonds on the carboxylside of proline in proteins of relatively small molecular mass (Kato *et al.* 1980*b*; Goossens *et al.* 1996). It has been suggested that PEP plays a role in the regulation of intracellular protein turnover and also in the final degradation and processing of mature peptide hormones and neuropeptides (Welches *et al.* 1993). PEP may

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degrade many neuropeptides or hormones, such as arginine vasopressin (AVP), luteinizing hormone–releasing hormone (LH–RH), thyrotropin releasing hormone (TRH), substance P, oxytocin, bradykinin, neurotensin and angiotensin (Welches *et al.* 1993).

DPP IV is a membrane bound serine protease that catalyses the cleavage of dipeptides from the amino-terminus of oligo- and polypeptides under definite structural conditions (Vanhoof *et al.* 1992; Yaron & Naider, 1993). DPP IV has a role in a variety of physiological functions, such as activation and inactivation of peptides, the processing of polypeptides and proteins, intestinal assimilation, renal handling of proline containing peptides, and adhesion and modulation of immune reactivity (Vanhoof *et al.* 1992; Yaron & Naider, 1993). DPP IV is responsible for the plasma degradation of substance P, growth hormone releasing hormone and the alpha chain of fibrin (Vanhoof *et al.* 1992). DPP IV may enhance the degradation of peptides derived from collagen in connective tissues (Kato *et al.* 1978). On the T cell surface, DPP IV has been proved to be identical with the CD26 molecule (Barton *et al.* 1990). Both, recombinant soluble CD26 and plasma DPP IV exhibit co-stimulatory functions for the response to recall antigens and play an important role in lymphocyte activation and production of cytokines such as interleukin-2 and interferon-γ (Schon *et al.* 1989; Tanaka *et al.* 1994; Duke-Cohan *et al.* 1995: Reinhold *et al.* 1996).

The above characteristics of PEP and DPP IV prompted us to examine whether the serum activities of these peptidases are altered in fibromyalgia. Fibromyalgia is a chronic, painful musculoskeletal disorder characterized by widespread musculoskeletal pain, low pain threshold to pressure (pressure hyperalgesia), non-restorative sleep, fatigue and morning stiffness (Wolfe *et al.* 1990). There is a strong comorbidity between major depression and fibromyalgia and an increased incidence of depressive symptoms in fibromyalgia patients (Hudson *et al.* 1992). Some, but not all authors, found a higher frequency of depression or depressive symptoms among patients with fibromyalgia and their first-degree relatives than among patients with rheumatoid arthritis (Hudson *et al.* 1985; Hawley & Wolfe, 1993; Krag *et al.* 1994). The biophysiology of fibromyalgia has remained elusive. There are some reports that antidepressants, tricyclics (TCAs) as well as selective serotonin reuptake inhibitors (SSRIs), have some efficacy in that condition (Gruber *et al.* 1996). It is thought that the diffuse myalgia, fatigue and psychological distress are related to disorders of the sleep–wake system or to immune and neuro-endocrine disorders (Moldofsky, 1995).

We anticipated finding lower serum PEP and DPP IV activity in fibromyalgia because of the following: (*i*) depression, which often accompanies fibromyalgia, is characterized by lower serum PEP and DPP IV activity (Maes *et al.* 1991, 1994*a*, 1995, 1996); and (*ii*) lower PEP and DPP IV activity may be accompanied by decreased inactivation of peptides, which are related to nociception, allodynia and hyperalgesia, e.g. bradykinin, substance P and pro-inflammatory cytokines, such as interleukin-1 (IL-1) and IL-6 (Lotz *et al.* 1988; Watkins *et al.* 1995). In this respect, it is important to note that very high activities of PEP are detected in skeletal muscle tissues, where PEP may function to degrade substance P (Kato *et al.* 1980*b*; Moriyama *et al.* 1988). DPP IV is identified on the cell surface of synovial cells, where it is responsible for the degradation of substance P (Bathon *et al.* 1992). DPP IV is also associated with microvessels of peripheral nerves (Barnes *et al.* 1991).

