Psychological Medicine, 2001, **31**, 585–591. Printed in the United Kingdom

Decreased tryptophan availability but normal post-synaptic 5-HT_{2C} receptor sensitivity in chronic fatigue syndrome

C. M. VASSALLO,[1] E. FELDMAN, T. PETO, L. CASTELL, A. L. SHARPLEY AND P. J. COWEN

From the University Department of Psychiatry, Warneford Hospital; Department of Biochemistry, University of Oxford, Department of Psychological Medicine and Nuffield Department of Medicine, John Radcliffe Hospital, Oxford

ABSTRACT

Background. Chronic fatigue syndrome (CFS) has been associated with increased prolactin (PRL) responses to the serotonin (5-HT) releasing agent fenfluramine. It is not known whether this abnormality is due to increased 5-HT release or heightened sensitivity of post-synaptic 5-HT receptors.

Methods. We measured the increase in plasma PRL produced by the directly acting 5-HT receptor agonist, m-chlorophenylpiperazine (mCPP), in patients with CFS and healthy controls. We also compared the ability of mCPP to lower slow wave sleep (SWS) in the sleep polysomnogram of both subject groups. Finally, we measured plasma amino-acid levels to determine whether tryptophan availability differed between CFS subjects and controls.

Results. mCPP elevated plasma PRL equivalently in patients with CFS and controls. Similarly, the decrease in SWS produced by mCPP did not differ between the two subject groups. Plasma-free tryptophan was significantly decreased in CFS.

Conclusions. The sensitivity of post-synaptic 5-HT_{2c} receptors is not increased in patients with CFS. This suggests that the increased PRL response to fenfluramine in CFS is due to elevated activity of pre-synaptic 5-HT neurones. This change is unlikely to be due to increased peripheral availability of tryptophan.

INTRODUCTION

Neuroendocrine studies provide some evidence that brain serotonin (5-HT) function may be abnormal in patients with chronic fatigue syndrome (CFS). Two studies have suggested that the prolactin (PRL) response to the 5-HT releasing agent, D-fenfluramine, is exaggerated in CFS although there are also contradictory findings (Cleare *et al*. 1995; Yatham *et al*. 1995; Sharpe *et al*. 1997). In addition, the PRL response to the 5-HT_{1A} receptor agonist, buspirone, is increased in CFS patients (Bakheit *et al*. 1992; Sharpe *et al*. 1996) although buspirone-mediated PRL release may involve dopaminergic rather than 5-HT mechanisms (Meltzer *et al*. 1991).

Studies with selective receptor antagonists suggest that the PRL response to D-fenfluramine involves indirect activation of post-synaptic 5-HT_{2C} receptors (Goodall *et al*. 1993; Albinsson *et al*. 1994; Park & Cowen, 1997). Thus, increased PRL responses to fenfluramine challenge could indicate increased pre-synaptic release of 5-HT or a supersensitivity of post-synaptic 5-HT_{2C} receptors. The aim of the present study was to distinguish between these possibilities by measuring the PRL response to m-chlorophenylpiperazine (mCPP) a direct 5-

[1] Address for correspondence: Professor P. J. Cowen, University Department of Psychiatry, Warneford Hospital, Oxford OX3 7JX.

HT_{2C} receptor agonist (Khan & Wetzler, 1991). We hypothesized that CFS patients would exhibit heightened PRL responses to mCPP indicating a supersensitivity of post-synaptic 5-HT_{2C} receptors.

In addition, 5-HT_{2C} receptors are involved in the regulation of slow-wave sleep (SWS) in the sleep polysomnogram (EEG) and we have previously demonstrated that mCPP lowers SWS in a dose-related fashion (Katsuda *et al.* 1993; Sharpley *et al.* 1994). In a separate study, therefore, we measured the decrease in SWS produced by mCPP in patients with CFS compared to controls. Again we hypothesized that patients with CFS would have a heightened reduction in SWS.

The synthesis of serotonin in the brain is dependent on the availability of its precursor, tryptophan. Tryptophan binds to albumin in the blood and is therefore present in a free and bound form in plasma. The brain entry of tryptophan depends in turn on the concentration that is free in plasma (plasma-free tryptophan) and the ratio of tryptophan to large neutral amino acids (NAAs) that compete for the same site of brain entry (see Curzon, 1988). In the present study we also measured plasma levels of tryptophan and other amino acids to confirm whether changes in tryptophan availability might underline the previously reported alterations in 5-HT-mediated neuroendocrine responses in CFS.

