Rheumatology 2001;40:290–296

Cerebrospinal fluid biogenic amine metabolites, plasma-rich platelet serotonin and [^{3}H]imipramine reuptake in the primary fibromyalgia syndrome

E. Legangneux, J. J. Mora[1], O. Spreux-Varoquaux[2], I. Thorin, M. Herrou[3], G. Alvado[4] and C. Gomeni[5]

Department of Psychiatry, University of Caen, [1]Department of Rheumatology, Hospital of Bayeux, [2]Department of Pharmacology, Hospital of Versailles, [3]Department of Pharmacology, University of Caen, [4]Department of Surgery, Hospital of Bayeux and [5]Biotrial, rue Jean-Louis Bertrand, Rennes, France

Abstract

Background. Primary fibromyalgia syndrome (PFS) is a chronic disorder commonly seen in rheumatological practice. The pathophysiological disturbances of this syndrome, which was defined by the American College of Rheumatology in 1990, are poorly understood. This study evaluated, in 30 patients, the hypothesis that PFS is a pain modulation disorder induced by deregulation of serotonin metabolism.

Objectives. To compare platelet [^{3}H]imipramine binding sites and serotonin (5-HT) levels in plasma-rich platelets (PRP) of PFS patients with those of matched healthy controls and to compare the levels of biogenic amine metabolites in the cerebrospinal fluid (CSF) of PFS patients with those of matched controls.

Methods. Platelet [^{3}H]imipramine binding sites were defined by two criteria, B_{max} for their density and K_d for their affinity. PRP 5-HT and CSF metabolites of 5-HT (5-hydroxyindoleacetic acid, 5-HIAA), norepinephrine (3-methoxy, 4-hydroxy phenylglycol, MHPG) and dopamine (homovanillic acid, HVA) were assayed by reversed-phase high-performance liquid chromatography with coulometric detection.

Results. [^{3}H]Imipramine platelet binding was similar ($P = 0.43$ for B_{max} and $P = 0.30$ for K_d) in PFS patients ($B_{max} = 901 \pm 83$ fmol/mg protein, $K_d = 0.682 \pm 0.046$) and in matched controls ($B_{max} = 1017 \pm 119$ fmol/mg protein, $K_d = 0.606 \pm 0.056$). PRP 5-HT was significantly higher ($P = 0.0009$) in PFS patients (955 ± 101 ng/10^9 platelets) than in controls (633 ± 50 ng/10^9 platelets). When adjusted for age, the levels of all CSF metabolites were lower in PFS patients. The CSF metabolite of norepinephrine (MHPG) was lower ($P = 0.003$) in PFS patients (8.33 ± 0.33 ng/ml) than in matched controls (9.89 ± 0.31 ng/ml) and 5-HIAA was lower ($P = 0.042$) in PFS female patients (22.34 ± 1.78 ng/ml) than in matched controls (25.75 ± 1.75 ng/ml). For HVA in females, the difference between PFS patients (36.32 ± 3.20 ng/ml) and matched controls (38.32 ± 2.90 ng/ml) approached statistical significance ($P = 0.054$).

Conclusion. Changes in metabolites of CSF biogenic amines appear to be partially correlated to age but remained diagnosis-dependent. High levels of PRP 5-HT in PFS patients were associated with low CSF 5-HIAA levels in female patients but were not accompanied by any change in serotonergic uptake as assessed by platelet [^{3}H]imipramine binding sites. These findings do not allow us to confirm that serotonin metabolism is deregulated in PFS patients.

KEY WORDS: Primary fibromyalgia syndrome, Matched controls, Serotonin, Norepinephrine, Dopamine, Cerebrospinal fluid.

Submitted 9 November 1999; revised version accepted 2 October 2000.

Correspondence to: E. Legangneux, Sanofi Synthelabo, 1 Av. Pierre Brossolette, F-91380 Chilly-Mazarin, France.