The aims of the present study were to examine: serum activities of PEP and DPP IV in fibromyalgia compared with normal subjects; the relationships between the serum activities of these peptidases and symptoms, such as pressure hyperalgesia and depression; and the effects of subchronic treatment with sertraline, an SSRI, on serum PEP and DPP IV activity in fibromyalgia.

METHOD

Subjects

Forty-nine subjects participated in this study, 21 fibromyalgia patients and 28 normal controls. Fibromyalgia patients fulfilled the American College of Rheumatology (ACR) criteria for fibromyalgia (Wolfe *et al.* 1990), i.e. a history of widespread pain for at least 3 months in all of the following regions – pain in the left side of the body, pain in the right side of the body, pain

above and below the waist, axial skeletal pain (cervical spine or anterior chest or thoracic spine or low back pain); and pain in 11 of the following 18 tender point sites – occiput left or right (R/L), low cervical L/R, trapezius L/R, supraspinatus L/R, second rib L/R, lateral epicondyle L/R, gluteal L/R and knee L/R. The presence of a major clinical condition other than fibromyalgia was excluded by physical examinations and routine blood and urine screening, including measurements of erythrocyte sedimentation, white blood cells and differentials, red blood cells, haematocrit and haemoglobin, baseline thyroid-stimulating hormone and antinuclear autoantibodies. Other exclusionary criteria for patients were: patients who had received fluoxetine within 6 weeks, MAO inhibitors within 2 weeks, other psychoactive drugs within 1 week, and anti-inflammatory drugs within 4 weeks before inclusion; subjects presenting with a significant risk of suicide; and patients treated with psychoactive medication (except lormetazepam) and any other medication for the symptoms of fibromyalgia (except paracetamol). Exclusionary criteria for fibromyalgia patients and normal controls were: immunocompromised patients and patients suffering from a neurological, inflammatory, endocrine or clinically significant chronic disease; subjects presenting with a recent or past history of psychiatric disorders, such as major depression, which were assessed by means of DSM-II-R criteria (APA, 1987) on the basis of the Structured Clinical Interview for the DSM-III-R (Spitzer *et al*. 1990); abnormal liver function tests, such as serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase (ALP), and gamma-glutamyl transpeptidase (γGT); and pregnant females. Exclusionary criteria for normal volunteers were: any of the above ACR criteria for fibromyalgia; regular drinkers; use of any medication the month prior to these studies; and recent or past use of any psychotropic drugs. After complete description of the study to the subjects, written informed consent was obtained.

Procedure

After the initial, baseline screening (physical examination, inclusion and exclusion criteria, blood samplings for routine screening), patients underwent a 1-week drug-free period. Hereafter, serum for the assay of PEP and DPP IV activity was collected at 7.30 a.m. ($\pm$30 min) after an overnight fast. Serum was centrifuged at 3500 ***g*** for 15 min and stored at -70 °C until thawed for assay. The preparation of the subjects prior to blood collection was carefully controlled and blood collections were performed in standardized conditions in order to minimize sources of pre-analytical variation. Since there is a significant seasonal variation in both serum DPP IV and PEP with orthophases between August–September (Maes *et al*. 1994*b*), we have collected serum from patients and healthy volunteers between November (1995) and July (1996). Seasonal effects, however, are not likely to be detected in cross-sectional studies, since the seasonal variations in serum PEP (28·1%) and DPP IV (6·7%) in healthy volunteers explain only a smaller part of the biological intra-individual variability, which, in turn, is only a part of the total biological variability. Nevertheless, we have adjusted the results of this study for possible effects of the seasons by means of regression analyses. All serum specimens from patients and controls were assayed the same day, in a single run with a single lot number of reagents and consumables employed by a single operator. Serum DPP IV was determined by means of a colorimetric method using the chromogenic substrate glycyl-L-proline-p-nitroanilide tosylate (Nagatsu *et al*. 1976). The substrate was purchased from Sigma (Bornem, Belgium). We adapted the method for direct continuous measurement on the centrifugal analyser Cobas Bio (Roche Diagnostics, Basel, Switzerland) (Vanhoof *et al*. 1992). One unit (U) of DPP IV activity is defined as the enzyme activity which produces 1 μmol of p-nitroaniline in 1 min under the above conditions. Under the conditions described here, there is no interference from other dipeptidyl peptidases or aminopeptidases present in the serum. The analytical imprecision of our DPP IV assay is 2·2%. PEP activity in serum has been determined by a fluorimetric method (Goossens *et al*. 1992). The synthetic substrate Z-glycyl-prolyl-4-methylcoumarinyl-7-amide was purchased from Bachem Feinchemikalien AG (Bubendorf, Switzerland). The assay is highly specific for PEP, since DPP IV and aminopeptidase P do not result in any breakdown of the substrate. Moreover, the possible interference from metalo-

