



β -Alanine and γ -aminobutyric acid in chronic fatigue syndrome

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Abstract

Background: Due to the occurrence of sleep disturbances and fatigue in chronic fatigue syndrome (CFS), an investigation was performed to examine if there is an abnormal excretion of γ -aminobutyric acid (GABA) and/or its structural analogue β -alanine in the urine from CFS patients. Both GABA and β -alanine are inhibitory neurotransmitters in the mammalian central nervous system.

Methods: The 24 h urine excretion of GABA and β -alanine was determined by isotope dilution gas chromatography mass spectrometry in 33 CFS patients and 43 healthy controls. The degree of symptoms in both patients and controls was measured by grading of three typical CFS symptoms using a Visual Analogue Scale.

Results: Men had a significantly higher excretion of both β -alanine and GABA than women. Comparing CFS patients with healthy controls showed no significant difference in excretion of neither β -alanine nor GABA. No correlation was found between the excretion of β -alanine or GABA and any of the three characteristic CFS symptoms measured. However, two female and two male CFS patients excreted considerably higher amounts of β -alanine in their 24 h urine samples than control subjects.

Conclusions: Increased excretion of β -alanine was found in a subgroup of CFS patients, indicating that there may be a link between CFS and β -alanine in some CFS patients.

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1. Introduction

Chronic fatigue syndrome (CFS) is a disabling illness, with major impact on quality of life. No single pathogenetic factor has as yet been convincingly demonstrated [1]. In the majority of CFS patients, the symptoms of fatigue and neurocognitive dysfunctions start after a flu-like illness, indicating that viral infections may play a pathogenic role [2].

Disabling fatigue is the most characteristic symptom of CFS. One of the neurotransmitters involved in regulating sleep and which also likely has a potential of evoking fatigue is γ -aminobutyric acid (GABA) by activating GABA_A receptors [3–5]. GABA is the major inhibitory neurotransmitter of the central nervous system (CNS), and GABA receptors can be found on 60–75% of its neurones [6]. Many hypnotics, including barbiturates and benzodiazepines, act by stimulating GABA re-

ceptors. β -Alanine is an amino acid and a structural analogue of GABA, differing only by having one less methylene group.

β -Alanine found in the CNS originates in part from the metabolism of the polyamines putrescine, spermidine and spermine [7,8].

In cases of elevated concentrations of β -alanine or GABA in CNS one can expect to find increased excretion of these amines in urine as there is an active transport of both β -alanine and GABA from CNS to blood over the blood–brain barrier. Studies by Komura et al. [9,10] and Kakee et al. [11] have demonstrated the active transfer of β -amino acids, including GABA and β -alanine, from CNS to blood. Patients with decreased levels of 4-aminobutyrate aminotransferase, also called GABA-transaminase, have extremely high CNS concentrations of β -alanine and GABA, which results in excretion of large amounts of the two substances in the urine [12,13]. GABA-transaminase deficiency also results in very high concentrations of β -alanine and GABA in the cerebrospinal fluid and plasma [12,13]. Among the most pronounced symptoms observed in these patients were lethargy, somnolence, seizures and retarded psychomotor development.

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Interestingly, McGregor et al. have shown that there is a positive correlation between typical CFS symptoms and an increased excretion of β -alanine in the urine of CFS patients [14]. In contrast, Jones et al. did not find such a connection [15].

The aim of the present study was to investigate, using state-of-the-art methodology, if increased amounts of β -alanine and/or GABA are found in the urine from CFS patients compared to healthy controls. The rationale for this approach was that GABA is an inhibitory neurotransmitter in CNS involved in sleep regulation [3,4]. β -Alanine, also activating GABA_A receptors [16,17], could likewise be involved in sleep regulation. Indeed, it has been demonstrated that intracerebroventricular injections of β -alanine in chicks strongly diminish spontaneous activity and induce sleep-like behaviour [18]. It has furthermore been shown that the same administration of β -alanine to rats causes inhibition of exploratory behaviour and of motility [19]. These animal model studies agree with our hypothesis that increased extracellular concentrations of β -alanine in the CNS may be among the pathogenic factors in CFS.

Using an isotope dilution gas chromatography mass spectrometry (ID GC–MS) method, we measured the excretion of β -alanine and GABA in the urine of 33 CFS patients (22 female and 11 male) and compared with a group of 42 healthy controls (24 female and 19 male). Typical CFS symptoms of all subjects were recorded and eventual relation between symptoms and excretion of β -alanine/GABA was determined.