METHOD

Neuroendocrine investigation

Subjects

We recruited 20 patients (16 women, four men), mean age 38·8 years (range 19–60 years). Their mean weight was 67·4 kg (range 51–94 kg). Most subjects ($N = 18$) were recruited from medical and psychiatric out-patient clinics; a further two subjects responded to a newspaper advertisement. Subjects all met criteria for ICD-10 neurasthenia. In addition, all but one met the Oxford criteria and Centre for Disease Control (CDC) criteria for CFS. Ratings on the Health Status Questionnaire (HSQ) (Claiborne *et al.* 1999) showed that on item 4 (problems with work and other daily activities) all subjects had impairment in either work or another activity (mean score 4·4, range 4·0–7·0). A score of 4 on the HSQ, represents most impaired while a score of 8 indicates no impairment.

On the basis of the Structured Clinical Interview for DSM-IV (SCID) patients were determined to have no current Axis I disorder on DSM-IV. Past Axis 1 disorders included major depressive disorder ($N = 9$), generalized anxiety disorder ($N = 2$), panic disorder ($N = 1$) and bulimia nervosa ($N = 1$). They had all been free of psychotropic medication for at least 1 month. With the exception of hormone therapy (see below) none were taking medication believed to interact with brain 5-HT pathways.

We also studied 21 healthy control subjects (16 women and five men). Their mean age was 37 years (range 19–60 years). Their mean weight was 68·3 kg (range 53–93 kg). On the basis of a SCID interview they were determined to be free of current Axis I disorder on DSM-IV. They had all been drug-free for at least 1 month. Two subjects in the control group and one in the patient group were post-menopausal.

Neuroendocrine testing

Each subject underwent a pair of neuroendocrine challenge tests on two mornings. These were separated by a mean interval of 13 days in chronic fatigue subjects and 16 days in healthy participants (minimum interval 2 days). The drug challenges consisted of mCPP and placebo to which subjects were randomly allocated in a double-blind, cross-over manner. Subjects who were menstruating or receiving hormonal therapy ($N = 3$ in each group) were tested in the early follicular stage of the menstrual cycle. If it was not possible to complete both tests in the same cycle, the second test was performed in a subsequent cycle. Subjects came to the laboratory after an overnight fast at 08.30 h and an indwelling venous cannula was inserted. After a 30-min baseline period, mCPP (0·25 mg/kg orally) or placebo was administered following which venous sampling continued for the next 180 min.

Biochemical measurements

Plasma was separated by centrifugation and stored at −30 °C. PRL concentration was measured using a standard immunoradiometric assay (reagents provided by Netria, London) with inter- and intra-assay coefficients of vari-

https://doi.org/10.1017/S0033291701003580 Published online by Cambridge University Press

ation of 4·8% and 2·4%. Plasma cortisol was determined by radioimmunoassay (RIA). The intra- and inter-assay coefficients of variation over the range encompassed by the standard curve were 4·3% and 5·8%. Measurement of mCPP in plasma was carried out using high performance chromatography (HPLC) (Franklin, 1992). The limit of detection of the assay was 0·2 ng/ml for 1 ml of plasma. Plasma neutral amino acid concentrations (leucine, isoleucine, phenylalanine, tyrosine and valine) were measured by an automated HPLC system with fluorescence end-point detection and pre-column sample derivatisation adapted from the method of Furst *et al.* (1990). Plasma total and free tryptophan were measured with HPLC and electrochemical detection.

Statistics

The PRL response to mCPP and placebo was measured as area under the curve (AUC) by the trapezoid method with extrapolation of baseline secretion from time '0'. The AUC data were analysed by a repeated measures analysis of variance (ANOVA) with 'mCPP' (mCPP *v.* placebo) as the within subject factor and 'diagnosis' (CFS *v.* control) as the between subjects factor. We also subtracted the AUC PRL response following placebo from that produced by mCPP to give a delta AUC PRL response. This was analysed by a factorial ANOVA. Plasma mCPP levels were measured as AUC and compared by student's unpaired *t* test (two-tailed). Plasma amino acids were similarly compared. In our earlier study of the PRL response to D-fenfluramine we found that patients with CFS had an approximately two-fold increase in their AUC PRL response relative to healthy controls (Sharp *et al.* 1997). Based on previous work with mCPP (Cowen *et al.* 1996) we calculated that the present study had a power of 0·9 to detect a two-fold increase in PRL response to mCPP in CFS subjects.