Primary fibromyalgia syndrome (PFS) is a chronic disorder commonly seen in rheumatological practice. Characteristics of this syndrome, described by Smythe [1], Yunus *et al*. [2] and Wolfe *et al*. [3], were defined by the American College of Rheumatology (ACR) [4]

in 1990. However, the pathophysiological disturbances of this syndrome, mainly observed in females, are poorly understood. Some studies suggest pathological changes in muscular tissues or immunological disturbances [5, 6]. However, because pain, fatigue, sleep disturbances and psychological disturbances are the main symptoms of PFS, it has been suggested that a deficiency in serotonin is involved in this syndrome, and previous studies seem to confirm this hypothesis [7–9]. Elevated levels of substance P, which are mediated by serotonin [10], have been reported [11]. Levels of metabolites of serotonin, norepinephrine and dopamine in the cerebrospinal fluid (CSF) were found to be lower in PFS patients than in controls [12]. Serum serotonin concentrations were also found to be lower in PFS patients than in controls, while the level of platelet [^{3}H]imipramine binding was higher [13].

Our study, based on the hypothesis that PFS is a pain modulation disorder [7], was designed to validate these previous results. In humans, platelets have been used extensively to assess neurotransmitter function because of their similarities to serotonin (5-HT) nerve terminals, particularly in the presence of amine storage granules and an identical high-affinity 5-HT transporter, with similar genetically determined activity of 5-HT uptake. The objectives of this study were to compare platelet [^{3}H]imipramine binding sites, which is an indicator of serotonergic uptake, and 5-HT levels in plasma-rich platelets (PRP) between PFS patients and matched healthy controls, and to compare the levels of biogenic amine metabolites in the cerebrospinal fluid (CSF) between PFS patients and matched controls.

Patients and methods

The study was approved by the local ethics committee and all subjects gave their informed consent before participation.

Patients

Thirty patients suffering from PFS according to the ACR diagnosis criteria [4] were recruited in the Department of Rheumatology at the hospital of Bayeux. They had experienced symptoms of PFS for at least the last 3 yr. Clinical examination was performed by a rheumatologist and a psychologist. Patients suffering from symptomatic osteoarthritis or depression were excluded from the study. None of the patients included in the study had any known current associated disorder. They had a normal biological check-up, including blood count, antinuclear antibodies, a Waaler–Rose latex test, creatine phosphokinase and erythrocyte sedimentation rate. To prevent interference with evaluation of platelet 5-HT content and platelet [^{3}H]imipramine binding sites, no patient took psychotropic medication for at least 3 weeks before the study. Analgesic drugs, except opiates, were allowed (paracetamol and salicylates), as 5-HT values were obtained from PRP rather than from serum. However, no patient reported the use of analgesics 48 h before hospitalization. The patients were admitted to the Caen hospital the day before the investigations. In the morning, blood samples were taken to determine PRP serotonin and platelet [^{3}H]imipramine binding sites. Two hours later the patients underwent lumbar puncture, and they were allowed to leave the hospital in the evening after a medical check. One patient refused lumbar puncture.

Healthy control subjects

Thirty subjects were matched in gender and age with the PFS patients. The healthy control subjects were included, after a medical and biological check-up, within 1 month after the inclusion of the corresponding patients in order to limit variation in serotonin due to seasonal effects. None of the controls was taking any psychotropic or analgesic medication before inclusion, or suffering from any psychiatric or painful disorder. Blood samples were taken in the morning for the determination of PRP serotonin and platelet [^{3}H]imipramine binding sites, at the same time as the patients, but healthy subjects were not subjected to lumbar puncture, which was considered unethical by the local ethics committee.

CSF control patients

Because of ethical considerations, CSF samples from control subjects were collected specifically from 69 patients undergoing rachianaesthesia for a surgical reason (prostate adenoma or hip fracture) in the Department of Surgery at the Bayeux hospital. These patients were selected, after clinical examination by an anaesthetist, on the basis of their previous medical history. None of them was suffering from any current psychiatric disease and none was taking any psychotropic drug before inclusion. To compensate for the variability induced by this kind of recruitment, the number of subjects was increased to 69 versus 30 in the PFS group.

CSF sampling

Three millilitres of CSF was collected from each subject by lumbar puncture. No subject experienced any adverse event except temporary headache. None of the samples was contaminated with blood. The samples were frozen in the dark at -70°C until analysis.