2. Study cohort and materials

This study was approved by the local Ethics Committees at the Karolinska University Hospital, Huddinge (Stockholm, Sweden) and at the University Hospital in Linköping (Sweden) with approval number 03-379. The CFS patients included in the study, 22 women and 11 men, fulfilled the 1994 case definition for CFS [20]. They were recruited from an outpatient clinic specialised for CFS patients at the Karolinska University Hospital, Huddinge. Age and sex matched healthy subjects were recruited among hospital staff as a control group, and consisted of 24 women and 19 men. All patients and healthy subjects gave informed consent before participating in the study. Patients and controls had similar age distribution (Table 1).

The patients and the healthy controls collected urine during a 24 h period in a plastic bottle containing 15 ml of hydrochloric acid (6.0 mol/l) for stabilisation of amines and preservation. All samples except five were collected during May to June 2004. Five of the patients participated in a pilot study and collected

their samples during September to October 2002. The participating subjects, except for the five pilot study patients, were also asked to fill out a questionnaire where any medication was noted together with grading of typical CFS symptoms. Three different symptoms, fatigue, muscle pain and cognitive difficulties, were recorded on a continuous Visual Analogue Scale (VAS), 0–10. Among these patients, 10 (36%) had a sudden onset, whereas 18 (64%) had gradual onset of their CFS. The mean duration of the illness was 7.4 years with a range of 1.5 to 25 years. In 14 (50%) of the patients the symptoms of CFS started after a flu-like infection.

All chemicals used were of analytical grade. GABA and β -alanine were purchased from Fluka Chemie (Buchs SG, Switzerland). β -Alanine (U-13C₃; 15N) was from Cambridge Isotope Laboratories, Inc (MA, USA), and GABA-2,2,3,3,4,4-D₆ from CDN Isotopes (Quebec, Canada). All chemicals used for derivatization, ethyl acetate, 1-hexanol, acetylchloride and heptafluorobutyric anhydride, were obtained from Fluka Chemie (Buchs SG, Switzerland).

3. Methods

The volume of the collected urine was determined. A portion of the urine was frozen and stored at $-20\text{ }^{\circ}\text{C}$ until analysed. The concentration of creatinine was assayed by an enzymatic method on a Bayer Advia 1650 instrument, according to the manufacturer's instructions. For the determination of the concentration of β -alanine and GABA, 25 μl urine was hydrolysed for 20 h at $120\text{ }^{\circ}\text{C}$ in hydrochloric acid, 6.0 mol/l. All urine samples were analysed blinded. The derivatization method used was a modification of the method described by MacKenzie and Tenaschuk [21,22]. After hydrolysis, 100 μl of an internal standard solution was added and the samples were taken to dryness under a stream of compressed air. Esterification was done by treating the samples with acetyl chloride, 1.5 mol/l, in 1-hexanol at $120\text{ }^{\circ}\text{C}$ for 15 min. After blowing air over the samples, the dry residues were dissolved in 200 μl of ethyl acetate and 50 μl of heptafluorobutyric anhydride was added. After 15 min at $90\text{ }^{\circ}\text{C}$, the reagent in the samples was taken to dryness with compressed air. Ethyl acetate, 200 μl , was added and the samples were analysed on a mass spectrometer. Isotope dilution technique was used for quantification of β -alanine and GABA. β -Alanine (U-13C₃; 15N) and γ -aminobutyric-2,2,3,3,4,4-D₆ acid were used as internal standards for β -alanine and GABA. To determine the between-run variation, three different urine samples with low, medium and high concentrations of β -alanine and GABA were

Table 1
Symptoms recorded by CFS patients and healthy controls on a 0–10 Visual Analogue Scale (VAS)

Group	Number	Age, years	Symptoms (VAS), mean (range)		
	F = female	mean (range)	Fatigue	Muscle	Cognition/ memory
	M = male				
CFS patients	20 F	39.6 (25.5–53.5)	7.1 ^a (2.2–9.6)	4.8 ^a (0.0–7.5)	5.7 ^a (0.4–9.2)
	8 M	40.4 (30.1–53.3)	6.3 ^a (0.7–9.7)	4.0 ^a (0.0–8.0)	4.5 ^a (0.0–9.6)
Controls	24 F	41.9 (28.9–54.1)	1.6 (0.0–5.7)	0.7 (0.0–4.7)	0.9 (0.0–3.2)
	19 M	40.6 (32.2–48.3)	1.4 (0.0–5.0)	0.9 (0.0–4.8)	1.0 (0.0–4.5)