Sleep study

Subjects

We studied 11 patients with CFS as diagnosed above. Their mean age was 34·3 years (range 20–48 years). They weighed 63·7 kg (50·5–75·9 kg). Eight of these patients also took part in the neuroendocrine study. We also tested 11 healthy controls, whose mean age was 36·8 years (23–55 years). The weight of healthy participants averaged 65·4 kg (55·3–76·2 kg).

Sleep EEG recording and drug administration

Home-based sleep recordings were made using a Medilog 9000-II cassette monitoring system. Standard sleep montage electrodes were applied at approximately 17.00 h on two study nights separated by a mean interval of 10 days (minimum interval 7 days). The subjects then returned home to sleep as usual. Subjects were instructed to keep their sleep routines constant for both study nights and the preceding nights. The records were analysed using the Oxford Medilog sleep stager (9200) which provides measures for all aspects of sleep architecture according to standard criteria (Rechtsschaffen & Kales, 1968). In addition the tapes were visually inspected and edited by a scorer blind to treatment status. Subjects received a fixed dose of mCPP (7·5 mg) or placebo in a double blind, randomized fashion. They took mCPP or placebo at 22.00 h.

Statistics

Sleep data were analysed as a repeated measures (ANOVA) with 'mCPP' (mCPP *v.* placebo) as the within subject factor and 'diagnosis' (CFS *v.* control) as the between subjects factor.

RESULTS

Amino acid levels

Baseline free tryptophan (at time '0' from the placebo challenge) was significantly lower in the CFS patients than the controls (Table 1). The concentration of large neutral amino acids (NAAs) did not differ with diagnosis and the ratio of free tryptophan to NAA was corre-

Table 1. *Plasma amino acids in CFS patients and controls*

	CFS ($N = 20$)	Controls ($N = 21$)
Free TRP (μg/ml)	0·35 ± 0·04*	0·55 ± 0·08
Total TRP (μg/ml)	8·1 ± 0·3	7·7 ± 0·2
NAAs (nmol/ml)	444 ± 13	433 ± 15
Ratio free TRP:NAA	0·0008 ± 0·0001†	0·0012 ± 0·0002

* $P = 0·033$.
† $P = 0·022$.

https://doi.org/10.1017/S0033291701003580 Published online by Cambridge University Press

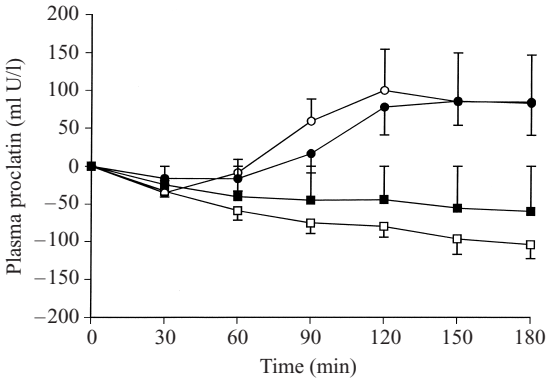

FIG. 1. Mean ± S.E.M. plasma prolactin (measured as change from baseline in 19 CFS patients (○, □) and 21 healthy controls (●, ■). Subjects were tested on two occasions receiving either mCPP (0·25 mg/kg) (○,●) or placebo (□,■). Prolactin response, measured as area under the curve, does not differ between patients and controls (ANOVA).

spondingly lowered. Total tryptophan did not differ between patients and controls (Table 1).

Endocrine responses

Relative to placebo mCPP produced a robust increase in plasma PRL ($F = 23{\cdot}7$; df = 1, 39; $P = 0{\cdot}001$) (Fig. 1). However, there was no main effect of diagnosis ($F = 0{\cdot}16$; df = 1, 39; $P = 0{\cdot}70$) or diagnosis by drug interaction ($F = 0{\cdot}92$; df = 1, 39; $P = 0{\cdot}34$). When the delta AUC PRL responses were measured by factorial ANOVA, the mean value in the patients was higher than that of the controls (301 ± 78 *v.* 214 ± 74 mIU × h/l) but the difference did not approach statistical significance ($F = 0{\cdot}66$; df = 1, 39; $P = 0{\cdot}42$).