proteases is prevented by the addition of EDTA to the incubation buffer. One unit (U) of PEP activity is defined as the enzyme catalytic activity that releases 1 μmol 7-amino-4-methylcoumarin in 1 min under the assay conditions described here. The analytical imprecision of our PEP assay is 4·8%.

Tenderness at tender points was evaluated by means of dolorimetry: a trained rheumatologist advanced the instrument at a rate of approximately 1 kg/s and the patient was instructed to say when this procedure became painful. The pressure was measured when painful for each of the 18 tender points listed above. The total fibromyalgic score (R + L) was used in the statistical analyses. A visual analogue scale (VAS) was employed to rate the following items: global fibromyalgic symptoms, stiffness, pain, anergy and sleep disturbances. The 17-item Hamilton Depression Rating Scale (HDRS) was used to measure depressive symptomatology (Hamilton, 1960). However, many symptoms of the 17-item HDRS scale reflect somatic or vegetative disorders. In order to have an index of the non-somatic depressive symptoms, we computed the sum of the cognitive symptoms of the HDRS, i.e. feelings of depression, loss of interest, suicidal ideation, feelings of guilt and psychic anxiety.

Fibromyalgia patients had repeated measurements of serum PEP and DPP IV activity and of the above rating scales both before and after repeated administration of sertraline or placebo for 12 weeks. Fibromyalgia patients took part in a double-blind, placebo-controlled, multicentre study on the clinical efficacy of sertraline in fibromyalgia (Pfizer, Belgium). Patients were randomized to received sertraline, 50–100 mg/daily, or placebo for 12 weeks. Most fibromyalgia patients were free of psychotropic drugs prior to the 1-week wash-out period. Four patients had been taking antidepressants prior to the 1-week wash-out period, i.e. amitriptyline (25–75 mg/day, $N = 3$) or trazodone (100 mg/day). During the study, four patients were treated with lormetazepam in case of severe sleep disorders and three patients were treated with paracetamol.

Statistics

Relationships between variables were checked with Pearson's product moment and by means of (multiple) regression analysis. The independence of classification systems was assessed by means of analysis of contingence (χ^2 test). Group mean differences were ascertained by means of analysis of variance (ANOVA) or covariance (ANCOVA). *A priori* differences between group means were checked with the Dunn test. Treatment effects of sertraline *versus* placebo were checked by means of repeated measure design ANOVAs and ANCOVAs taking into account the time × type of treatment interaction. Transformations were used in order to reach normality of distribution and to adjust for heterogeneity of variance between the study groups. The diagnostic performance of an abnormal test result was assessed by means of computation of the sensitivity, specificity and predictive value of a positive (PV^+) test result and the κ statistic (Zweig & Campbell, 1993).