^a Significantly more symptoms in patients than in controls ($p < 0.001$).

used as controls and were included in each urine analysis. The mass spectrum of β -alanine, GABA and the two internal standards were determined (Fig. 1) and quantification was done

using a Hewlett Packard 5970 MSD mass spectrometer coupled to a HP 5890 gas chromatograph using a HP 1-MS column, 30 m \times 0.25 mm with a film thickness of 0.25 μ m. After sample

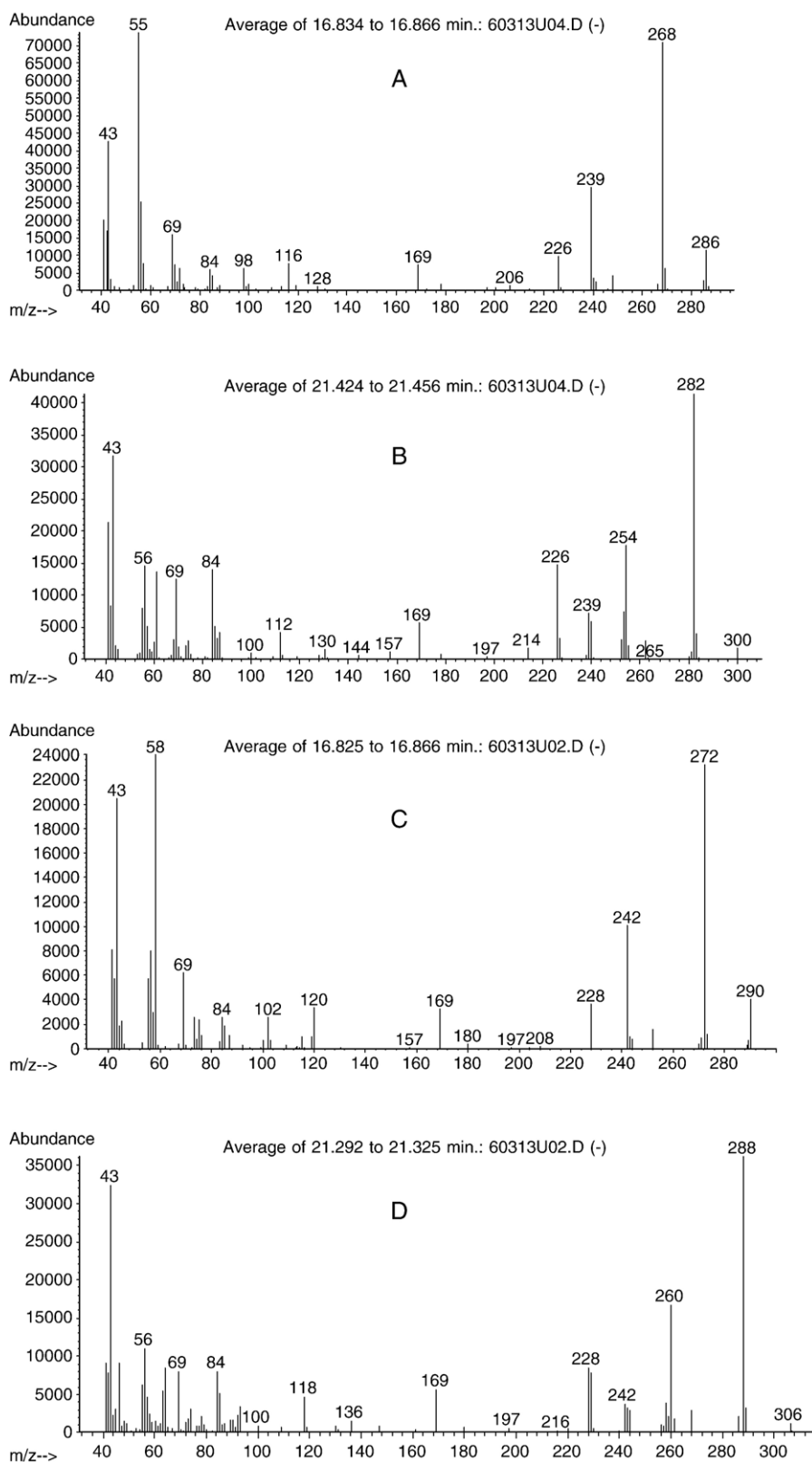


Fig. 1. Mass spectra of the heptafluorobutryl-*n*-hexyl derivatives together with the mass fragment used for quantification: A) β -alanine; m/z 268, B) β -alanine (U- $^{13}\text{C}_3$; ^{15}N), used as internal standard; m/z 282, C) γ -aminobutyric acid (GABA); m/z 272, D) γ -aminobutyric-2,2,3,3,4,4-D $_6$ acid, used as internal standard; m/z 288.