Plasma mCPP levels, measured as AUC were significantly higher in patients than controls (35·2 ± 5·3 *v.* 22·8 ± 1·8 ng × h/ml, $P = 0{\cdot}033$). The correlation between mCPP plasma levels and delta AUC PRL response to mCPP was not significant when assessed by Pearson's product moment ($r = 0{\cdot}20$, $P = 0{\cdot}22$). However, with Spearman's rho, a significant positive correlation was obtained (rho = 0·41, $P = 0{\cdot}008$). When the factorial ANOVA was repeated with rank transformed mCPP levels as a covariate, the adjusted mean difference between patients and controls in delta AUC PRL response was less (262 ± 73 *v.* 251 ± 73 mIU × h/l; $F = 0{\cdot}01$; df = 1, 38; $P = 0{\cdot}92$). This suggests that when the difference in mCPP levels is taken into account, PRL responses to mCPP are very similar in patients and controls.

Plasma mCPP levels were a significant covariate of the delta PRL response in the ANCOVA ($F = 6{\cdot}11$; df = 1, 38; $P = 0{\cdot}018$). There was a wide variation in AUC plasma levels of mCPP ranging from 4·4–85·2 ng × h/ml. However, when two subjects with the lowest mCPP levels were excluded, the variation in plasma level was reduced to four-fold (data not shown).

Baseline plasma cortisol levels did not differ between patients with CFS and controls (21·8 ± 2·5 *v.* 23·1 ± 3·1 μg/100 ml, $P = 0{\cdot}75$). In

Table 2. *Selected sleep EEG measures (in minutes) in CFS patients and controls*

	Patients		Controls	
	Placebo	CPP	Placebo	mCPP
Sleep continuity				
Total sleep period*	476·0 ± 24·3	446·5 ± 22·9	447·7 ± 9·6	437·4 ± 10·9
Actual sleep time*	434·0 ± 21·3	393·2 ± 18·2	422·0 ± 9·1	390·5 ± 16·5
Sleep efficiency %*†	85·9 ± 2·2	78·4 ± 3·1	91·8 ± 1·0	84·8 ± 2·8
Wake after sleep onset*	40·9 ± 6·5	59·0 ± 10·3	20·9 ± 3·0	44·3 ± 9·3
Sleep latency‡	19·1 ± 4·4	39·2 ± 13·9	8·7 ± 2·0	20·5 ± 6·7
NREM measures				
Stage 1	42·4 ± 7·5	35·5 ± 6·1	36·9 ± 3·8	39·6 ± 3·9
Stage 2	197·9 ± 17·5	184·4 ± 13·5	185·9 ± 7·1	178·6 ± 12·3
Slow-wave sleep*	90·1 ± 17·8	75·9 ± 12·0	104·4 ± 10·3	78·1 ± 7·8
REM measures				
REM sleep	103·7 ± 7·5	97·3 ± 9·7	94·8 ± 6·5	94·1 ± 9·6
REM latency	75·4 ± 5·9	72·3 ± 6·7	87·1 ± 14·4	85·4 ± 13·9

* Main effect of mCPP ($P < 0{\cdot}01$).
† Main effect of diagnosis ($P < 0{\cdot}05$).
‡ Main effect of mCPP ($P < 0{\cdot}05$).

https://doi.org/10.1017/S0033291701003580 Published online by Cambridge University Press

addition there was no correlation between baseline cortisol levels and the delta PRL response to mCPP ($r = 0{\cdot}076$, $P = 0{\cdot}64$; rho = 0·18, $P = 0{\cdot}27$).

Sleep measures

The ANOVA showed that CFS patients had a lower sleep efficiency than controls after both mCPP and placebo. Relative to placebo, mCPP significantly reduced total sleep period, actual sleep time, sleep efficiency, SWS, and increased sleep latency and wake after sleep onset. These changes were seen to an equivalent extent in both patients and controls (Table 2). There were no significant correlations between sleep efficiency and slow-wave sleep in either patients or controls following placebo or mCPP treatment (all P values $> 0{\cdot}2$ with both Pearson and Spearman tests).

DISCUSSION

Our findings indicate that, contrary to our hypothesis, patients with CFS do not exhibit increased sensitivity of post-synaptic 5-HT$_{2C}$ receptors in either neuroendocrine or sleep EEG models. This suggests that the increases in 5-HT neuroendocrine responses revealed by fenfluramine challenge are probably due to enhanced presynaptic release of 5-HT.