RESULTS

Table 1 shows the demographic data of the subjects and the measurements of serum PEP and DPP IV activity. There were no significant differences either in the male/female ratio or in age between the fibromyalgia patients and normal controls. There were no significant correlations between age and serum PEP ($r = -0{\cdot}02$, $P = 0{\cdot}9$) or DPP IV ($r = -0{\cdot}05$, $P = 0{\cdot}7$) activity (results of intraclass regression analyses pooled over both study groups). By means of ANOVAs, factorial design with gender as second factor, no significant differences between females and males were found in serum PEP ($F = 0{\cdot}1$, df = 1/45, $P = 0{\cdot}7$) or DPP IV ($F = 0{\cdot}08$, df = 1/45, $P = 0{\cdot}8$) activity. In any case, we have controlled subsequent statistical analyses for age and sex, by entering these variables as covariates in regression analyses.

Table 1 shows that serum PEP activity was significantly lower in fibromyalgia patients than in normal controls. ANCOVA with the seasons as covariates (entered as dummy variables), did not change these results ($F = 4{\cdot}6$, df = 1/44, $P = 0{\cdot}03$). ANOVA, factorial design with diagnosis as second factor, did not show significant differences in serum PEP between the seasons ($F = 0{\cdot}7$, df. = 1/2, $P = 0{\cdot}5$). At a cut-off value of PEP < 0·19 U/l, we found a significant separation of fibromyalgia patients from normal volunteers ($\kappa = 0{\cdot}3$, $t = 2{\cdot}1$, $P = 0{\cdot}04$); the di-

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Table 1. *Demographic data and measurement of serum prolyl endopeptidase (PEP) and dipeptidyl peptidase IV (DPP IV) activity in patients with fibromyalgia* versus *normal controls*

Groups	Men:Women	Age (years)	PEP (U/L)*	DPP IV (U/l)*
Normal volunteers	5:23	47·8 (8·1)	0·235 (0·063)	39·8 (10·0)
Fibromyalgia patients	4:17	50·1 (7·0)	0·203 (0·049)	39·3 (6·3)
χ^2 or F	$\chi^2 = 0{\cdot}01$	$F = 1{\cdot}1$	$F = 4{\cdot}1$	$F = 0{\cdot}03$
df	1	1/47	1/45	1/45
P	0·9	0·3	0·04	0·9

All results are shown as mean (±S.D.).
* Results of analyses of covariance with age and gender as covariates.

Table 2. *Measurement of serum prolyl endopeptidase (PEP) and dipeptidyl peptidase IV (DPP IV) activity in normal volunteers (NV) and fibromyalgic patients (F), divided into those with or without severe pressure hyperalgesia (±PH) or cognitive symptoms of depression (±CD)*

Groups	Men:Women	Age (years)	PEP (U/l)	DPP 4 (U/l)
NV	5:23	47·8 (8·1)	0·235 (0·063)	39·8 (10·0)
F−PH	2:9	48·5 (7·2)	0·229 (0·039)	38·3 (5·0)
F+PH	2:8	52·0 (6·5)	0·179 (0·044)*	40·5 (7·6)
F−CD	2:9	48·2 (7·6)	0·218 (0·044)	40·4 (7·3)
F+CD	2:8	52·3 (5·8)	0·187 (0·051)†	38·2 (5·0)

All results are shown as mean (±S.D.).
* Significantly different from NV ($t = 3{\cdot}6$, $P = 0{\cdot}001$, and F−PH($t = 2{\cdot}9$, $P = 0{\cdot}005$); results of Dunn test performed after ANCOVA with age and sex as covariates ($F = 6{\cdot}6$, df = 2/44, $P = 0{\cdot}003$).
† Significantly different from NV ($t = 2{\cdot}7$, $P = 0{\cdot}009$); results of Dunn test performed after ANCOVA with age and sex as covariates ($F = 3{\cdot}4$, df = 2/44, $P = 0{\cdot}04$).

agnostic performance was, sensitivity = 42·8%, specificity = 85·7%, and PV+ = 75·0%. There were no significant differences in serum DPP IV activity between both groups.