[^{3}H]Imipramine receptor assay

The method of Mellerup and Langer [14] was used. Briefly, about 20 ml of blood was collected in the morning in plastic tubes containing anticoagulant (citrate–EDTA). After gentle mixing, centrifugation was performed at 200 *g* for 20 min at room temperature. The PRP were collected in the morning between 9 and 10 a.m. and centrifuged at 10 000 *g* for 10 min at 4°C. The supernatant was discarded. The drained platelet pellet was stored at -80°C until analysis or transportation in dry ice.

Preparation of the platelet membranes

The isolated platelets were washed twice with 8 ml buffer (Tris–EDTA, pH 7.5), then lysed with an Ultra-Thurrax homogenizer (T25, Bioblock Scientific, Illkirch, France). The membranes were precipitated (30 000 r.p.m. for 10 min at 4°C), and washed twice with 8 ml buffer. The membranes were resuspended in 4 ml of the latter buffer before analyses were carried out in the final suspension.

Binding assay

Platelet membrane suspension (100 μl) was incubated with increasing concentrations of [^{3}H]imipramine (0.2–2.0 nM) in a total volume of 500 μl for 3 h at 0°C. Incubation was terminated by addition of 5 ml ice-cold incubation buffer (Tris–EDTA, pH 7.5) and rapid filtration through GF/F glass-fibre filters. The filters were washed with 20 ml ice-cold buffer and counted in a scintillation counter. Non-specific binding was determined in the presence of 1 μM desmethylimipramine. Specific binding was calculated from the difference between total binding and non-specific binding.

PRP 5-HT determination

About 10 ml blood was collected in the morning at the same time as for the determination of platelet [^{3}H]imipramine binding. After centrifugation at 200 *g* for 20 min at room temperature, PRP was collected. The platelet count of the PRP was measured manually with a Coulter counter. PRP was then kept frozen in the dark at -70°C until analysis. PRP 5-HT was assayed blindly by a reversed-phase HPLC method with coulometric detection [15]. Coulometric detection is known to minimize analytical interference and to allow selective chromatographic detection. The determinations used 1 ml PRP. The results correspond to platelet 5-HT levels plus plasma unconjugated 5-HT levels (corresponding to 2–4% of platelet 5-HT). The minimum quantifiable level was 0.2 ng/ml. The interassay coefficients of variation were 2.0, 3.0 and 2.7% for 30, 120 and 300 ng/ml 5-HT concentrations respectively.

Determination of biogenic amine metabolites in CSF

The substances measured were 3-methoxy, 4-hydroxy phenylglycol (MHPG, a norepinephrine metabolite), 5-hydroxyindoleacetic acid (5-HIAA; from serotonin) and homovanillic acid (HVA; from dopamine). The tubes containing CSF samples were centrifuged, aliquoted and frozen on the day of lumbar puncture, then kept at -70°C in the dark. Just before analysis, they were thawed in ice-cold water, 50 μl of the vortexed content was measured in an Eppendorf tube containing 5 μl of the internal standard solution [4 ng of 5-hydroxyindolecarboxylic acid (5-HICA)], vortexed again and injected into a high-performance liquid chromatography system using a 20 μl sample loop [16]. The mobile phase was a 100 mM phosphoric acid buffer containing 2 mM sodium octyl sulphonate adjusted to pH 2.56 with NaOH. Methanol (40 ml) was added to 500 ml of buffer. The flow rate was 1 ml/min through a reversed-phase column (Lichrospher 60 RP-select B, 4 × 125 mm, 5 μm; Merck). For coulometric detection (Coulochem II; ESA, Bedford, MA, USA) a dual electrochemical cell (ESA 5011) was used. The first detector was set at + 50 mV and the second was set to a reporting integrator (D2000; Merck) at + 360 mV. A guard cell (ESA 5020) was inserted between the pump and the injector and set at + 370 mV. The substances analysed were, in order of elution, MHPG, 5-HIAA, HVA and 5-HICA. Relative response factors to the internal standard of each component were obtained from known amounts in a standard solution. Concentrations of unknown compounds were calculated from the response factor and areas of each metabolite relative to the internal standard area. The results were corrected for the initial dilution with internal standard solution. All assays were conducted blind on numbered samples.