Table 2
Between-run variation in determination of total β -alanine and GABA in three different control urine samples

	β -Alanine ($\mu\text{mol/l}$)			GABA ($\mu\text{mol/l}$)		
	Control sample no.			Control sample no.		
	1	2	3	1	2	3
Mean	71.9	125	350	28.3	43.7	117
Minimum	68.9	120	338	26.7	39.8	110
Maximum	75.4	129	360	30.3	46.0	123
SD	1.87	2.93	6.91	1.34	2.05	4.32
CV (%)	2.60	2.35	1.98	4.74	4.68	3.69

Number of analysis of each control sample = 9.

SD = standard deviation. CV = coefficient of variation.

injection, the oven temperature was held at 100 °C for 1 min and then ramped to 285 °C at 3 °C/min. Under these conditions, the derivatives of β -alanine and its internal standard eluted after around 17 min. GABA and its internal standard eluted after about 22 min. The analyses and quantification on the mass spectrometer were carried out by selected ion monitoring, with acquisition of ions m/z 268.1 and m/z 282.1 for β -alanine and GABA, respectively. Acquisition of the internal standards β -alanine (U-13C3; 15N) and γ -aminobutyric-2,2,3,3,4,4-D6 acid was done with m/z 272.1 and m/z 288.1, respectively. All these four fragments are the result from loss of the neutral fragment O (CH₂)₅CH₃ from the molecular ion. Standard solutions with five different concentration levels, between 10 and 400 $\mu\text{mol/l}$ for β -alanine and between 5.0 and 200 $\mu\text{mol/l}$ for GABA, were used for quantification. To establish the proportion between free and conjugated β -alanine and GABA in urine, samples from twelve healthy controls were analysed both with and without acid hydrolysis.

Both β -alanine and GABA were quantified by ID GC–MS. This is an accurate and precise technique often used to establish reference or definitive methods for different analytes, as is done for example for glucose by National Institute of Standards and Technology (Gaithersburg, MD, USA). Data were analysed using the GLM-module of Systat 11 for Windows (<http://www.systat.com>) using the urinary excretion of β -alanine and GABA as dependent variables and sex, age and measures of tiredness, muscle ache and cognitive problems as independent variables. The analysis was performed both using non-transformed values

for the 24 h amounts and the natural logarithm of the same values (to correct for skewness).

4. Results

The results from grading of symptoms using VAS showed that the patients – as expected – had significantly more of the CFS related symptoms including fatigue, muscle problems and cognition/memory complaints, compared to the healthy controls (Table 1). The symptoms of fatigue and cognition/memory problems were most pronounced. All CFS patients and healthy controls except two (not included in the study) collected a full 24 h urine sample. One of the subjects admitted uncomplete urine collection and the other was excluded because of unreasonably small amounts of creatinine excreted. The between-day variation of the ID GC–MS method for the three concentration levels of the control samples is shown in Table 2. The between-run coefficient of variation ranged from 1.98–2.60% for β -alanine and 3.69–4.74% for GABA.

When measuring the 24 h excretion of β -alanine and GABA for the patients and control subjects, we found that men, as seen among the control subjects, excreted a significantly larger amount of both β -alanine and GABA during 24 h than women ($p < 0.05$). The mean excretion of β -alanine in the control group was for men 356 $\mu\text{mol}/24$ h (133–772 $\mu\text{mol}/24$ h, $n = 19$), and for women 261 $\mu\text{mol}/24$ h (55–652 $\mu\text{mol}/24$ h, $n = 24$). The mean excretion of GABA in the control group was 98 $\mu\text{mol}/24$ h (38–143 $\mu\text{mol}/24$ h, $n = 19$) for men and 83 $\mu\text{mol}/24$ h (52–121 $\mu\text{mol}/24$ h, $n = 24$) for women. Male patients had a mean excretion of β -alanine of 512 $\mu\text{mol}/24$ h (155–2107 $\mu\text{mol}/24$ h, $n = 11$) while the corresponding excretion for female patients was 228 $\mu\text{mol}/24$ h (103–652 $\mu\text{mol}/24$ h, $n = 22$). The mean excretion of GABA among CFS patients was 85 $\mu\text{mol}/24$ h (67–111 $\mu\text{mol}/24$ h, $n = 11$) for men and 69 $\mu\text{mol}/24$ h (38–114 $\mu\text{mol}/24$ h, $n = 22$) for women. When comparing male and female CFS patients with male and female controls respectively, no significant difference ($p > 0.05$) in excretion of neither β -alanine nor GABA was found. However, as can be seen in Fig. 2, two of the female and two of the male CFS patients excreted more of β -alanine than the healthy controls and other CFS patients. In contrast to β -alanine, no extraordinary high excretion of GABA could be seen among the patients compared to the controls (Fig. 3).