In fibromyalgia patients, there were significant and positive correlations between serum PEP, but not DPP IV, activity and the total myalgic scores ($r = 0{\cdot}58$, $P = 0{\cdot}005$). There were no significant correlations between serum PEP or DPP IV activity and: (*i*) the 17-item HDRS score; (*ii*) any of the subjective VAS scale ratings, i.e. global fibromyalgia symptoms, pain, stiffness, anergy and sleep disturbances; (*iii*) duration of illness or age at onset. There were significant and negative relationships between serum PEP, but not DPP IV, activity and the cognitive symptoms of the HDRS ($r = -0{\cdot}60$, $P = 0{\cdot}005$). By means of multiple regression analysis, it was found that 49·6% of the variance in serum PEP activity could be explained by the multiple regression on the myalgic score ($F = 7{\cdot}3$, $P = 0{\cdot}01$) and the cognitive HDRS symptoms ($F = 8{\cdot}4$, $P = 0{\cdot}009$) ($F = 8{\cdot}3$, df = 1/19, $P = 0{\cdot}002$).

The above results show that serum PEP activity is diminished in those fibromyalgia patients with more severe pressure hyperalgesia and cognitive symptoms of depression. Therefore, we have examined the differences in PEP (and DPP IV) activity between normal controls and fibromyalgia patients with or without severe pressure hyperalgesia (i.e. myalgic score < 50) and with or without cognitive depressive symptoms (sum of the cognitive items > 6). Table 2 shows that fibromyalgia subjects with severe myalgic tender points had a significantly lower serum PEP activity than normal controls and patients with less severe tenderness. Covarying for seasonal effects did not change these results ($F = 7{\cdot}5$, df = 2/43, $P = 0{\cdot}002$). Table 2 shows that fibromyalgia patients with the more severe cognitive depressive symptoms had significantly lower serum PEP activity than normal volunteers. Covarying for seasonal effects did not change these results ($F = 6{\cdot}3$, df = 2/43, $P = 0{\cdot}004$). There were no significant differences in serum DPP IV activity according to severity of myalgic pain and cognitive depressive symptoms.

Table 3 shows the repeated measurements of the myalgic score, the 17-tem HDRS score, serum PEP and DPP IV activity in the 19

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Table 3. *Measurements of the myalgic score (MS), the Hamilton Depression Rating Scale (HDRS) – score, serum activity of prolyl endopeptidase (PEP) and dipeptidyl peptidase IV (DPP IV) in fibromyalgia patients both before and after treatment with sertraline/placebo*

	MS		HDRS		PEP (U/l)		DPP IV (U/l)	
Treatment	Baseline	End point	Baseline	End point	Baseline	End point	Baseline	End point
Placebo	49·2 (14·4)	55·3 (15·0)	16·4 (5·0)	13·4 (6·4)	0·17 (0·048)	0·188 (0·030)	37·8 (3·7)	37·8 (9·8)
Sertraline	49·4 (14·5)	54·3 (23·9)	13·8 (4·7)	10·5 (6·9)	0·212 (0·043)	0·196 (0·029)	40·7 (7·4)	39·3 (8·2)
*F**	0·02	—	0·01	—	1·95	—	0·5	—
f	1/18	—	1/18	—	1/16	—	1/16	—
P	0·9	—	0·9	—	0·2	—	0·5	—

All results are shown as mean (±S.D.).
*F** All results of repeated measures design ANOVA showing the interaction pattern time × type of treatment.