Statistical analysis

SAS (release 6.12; SAS Institute Inc, Cary, NC, USA) statistical software was used for all analyses. Platelet [^{3}H]imipramine binding parameters assessed in PFS patients and matched controls were compared using Student's *t*-test. For PRP 5-HT, assessed in PFS patients, matched controls and CSF controls, and for CSF metabolites, assessed in PFS patients and CSF controls, comparisons were performed by analysis of covariance including the factors age, gender and age plus gender, in order to investigate the potential effect of these factors on the variables studied. The analysis of PRP 5-HT, measured in the three groups, was completed by the Bonferroni multiple comparison test. The relationships between parameters of interest were studied using the Pearson correlation coefficient.

The number of subjects required was determined before the start of the study. The density of platelet [^{3}H]imipramine binding sites was used as the main variable, in accordance with Poirier *et al*. [17]. Thirty subjects in each group were needed to show a difference of 20% in B_{max} ($\alpha = 5\%$, $\beta = 5\%$; bilateral test).

Results

A summary of the demographic characteristics of the subjects is shown in Table 1. PFS patients and healthy control subjects were matched in sex and age; the sex ratio and the mean age were therefore the same in the two groups. On the contrary, due to the inclusion criteria for the CSF controls, these subjects were significantly

TABLE 1. Demographic characteristics of participants in the study

	PFS patients	Healthy controls	CSF controls
Age (yr), mean ± S.E.M.	47.6 ± 1.8	47.3 ± 1.8	66.7 ± 1.4
No. of subjects	30	30	69
Females, males	24, 4	26, 4	31, 38
Erythrocyte sedimentation rate (mm/h), mean ± S.E.M.	9.2 ± 1.2	6.8 ± 1.1	ND

ND, not done.

older ($P = 0.0001$) and their sex ratio was different ($P = 0.001$). For these reasons, analyses involving the CSF controls were adjusted for age and sex. In addition, because of the limited number of male patients in the PFS group, confirmatory analysis was restricted to female patients. Table 2 shows which investigations were performed in the three populations (PFS patients, matched healthy controls and CSF controls) and the numbers of subjects involved in each investigation.

Imipramine binding sites on the serotonin transporter of the platelet membranes were used as a marker of serotonergic function. Table 3 shows the mean B_{max} values for the density and K_d for the affinity of [^{3}H]imipramine binding on platelets in the patients and in healthy controls (the results of one PFS patient were not available due to a laboratory error). No statistically significant difference was found between the two populations for B_{max} and K_d.

Scatter-plots of the individual concentrations of PRP 5-HT in each subject in the three populations are shown in Fig. 1. The analysis was performed with and without covariant adjustment. Covariants did not affect the variability of the outcome parameters significantly, and unadjusted analysis was used. This indicated that the mean values of PRP 5-HT were similar in healthy controls and CSF control subjects, while both were significantly lower ($P = 0.0009$) than the values obtained in PFS patients (Table 4). These results were confirmed by an analysis restricted to females. PRP 5-HT values were not correlated with the B_{max} ($P = 0.540$) or K_d ($P = 0.643$) values for [^{3}H]imipramine binding on platelets.

Table 5 shows the mean CSF concentration of each metabolite in the PFS patients and CSF controls. The covariant age was shown to have an effect on the variability of the metabolites. However, when adjusted for age, MHPG concentration remained significantly lower ($P = 0.003$) in PFS patients than in CSF controls. When males and females were considered separately, this difference was no longer statistically significant. However, when the analysis was restricted to the female population the CSF 5-HIAA values were significantly

TABLE 2. Investigations performed and numbers of subjects

	PFS patients (*n*)	Matched healthy controls (*n*)	CSF controls (*n*)
[^{3}H]Imipramine binding	2	30	ND
PRP 5-HT	30	30	69
CSF 5-HIAA	29	ND	69
CSF MHPG	29	ND	69
CSF HVA	29	ND	69

ND, not done.

TABLE 3. [^{3}H]Imipramine platelet binding

	PFS patients ($n = 29$)	Healthy controls ($n = 30$)	P (Student's t-test)
B_{max} (fmol/mg protein)	901 ± 83	1017 ± 119	0.43
K_d	0.682 ± 0.046	0.606 ± 0.056	0.30

Data are mean ± S.E.M.