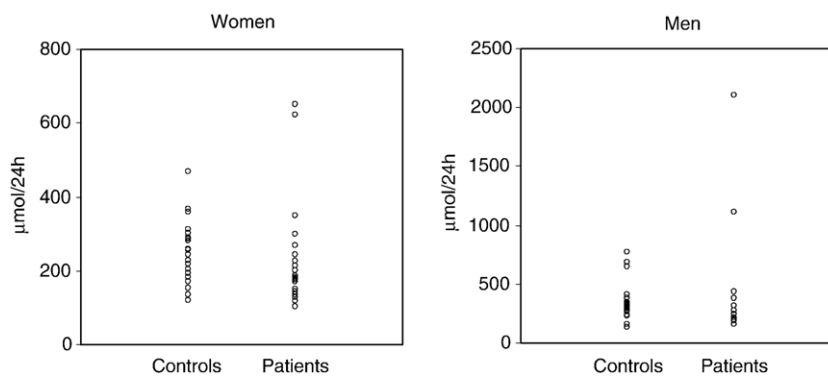


Fig. 2. Excretion of β -alanine in 24 h urine for female and male CFS patients and healthy controls.

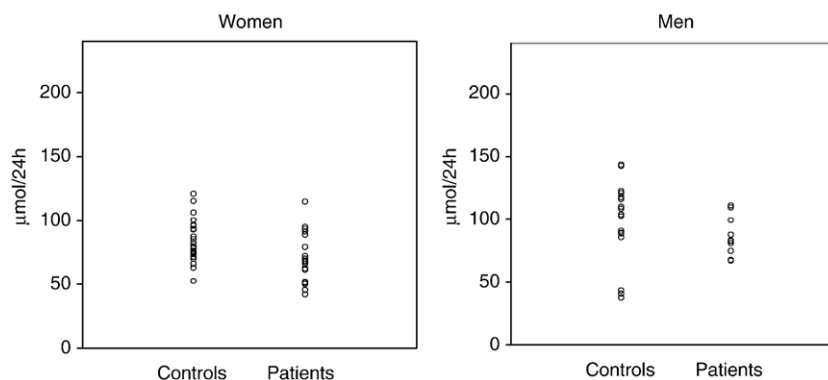


Fig. 3. Excretion of GABA in 24 h urine for female and male CFS patients and healthy controls.

The concentration of the free forms of β -alanine and GABA, together with the concentration of total β -alanine and GABA in urine was determined by analysis of twelve urine samples from healthy controls, both with and without acid hydrolysis. We found that about 30% of these amines existed in their free form and that about 70% were conjugated. The mean proportion of free β -alanine in urine was 35.2% (3.4–47.5%) and for GABA the mean was 29.4% (14.1–52.9%).

5. Discussion

We were unable to demonstrate significantly higher amounts of β -alanine and/or GABA excreted in the urine of CFS patients as a group compared to a group of healthy controls. However, two of the female and two of the male CFS patients excreted considerably more (250%, 239% and 580%, 306%, respectively more than the mean for healthy controls) of β -alanine compared to the healthy controls, indicating a possible link between high β -alanine excretion and CFS in a subgroup of patients. The mean duration of the illness in these four patients was 3.1 years (range 1.5–5.5 years). Three of these four patients had a gradual onset of CFS, the fourth a sudden onset. The symptoms of the four patients did not deviate significantly from the rest of the patients. Two of them had a flu-like onset of their CFS, two had not. However, the increased excretion of β -alanine in these patients supports the results of McGregor et al. [14], who found a relation between excretion of β -alanine and CFS symptoms. In contrast Jones et al. [15] found no abnormal excretion of β -alanine among CFS patients. In agreement with Jones et al., we were unable to detect the two substances CFSUM1 and CFSUM2, described by McGregor et al. [14], in the urine of patients or controls. Chalmers et al. [23] claim that CFSUM1 and CFSUM2 are artefacts from derivatization and not true endogenous metabolites.