fibromyalgia subjects who were treated with placebo/sertraline. Of the patients who did not complete the study, two were randomly assigned to placebo. One patient withdrew voluntarily, while the study treatment was suspended in another patient due to suicidal ideation. Of the patients who did complete the study, seven had been randomly assigned to placebo and 12 to sertraline. Serum PEP activity was significantly lower in fibromyalgia patients at the end of the study after treatment with sertraline (mean PEP $= 0{\cdot}194 \pm 0{\cdot}029$ U/l) than in healthy volunteers ($F = 8{\cdot}5$, df $= 1/42$, $P = 0{\cdot}006$). Repeated measure design ANOVAs did not disclose significant interaction patterns between time and type of treatment. These results suggest that there were no significant effects of sertraline on serum PEP and DPP IV activity. Repeated measure design ANCOVAs, with the seasons as covariates, did not change these results (PEP, $F = 1{\cdot}6$, df $= 1/16$, $P = 0{\cdot}2$; DPP IV, $F = 1{\cdot}2$, df $= 1/16$, $P = 0{\cdot}3$). Repeated measure design ANOVAs did not show significant interaction patterns between time and type of treatment for the myalgic score or the HDRS score. There were no significant differences in the effects of sertraline on the myalgic score in those patients who showed severe hyperalgesia *versus* those with reduced hyperalgesia ($F = 1{\cdot}2$, df $= 1/14$, $P = 0{\cdot}3$). There were no significant differences in the effects of sertraline on the cognitive HDRS symptoms in those patients with elevated cognitive depressive symptoms *versus* those with relatively low cognitive depressive symptoms ($F = 1{\cdot}8$, df $= 1{\cdot}14$, $P = 0{\cdot}2$). It should be understood, however, that the present study was not designed to evaluate the efficacy of sertraline in fibromyalgia. This was the objective of a double-blind, parallel group, placebo-controlled, multi-centre study performed by Pfizer, Belgium. The patient group included in the present study is part of that multi-centre comparison study group.

DISCUSSION

The main findings of this study are that subjects with fibromyalgia have significant lower serum PEP activity than normal volunteers and that the lowest serum PEP activity was observed in those fibromyalgia patients with more severe cognitive symptoms of depression and with more severe pressure hyperalgesia.

In the present study there was a highly significantly inverse relationship between serum PEP activity and severity of the cognitive symptoms of depression. In this respect, it is interesting to note that lower PEP activity has been found in depression (Maes *et al.* 1994*a*, 1995). Lower PEP activity in depression is in agreement (Abassi *et al.* 1992; Watanabe *et al.* 1993) with other findings that a low molecular weight hyperpeptiduria may occur in that illness (Saelid *et al.* 1985). As described in the Introduction, there is also a significant magnitude of depressive symptoms in fibromyalgia patients and a strong co-morbidity between both disorders (Hudson *et al.* 1992). We have argued that lower PEP activity in major depression could, in theory, be involved in many of the neuroendocrine perturbations in that condition, such as hypothalamic–pituitary–adrenal (HPA) axis hyperactivity, and HP–thyroid axis dysfunctions (Maes *et al.* 1994*a*). Indeed, PEP may cleave many neuropeptides or hormones that have been shown to be involved in the

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pathophysiology of depression, e.g. TRH and AVP (see Introduction) and maybe corticotropin releasing hormone (CRH) (Welches *et al*. 1993; Maes *et al*. 1994*a*). We have reviewed elsewhere the evidence that peripheral PEP may have relevance for the neuroendocrine function in the brain (Maes *et al*. 1994*a*, 1995). Therefore, it is tempting to hypothesize that: the depressive symptoms in fibromyalgia may be related to decreased PEP activity; and the co-morbidity between fibromyalgia and depression and the increased incidence of depressive symptoms in fibromyalgia patients may be ascribed to a common pathophysiology, e.g. lowered PEP activity.