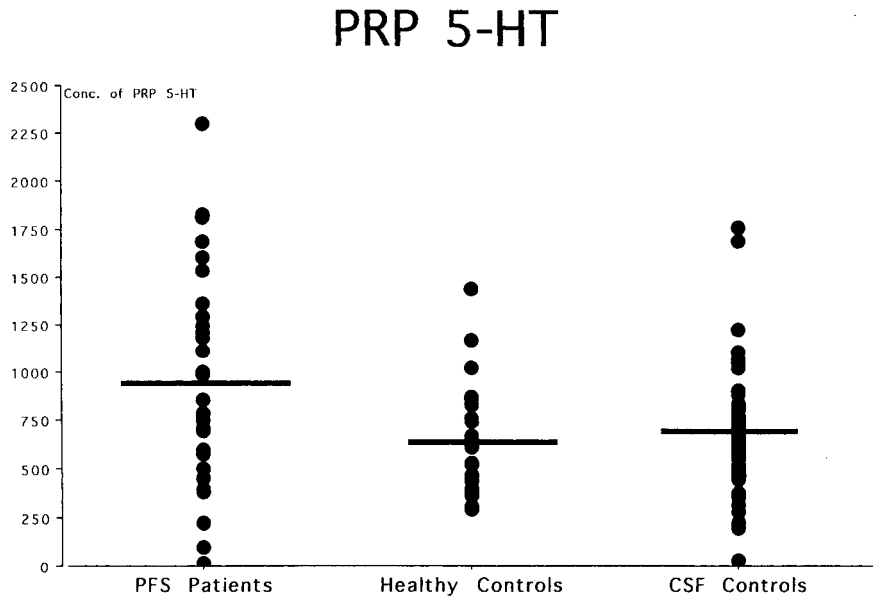

FIG. 1. Scatter-plots of concentrations of PRP 5-HT in individuals of the three populations studied.

Table 4. PRP 5-HT levels (ng/10^9 platelets) in the three populations

	PFS patients (4 males, 26 females)	Matched controls (4 males, 26 females)	CSF controls (38 males, 31 females)
Male	926 ± 409	739 ± 114	703 ± 49
Female	960 ± 103	617 ± 55	657 ± 48
Whole population	955 ± 101	633 ± 50*	682 ± 34*

Data are mean ± S.E.M.
*$P = 0.0009$ compared with PFS patients.

Table 5. Biogenic amine metabolites in CSF (ng/ml)

	MHPG	5-HIAA	HVA
PFS patients ($n = 29$)	8.33 ± 0.33	21.54 ± 1.58	35.01 ± 2.87
CSF controls ($n = 69$)	9.89 ± 0.31	25.46 ± 1.27	36.37 ± 1.66
P (ANCOVA)	0.003	0.618	0.172
Males			
PFS patients ($n = 4$)	9.23 ± 1.45	16.58 ± 1.15	26.83 ± 4.48
CSF controls ($n = 38$)	10.08 ± 0.43	25.23 ± 1.82	34.78 ± 2.84
Females			
PFS patients ($n = 25$)	8.19 ± 0.32	22.34 ± 1.78	36.32 ± 3.20
CSF controls ($n = 31$)	9.67 ± 0.43	25.75 ± 1.75	38.32 ± 2.90
P (ANCOVA)	0.915	0.042	0.054

Data are mean ± S.E.M. (adjusted for age, whenever age was significant as a covariate).
ANCOVA, analysis of covariance.

lower ($P = 0.042$) in PFS patients than in CSF controls, and only a borderline difference was found for CSF HVA levels.

The correlation between age and biogenic metabolites in CSF was of interest because age differed significantly between the PFS patients and the CSF controls. The correlation coefficients are reported in Table 6. Most of them are statistically significant, particularly those involving 5-HIAA concentration. However, the correlation between age and PRP 5-HT levels was not significant ($r = 0.025$, $P = 0.78$).

We also tested for correlations among biogenic amine metabolite concentrations in CSF. CSF 5-HIAA levels appeared to be correlated with CSF HVA and CSF MHPG levels, both in PFS patients ($P < 0.001$ and $P < 0.003$ respectively) and in CSF controls ($P < 0.001$ and $P < 0.01$), and the correlation between CSF MHPG and CSF HVA levels was also statistically significant ($P = 0.007$ in PFS patients and $P < 0.001$ in CSF controls).