Given its pronounced biological effects, it is possible that even a small increase in the concentration of β -alanine, in parallel with GABA, in the extracellular fluid of the CNS can result in symptoms of CFS [24]. This is analogous to the pronounced clinical effects of antidepressants despite the modest increases in the extracellular concentrations of monoamines they induce [25]. In the earlier studies, mentioned above, two patients with GABA-transaminase deficiency [12,13] had 5-fold elevated levels of β -alanine in cerebrospinal fluid and urine. Some of the CNS symptoms, i.e. pronounced somnolence and lethargy, seen in these

patients resemble those seen in CFS. This is in accordance with the hypothesis that in a subset of CFS patients the typical symptoms may be caused by high concentrations of β -alanine and possibly GABA in extracellular fluid of the CNS.

However, it is likely that minor elevations of the concentration of β -alanine in extracellular fluid of the CNS are difficult to detect by measuring urine excretion. The normal excretion of β -alanine is about 100–300 $\mu\text{mol}/24\text{ h}$, suggesting that a minor increased excretion caused by CFS can be masked by the normal variations in excretion of β -alanine. Thus periods of sufficiently high concentration of β -alanine in CNS to be reflected in urine are probably transient and difficult to capture, which might explain why we found so few patients with increased urinary excretion of β -alanine. Likewise this could explain the discrepancy in the findings of McGregor et al. [14] and Jones et al. [15] discussed above. There are differences in the protocols between these two studies and the present study, making it difficult to compare the results. In the current study the patients and healthy controls collected a 24 h urine sample and the concentration of β -alanine and GABA, including conjugated forms, was determined after acid hydrolysis of the urine samples. In the two other studies the study subjects did not collect a full 24 h urine sample, no hydrolysis was performed, and thus only the free form of β -alanine was determined. Also, the two previous studies did not report the results separately according to gender.

A possible source of the increased urine excretion of β -alanine seen in some CFS patients may be a low grade catabolic process (e.g. an ongoing virus infection in CNS), producing increased amounts of polyamines. Ornithine decarboxylase (ODC) is a highly inducible and strongly regulated enzyme that is rate-limiting in the production of polyamines [26–28]. Hence, an induction of ODC by an exogenous agent could result in enhanced levels of polyamines and β -alanine. Polyamines, and especially spermine, are known to be immunosuppressants. Spermine inhibits monocyte activation and natural killer cell activity [29–32], and therefore increased levels of spermine in the CNS could locally affect the immune system.

GABA and β -alanine also play a role in the effect of special drugs. There are antiepileptic drugs (e. g. vigabatrin) that inhibit GABA-transaminase and increase the amounts of these two inhibitory neurotransmitters in CNS [33,34] whereas the benzodiazepines act by stimulating GABA receptors [35]. The side effects

of these drugs resemble the typical symptoms of CFS, including fatigue, headache, dizziness and memory disturbances.

A phenomenon sometimes found in CFS, which also could be related to β -alanine/GABA, is the disturbance of the hypothalamo-pituitary-adrenal (HPA) axis [36–38]. Recently, mutations in 3 genes have been found which are involved in the HPA-axis activity [39]. It has also been shown that there is a connection between the activity of the GABA receptors and the function of the HPA axis. Indeed, benzodiazepines, which enhance the activity of β -alanine give HPA axis dysregulation as a side effect [40–44].

The sex difference observed in the present study was the finding that men excreted significantly more of both β -alanine and GABA than women. This difference has to our knowledge not been reported before and the reason for this difference is unclear.

Previous studies of the possible involvement of β -alanine in CFS have resulted in conflicting evidence. This may be due to a lack of standardisation in sampling and methodological inadequacies. Our study of patients with CFS and controls using standardised sampling procedures and an ID GC–MS analysis method, showed no overall difference between the groups regarding excretion of β -alanine and/or GABA. However, 2 female and 2 male patients of 33 CFS patients had markedly elevated excretion of β -alanine, suggesting a possible involvement of this inhibitory neurotransmitter in CFS.

These findings indicate that β -alanine, possibly derived from polyamines, may play a role in the pathogenesis of CFS in a subgroup of patients.