A second major finding of this study is that there was a highly significantly inverse relationship between serum PEP activity and severity of tenderness at tender points. These findings suggest that lower PEP activity may be related to the pressure hyperalgesia which occurs in fibromyalgia. It has been shown that PEP may degrade peptides, which are potent algesic agents, e.g. substance P and bradykinin (Moriyama *et al*. 1988; Bhoola *et al*. 1992). Recent evidence suggest an important role for bradykinin in the generation of pain and hyperalgesia associated with tissue damage and inflammation, e.g. inflammatory joint disease (Bhoola *et al*. 1992; Dray & Perkins, 1993; Tracey & Walker, 1995). Bradykinin is made *de novo* from kininogen precursors, which are activated following injury, anoxia, and inflammation (Dray & Perkins, 1993). These components of the kinin system may enter tissues, such as the synovial joint space, by transudation from the plasma or from neutrophils attracted into the synovium (Bhoola *et al*. 1992). PEP is also present and active in the cytoplasmic fraction of neutrophils (Rauner *et al*. 1976). PEP degrades bradykinin to produce inactive species (Bhoola *et al*. 1992). Substance P is a mediator of pain transmission and pain sensation and, as a result, is involved in nociception, hyperalgesia and neurogenic inflammation (Pernow, 1983; Piercey *et al*. 1986; Saria, 1987). One likely mechanism for inactivation of substance P is cleavage by PEP into a biologically active fragment with subsequent removal by reuptake or degradation by exopeptidases (Sandberg & Iversen, 1982). Thus, it may be hypothesized that lower serum PEP may cause a diminished degradation of bradykinin and substance P and that this could play a role in the tenderness, chronic pain and hyperalgesia in fibromyalgia. However, while high substance P concentrations were repeatedly found in the synovial fluid of patients with rheumatoid arthritis (RA), the results in fibromyalgia were more controversial (Menkes & Renoux, 1994).

Interestingly, major depression is often associated with pain, hyperesthesia, increased nociception and a variety of bodily complaints (Blumer & Heilbronn, 1987). Therefore, it may be hypothesized that an altered PEP activity in fibromyalgia and depression is related to the hyperesthesia or pain hypersensitivity in those patients. The results of the present study suggest that disturbances in PEP may play a role in fibromyalgia and that future research should focus on the role of peptidases in the pathophysiology of fibromyalgia. This research should include, for example, measurements of PEP activity in skeletal muscles, joints, and cerebrospinal fluid.

This study was unable to find any significant changes in serum DPP IV activity in fibromyalgia patients. Decreased DPP IV activity, on the other hand, was reported in major depression (Maes *et al*. 1991). DPP IV plays an important role in cell-mediated immunity. On the T cell surface, DPP IV is identical with the CD26 molecule and its expression is increased during T cell activation (Barton *et al*. 1990). Serum soluble CD26/DPP IV is a potent T cell co-stimulator (Duke-Cohan *et al*. 1995), which plays an important role in lymphocyte activation (Schon *et al*. 1989; Tanaka *et al*. 1994; Reinhold *et al*. 1996). In RA, for example, serum DPP IV activity is decreased whereas the expression of CD26 on T cells is increased (De Meester *et al*. 1993). Our negative DPP IV findings suggest that there are no disturbances in DPP-IV-related T cell activation in fibromyalgia or that the changes are not measurable in the plasma compartment.

Another finding of this study is that repeated administration of sertraline had no discernable effects on serum PEP and DPP IV activities. In a previous study in major depression, we found that repeated administration of fluoxetine, another SSRI, and TCAs significantly increased serum PEP activity (Maes *et al*. 1995). The present study showed that sub-chronic treatment

with sertraline did not have any significant effects on pressure hyperalgesia and the HDRS score. Previous reports have shown that TCAs and fluoxetine have some efficacy in the treatment of fibromyalgia (Goldenberg *et al*. 1996; Gruber *et al*. 1996).

In conclusion, the results of the present study show that lower PEP activity may be related to symptoms of fibromyalgia, such as pressure hyperalgesia and the cognitive depressive symptoms.

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