Table 6. Correlation coefficient (*r*) between age and biogenic amine metabolites in CSF

	All subjects	PFS patients	CSF controls
5-HIAA	0.362 ($P < 0.001$)	0.533 ($P = 0.002$)	0.265 ($P = 0.027$)
MHPG	0.275 ($P = 0.006$)	0.173 ($P = 0.370$)	0.121 ($P = 0.321$)
HVA	0.245 ($P = 0.014$)	0.460 ($P = 0.012$)	0.220 ($P = 0.069$)

Discussion

The main findings of our study are the high levels of PRP 5-HT associated with low levels of CSF 5HIAA in the PFS patients compared with the controls, with no change in peripheral serotonergic uptake function assessed by the level of platelet [^{3}H]imipramine binding.

Binding of [^{3}H]imipramine, a biochemical indicator of platelet serotonin uptake or transporter sites, has been investigated previously in depressed patients, with discordant results. The platelet is considered to be a peripheral model of neuronal activity with respect to 5-HT function. Some studies found low levels of imipramine binding sites on platelets from depressed individuals [18], and this has been used as a biological marker of depression [19]. However, a decreased number of platelet 5-HT transporter sites has become one of the most reproducible parameters in the biology of affective disorders [20]. On the basis of the hypothetical [21–23] link between PFS and depression, Russel *et al.* [13] found a higher number of platelet [^{3}H]imipramine binding sites in PFS patients than in healthy controls, whereas Kravitz *et al.* [24] found no significant difference. However, Magni *et al.* [25] showed that the number of imipramine binding sites was decreased in the platelets of patients with the chronic pain syndrome. According to the results of Kravitz *et al.* [24], our data do not confirm changes in the number of imipramine binding sites in PFS despite the fact that, as in the work of Russel *et al.* [13] and Magni *et al.* [25], patients were selected by the use of the same criteria as those defined by the ACR. We checked carefully that no patient took medication that might interfere with the number or

affinity of imipramine binding sites. Matched control subjects were selected at the same time as PFS patients in order to exclude any seasonal effect.

Serotonin is known to be a chemical mediator of pain perception, psychiatric disturbances, deep sleep and bowel transit, which are the main clinical characteristics of PFS. The similar PRP 5-HT concentrations obtained in the two populations of controls confirmed the validity of the CSF control group and gave more importance to the higher mean values observed in the PFS patients. Aspirin intake, which was not controlled in the CSF control subjects, could have influenced the assessment of 5-HT if it had been done in serum, which needs a total clotting process for sample preparation, but was not applicable in our study, which used PRP. However, the observed high level of PRP 5-HT should influence the uptake of serotonin in the platelet membrane, but no correlation was found between B_{max} values and PRP 5-HT levels. Our findings do not confirm the results of Russel *et al*. [13], who showed a low serum serotonin concentration and up-regulation of [^{3}H]imipramine binding. However, values of serum 5-HT found in the work of Russel *et al*. [13] were within the normal range, their numbers of patients and healthy controls were very low ($n = 9$), they did not use seasonal matching and used a less accurate method of analysis. In our study, the greater variability of the PRP 5-HT levels obtained in PFS patients compared with CSF controls and healthy controls could have resulted from deregulation of 5-HT metabolism, or could have been related to differences in the populations studied, as suggested by Houvenagel *et al*. [26]. In a recent study, Wolfe *et al*. [27] found a significantly lower level of serum serotonin in persons with fibromyalgia compared with those without fibromyalgia in the general population. However, comparison between subjects suffering from fibromyalgia and pain-free subjects did not demonstrate a statistically significant difference even with 292 subjects, and the authors noticed a wide range of values. Schwarz *et al*. [28] showed that there was a correlation between high serum concentration of 5-HIAA and low pain score in patients suffering from fibromyalgia. Stratz *et al*. [29] also showed a lower serum concentration of serotonin in patients suffering from fibromyalgia than in healthy volunteers, but the authors did not report either the matching of the subjects by age, sex, season and time of sampling, which are known to be factors of variability, or the eventual use of aspirin, despite the fact that the serotonin was assessed in serum.