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References

- [1] Prins JB, van der Meer JWM, Bleijenberg G. Chronic fatigue syndrome. *Lancet* 2006;367:346–55.
- [2] Komaroff AL. Chronic fatigue syndromes: relationship to chronic viral infections. *J Virol Methods* 1988;21:3–10.
- [3] Gottesmann C. GABA mechanisms and sleep. *Neuroscience* 2002;111:231–9.
- [4] Siegel JM. The neurotransmitters of sleep. *J Clin Psychiatry* 2004;65(suppl 16):4–7.
- [5] Ketter TA, Post RM, Theodore WH. Positive and negative psychiatric effects of antiepileptic drugs in patients with seizure disorders. *Neurology* 1999;53:S53–67.
- [6] Schwartz RD. The GABAA receptor-gated ion channel: biochemical and pharmacological studies of structure and function. *Biochem Pharmacol* 1988;37:3369–75.
- [7] Morgan DML. Polyamines. An overview. *Mol Biotechnol* 1999;11:229–50.
- [8] Van den Berg GA, Elzinga H, Nagel GT, Kingma AW, Muskiet FJ. Catabolism of polyamines in the rat. Polyamines and their non- α -amino acid metabolites. *Biochim Biophys Acta* 1984;802:175–87.
- [9] Komura J, Tamai I, Senmaru M, Terasaki T, Sai Y, Tsuji A. Brain-to-blood active transport of β -alanine across the blood–brain barrier. *FEBS Lett* 1997;400:131–5.
- [10] Komura J, Tamai I, Senmaru M, Terasaki T, Sai Y, Tsuji A. Sodium and chloride ion-dependent transport of β -alanine across the blood–brain barrier. *J Neurochem* 1996;67:330–5.
- [11] Kakee A, Takanaga H, Terasaki T, Naito M, Tsuruo T, Sugiyama Y. Efflux of a suppressive neurotransmitter, GABA, across the blood–brain barrier. *J Neurochem* 2001;79:110–8.
- [12] Medina-Kauwe LK, Tobin AJ, De Meirleir L, et al. 4-Aminobutyrate aminotransferase (GABA-transaminase) deficiency. *J Inher Metab Dis* 1999;22:414–27.
- [13] Scriver CR, Pueschel S, Davies E. Hyper- β -alaninemia associated with β -aminoaciduria and γ -aminobutyricaciduria, somnolence and seizures. *N Engl J Med* 1966;274:635–43.
- [14] McGregor NR, Dunstan RH, Zerbes M, Butt HL, Roberts TK, Klineberg II. *Biochem Mol Med* 1996;57:73–80.
- [15] Jones MG, Cooper E, Amjad S, Goodwin CS, Barron JL, Chalmers RA. Urinary and plasma organic acids and amino acids in chronic fatigue syndrome. *Clin Chim Acta* 2005;361:150–8.
- [16] Steinbach JH, Akk G. Modulation of GABA(A) receptor channel gating by pentobarbital. *J Physiol* 2001;537:715–33.
- [17] Wang DS, Zhu HL, Li JS. β -Alanine acts on glycine receptors in the rat sacral dorsal commissural neurons. *Int J Neurosci* 2003;133:293–305.
- [18] Tomonaga S, Tachibana T, Takagi T, et al. Effect of central administration of carnosine and its constituents on behavior in chicks. *Brain Res Bull* 2004;63:75–82.
- [19] Mena Gomez MA, Carlsson A, Garcia de Yebenes J. The effect of β -alanine on motor behavior, body temperature and cerebral monoamine metabolism in rat. *J Neural Transm* 1978;43:1–9.
- [20] Fukuda K, Straus SE, Hickie I, Sharpe MC, Dobbins JG, Komaroff A. International Chronic Fatigue Syndrome Study Group. *Ann Intern Med* 1994;121:953–9.
- [21] MacKenzie SL, Tenaschuk D. Quantitative formation of N(O,S)-heptafluorobutyryl isobutyl amino acids for gas chromatographic analysis, I. Esterification. *J Chromatogr* 1979;171:195–208.
- [22] MacKenzie SL, Tenaschuk D. Quantitative formation of N(O,S)-heptafluorobutyryl isobutyl amino acids for gas chromatographic analysis, II. Acylation. *J Chromatogr* 1979;173:53–63.
- [23] Chalmers RA, Jones MG, Goodwin CS, Amjad S. CFSUM1 and CFSUM2 in urine from patients with chronic fatigue syndrome are methodological artefacts. *Clin Chim Acta* 2005;364:148–58.
- [24] Petroff OAC, Hyder F, Rothman DL, Mattson RH. Effects of gabapentin on brain GABA, homocarnosine, and pyrrolidone in epilepsy patients. *Epilepsia* 2000;41:675–80.
- [25] Wikell C, Hjorth S, Apelqvist G, et al. Sustained administration of the antidepressant venlafaxine in rats: pharmacokinetic and pharmacodynamic findings. *Naunyn-Schmiedeberg's Arch Pharmacol* 2001;363:448–55.
- [26] Wallace HM. Polyamines in human health. *Proc Nutr Soc* 1996;55:419–31.
- [27] Jänne J, Alhonen L, Leinonen P. Polyamines: from molecular biology to clinical applications. *Ann Med* 1991;23:241–59.
- [28] Schipper RG, Verhofstad AAJ. Distribution patterns of ornithine decarboxylase in cells and tissues: facts, problems, and postulates. *J Histochem Cytochem* 2002;50:1143–60.
- [29] Zhang M, Borovikova LV, Wang H, Metz C, Tracey KJ. Spermine inhibition of monocyte activation and inflammation. *Mol Med* 1999;5:595–605.
- [30] Evans CH, Lee TS, Flugelman AA. Spermine-directed immunosuppression of cervical carcinoma cell sensitivity to a majority of lymphokine-activated killer lymphocyte cytotoxicity. *Nat Immun* 1995;14:157–63.
- [31] Chamaillard L, Quemener V, Havouis R, Moulinoux JP. Polyamine deprivation stimulates natural killer cell activity in cancerous mice. *Cancer Res* 1993;13:1027–33.
- [32] Seiler N, Atanassov CL. The natural polyamines and the immune system. *Prog Drug Res* 1994;43:87–141.
- [33] Schechter PJ. Clinical pharmacology of vigabatrin. *Br J Clin Pharmacol* 1989;27:19S–22S.
- [34] Lahat E, Ben-Zeev B, Zlotnik J, Sela B-A. Aminoaciduria resulting from vigabatrin administration in children with epilepsy. *Pediatr Neurol* 1999;21:460–3.
- [35] Sigel E, Stephenson FA, Mamalaki C, Barnard EA. A γ -aminobutyric acid/benzodiazepine receptor complex of bovine cerebral cortex. *J Biol Chem* 1983;258:6965–71.
- [36] Parker AJR, Wessely S, Cleare AJ. The neuroendocrinology of chronic fatigue syndrome and fibromyalgia. *Psychol Med* 2001;31:1331–45.