Our results concerning CSF biogenic amine metabolites confirm those of Russel *et al*. [12] except for MHPG in female PFS patients; our data reached statistical significance only for the total population. Despite the fact that the changes in CSF biogenic amines appeared to be related to age, they were also diagnosis-dependent. Indeed, the results were adjusted for age, so that age cannot account for the significant differences between subgroups in the levels of neurotransmitter metabolites in CSF. Because of the method of recruitment of the controls, the CSF control group differed from the PFS patient group in sex ratio and age (Table 1). A positive correlation with age has also been shown by Russel *et al*. [12], Houvenagel *et al*. [26] and others [30–32]. The strong correlation between CSF 5-HIAA and CSF HVA described by Russel *et al*. [12] was also obtained with our data; it seems to be physiological rather than related to PFS. However, the correlation of age with CSF 5-HIAA or HVA appears to be more important in PFS patients than in controls (Table 6). The concentration of substance P, which is known to have a role in pain transmission and which is influenced by serotonin, has been shown to be three times higher in the CSF of patients with fibromyalgia than in normal controls [33].

This study does not allow confirmation of the involvement of serotonin in the pathophysiological process underlying the symptoms of PFS patients. Changes in the concentrations of biogenic amine metabolites in the CSF, after controlling for age, remained statistically different and were similar to those observed by Russel *et al*. [13]. High levels of PRP 5-HT were detected in PFS patients, contrary to previous findings with regard to serum serotonin [13, 28, 29], but they were not accompanied by any change in peripheral serotonergic function, assessed by the number of platelet [^{3}H]imipramine binding sites. Further investigation of plasma 5-HIAA levels could be of interest in relation to the possibility of peripheral deregulation of serotonin metabolism similar to that found in CSF.

Acknowledgements

This work was supported by a State grant for clinical pharmacology research in France.

References

1. Smythe HA. 'Fibrositis' as a disorder of pain modulation. Clin Rheum Dis 1979;5:823–32.
2. Yunus MA, Masi AT, Calabro JJ *et al*. Primary fibromyalgia (fibrositis): Clinical study of 50 patients with matched normal controls. Semin Arthritis Rheum 1981; 11:151–71.
3. Wolfe F, Hawley D, Cathey MA. Fibrositis: symptoms, frequency and criteria for diagnosis. J Rheumatol 1985; 12:1159–63.
4. Wolfe F, Smythe HA, Yunus MB *et al*. The American College of Rheumatology 1990 criteria for the classification of fibromyalgia: report of the multicenter criteria committee. Arthritis Rheum 1990;33:160–72.
5. Bartels EM, Danneskiold-Samsoe B. Histological abnormalities in muscle from patients with certain types of fibrositis. Lancet 1984;1:755–7.
6. Bengtsson A, Henriksson KG, Larsson J. Muscle biopsy in primary fibromyalgia: light microscopal and histochemical findings. Scand J Rheumatol 1986;15:1–6.
7. Moldofsky H, Warsh JJ. Plasma tryptophan and musculoskeletal pain in non-articular rheumatism ('fibrositis syndrome'). Pain 1978;5:65–71.
8. Moldofsky H. Rheumatic pain modulation syndrome: the interrelationships between sleep, central nervous system serotonin and pain. Adv Neurol 1982;33:51–7.