- [37] Cleare AJ. The HPA axis and the genesis of chronic fatigue syndrome. *Trends Endocrinol Metab* 2004;15:55–9.
- [38] Cleare AJ. The neuroendocrinology of chronic fatigue syndrome. *Endocr Rev* 2003;24:236–52.
- [39] Smith AK, White PD, Aslakson E, Vollmer-Conna U, Rajeevan MS. Polymorphisms in genes regulating the HPA axis associated with empirically delineated classes of unexplained chronic fatigue. *Pharmacogenomics* 2006;7:387–94.
- [40] Grotoli S, Maccagno B, Ramunni J, et al. Alprazolam, a benzodiazepine, does not modify the ACTH and cortisol response to hCRH and AVP, but blunts the cortisol response to ACTH in humans. *J Endocrinol Invest* 2002;25:420–5.
- [41] Grotoli S, Giordano R, Maccagno B, Pellegrino M, Ghigo E, Arvat E. The stimulatory effect of canrenoate, a mineralocorticoid antagonist, on the activity of the hypothalamus–pituitary–adrenal axis is abolished by alprazolam, a benzodiazepine, in humans. *J Clin Endocrinol Metab* 2002;87:4616–20.
- [42] Hausler A, Monnet G, Peter O. Involvement of GABAB receptors in the regulation of the hypothalamo-pituitary-adrenocortical (HPA) axis in rats. *J Steroid Biochem Mol Biol* 1993;46:767–71.
- [43] Arvat E, Giordano R, Grotoli S, Ghigo E. Benzodiazepines and anterior pituitary function. *J Endocrinol Invest* 2002;25:735–47.
- [44] Pivac N, Pericic D. Inhibitory effect of diazepam on the activity of the hypothalamic–pituitary–adrenal axis in female rats. *J Neural Transm Gen Sect* 1993;92:173–86.