9. Russell IJ, Michalek JE, Vipriao GA, Flechter EM, Wall K. Serum amino acids in fibrositis/fibromyalgia syndrome. J Rheumatol 1989;16(Suppl. 19):158–63.
10. Murphy RM, Zemla FP. Differential effects of substance P on serotonin-modulated spinal nociceptive reflexes. Psychopharmacology 1987;93:118–21.
11. Vaeroy H, Helle R, Firre I, Kass E, Terenius L. Elevated CSF levels of substance P and high incidence of Raynaud's phenomenon in patients with fibromyalgia: new features for diagnosis. Pain 1988;32:21–6.
12. Russel IJ, Vaeroy H, Javors M, Nyberg F. Cerebrospinal fluid biogenic amine metabolites in fibromyalgia/fibrositis syndrome and rheumatoid arthritis. Arthritis Rheum 1992; 35:550–6.
13. Russel IJ, Michalek JE, Vipraio GA, Flechter EM, Javors MA, Bowden CA. Platelet 3H-imipramine uptake receptor density and serum serotonin levels in patients with fibromyalgia/fibrositis syndrome. J Rheumatol 1992; 19:104–9.
14. Mellerup E, Langer SZ. A World Health Organization collaborative study: Validity of imipramine platelet binding sites as a biological marker of endogenous depression. Pharmacopsychiatry 1990;23:113–7.
15. Spreux-Varoquaux O, Gailledreau J, Vanier B *et al.* Initial increase of plasma free serotonin: a biological predictor for the antidepressant response to clomipramine. Biol Psychiatry 1996;40:465–73.
16. Matson WR, Langlais P, Volicer L, Gamache PH, Bird E, Mark KA. n-Electrode three dimensional liquid chromatography with electrochemical detection for determination of neurotransmitter. Clin Chem 1984;30:1477–88.
17. Poirier MF, Benkelaft C, Loo H *et al.* Reduced Bmax of 3H-imipramine binding to platelets of depressed patients free of previous medication with 5HT uptake inhibitors. Psychopharmacology 1986;89:456–61.
18. Briley MS, Langer SZ, Raisman R, Sechter D, Zarifian E. Tritiated imipramine binding sites are decreased in platelets of untreated depressed patients. Science 1980;209:303–5.
19. Carsten ME, Engelbrecht AH, Russel VA *et al.* Imipramine binding sites on platelets of patients with major depressive disorder. Psychiatry Res 1986;18:333–42.
20. Owens MJ, Nemeroff CB. Role of the serotonin in the pathophysiology of depression: focus on the serotonin transporter. Clin Chem 1994;40:288–95.
21. Hudson JI, Hudson MB, Pliner LF, Goldenberg DL, Pope HG. Fibromyalgia and major affective disorder: a controlled phenomenology and family history study. Am J Psychiatry 1985;142:441–6.
22. Goldenberg DL. Psychiatric and psychological aspects of fibromyalgia syndrome. Rheum Dis Clin North Am 1989;15:105–15.
23. Ahles TA, Yunus MB, Masi AT. Is chronic pain a variant of depressive disease? The case of primary fibromyalgia syndrome. Pain 1987;29:105–11.
24. Kravitz MH, Katz R, Kot E, Helmke N, Fawcett J. Biochemical clues to a fibromyalgia–depression link: imipramine binding in patients with fibromyalgia or depression and in healthy controls. J Rheumatol 1992; 19:1428–32.
25. Magni G, Andreoli F, Arduino C. 3H-Imipramine binding sites are decreased in platelets of chronic pain patients. Acta Psychiatr Scand 1987;75:108–10.
26. Houvenagel E, Forzy G, Cortet B, Vincent G. 5-Hydroxyindol acetic acid in cerebrospinal fluid in fibromyalgia [abstract]. Arthritis Rheum 1990;33(Suppl. 9):S55.
27. Wolfe I, Russel IJ, Vipraio G, Ross K, Anderson J. Serotonin levels, pain threshold and fibromyalgia symptoms in the general population. J Rheumatol 1997; 24:555–9.
28. Schwarz MJ, Spath M, Muller-Bardorff H, Pongratz DE, Bondy B, Ackenheil M. Relationship of substance P, 5-hydroxyindole acetic acid and tryptophan in serum of fibromyalgia patients. Neurosci Lett 1999;259:196–8.
29. Stratz T, Samborski W, Hrycaj P *et al.* Serum serotonin concentration in patients with generalized tendomyopathy (fibromyalgia) and rheumatoid arthritis. Med Klin 1993; 88:458–62.
30. Blennow K, Wallin A, Gottfries CG *et al.* Cerebrospinal fluid monoamine metabolites in 114 healthy individuals 18–88 years of age. Eur Neuropsychopharmacol 1993; 3:55–61.
31. Bowers M, Gerbode F. The relationship of monoamine metabolite in human CSF to age. Nature 1968; 219:1256–7.
32. Gottfries CG, Gottfries I, Johansson B *et al.* Acid monoamine metabolites in human CSF and their relationship to age and gender. Neuropharmacology 1971;10:665–72.
33. Russel IJ, Orr MD, Littman B *et al.* Elevated cerebrospinal fluid levels of substance P in patients with the fibromyalgia syndrome. Arthritis Rheum 1994;37:1593–601.