Previous neuroendocrine studies in CFS have yielded some evidence of increased 5-HT neurotransmission as judged by the elevated PRL response to the 5-HT releasing agent, D-fenfluramine (Cleare *et al*. 1995; Sharpe *et al*. 1997). Increased PRL responses have also been seen in response to the 5-HT$_{1A}$ receptor agonist, buspirone (Bakheit *et al*. 1992; Sharpe *et al*. 1996), but the lack of selectivity of this agent makes interpretation of its endocrine responses problematical (Meltzer, 1991). Indeed, patients with CFS appear to have blunted ACTH responses to the more selective 5-HT$_{1A}$ receptor ligand, ipsapirone (Dinan *et al*. 1997). However, the fact that CFS patients may also have underlying dysregulation of the HPA axis (Demitrack *et al*. 1991) means that deficient ipsapirone-mediated ACTH release may not necessarily be due to a primary deficit in 5-HT$_{1A}$ receptors.

In the present study we investigated PRL responses to the 5-HT agonist, mCPP. mCPP has a number of pharmacological actions including a significant affinity for α_2-adrenoceptors (see Khan & Wetzler, 1991). However, mCPP-induced PRL release in humans is abolished by the selective 5-HT$_{2A/C}$ receptor antagonist, ritanserin (Seibyl *et al*. 1991). In addition, mCPP appears to be an antagonist at 5-HT$_{2A}$ receptors. Taken together the data suggest that the ability of mCPP to increase plasma PRL is mediated via activation of 5-HT$_{2C}$ receptors (see Cowen, 1993). Like Gijsman *et al*. (1998) we found a wide inter-subject variation in plasma levels of mCPP, although a substantial degree of this variance was accounted for by two subjects with the lowest mCPP levels. This variation in mCPP kinetics makes it worthwhile measuring plasma mCPP concentrations after oral challenge tests, particularly since plasma levels of mCPP may correlate with PRL response (Cowen *et al*. 1996).

We have previously found that the dose of mCPP we employed (0·25 mg/kg) is capable of detecting an increase in 5-HT receptor sensitivity, found for example during dieting (Cowen *et al*. 1996). Taken with the data above, our findings suggest that increased PRL responses to fenfluramine in CFS patients are not caused by heightened sensitivity of post-synaptic 5-HT$_{2C}$ receptors. Accordingly, elevated fenfluramine-mediated PRL release in CFS most probably reflects increased pre-synaptic 5-HT function. It is conceivable, however, that in CFS, part of the PRL response to D-fenfluramine is mediated via recruitment of other post-synaptic 5-HT receptors (for example, 5-HT$_{1A}$ and 5-HT$_{2A}$) that do not appear to play a role in D-fenfluramine-induced-PRL release under normal conditions (Albinsson *et al*. 1994; Goodall *et al*. 1994; Park & Cowen, 1997).

Consistent with our previous work, mCPP significantly lowered SWS in the sleep EEG (Katsuda *et al*. 1993). However, this reduction was similar in patients and controls, again suggesting a normal sensitivity of 5-HT$_{2C}$ receptors in CFS. Interestingly, sleep efficiency was significantly lower in CFS subjects than controls both after placebo and mCPP. Lowered sleep efficiency is a fairly consistent finding in CFS (Morriss *et al*. 1993). Whether it contributes to the clinical symptomatology or results from it, is presently unclear. Baseline SWS did not differ between patients and controls and sleep

https://doi.org/10.1017/S0033291701003580 Published online by Cambridge University Press

efficiency did not correlate with SWS either under baseline or mCPP conditions. This makes it unlikely that differences in sleep architecture between patients and controls could have confounded the effect of mCPP on slow-wave sleep.

Analysis of plasma amino acid levels indicated a decrease in fasting plasma-free TRP and a corresponding diminution in the ratio of free TRP to NAAs. This contrasts with an earlier report (Castell *et al*. 1999). There is no obvious explanation for this discrepancy; it may be relevant that the subjects in the earlier investigation were studied before an exercise challenge that may have been a stressful prospect for CFS subjects. Both total TRP and free TRP probably contribute to brain 5-HT synthesis and, interestingly, elevations in plasma-free TRP are thought to be responsible for the increase in brain TRP and brain 5-HT synthesis produced by exercise (Chaouloff *et al*. 1986; Curzon, 1988). Our data, however, make it unlikely that increases in plasma-free TRP can be a general explanation for enhanced 5-HT neuroendocrine responses in CFS.

In a previous study of healthy individuals who dieted, we found that low levels of plasma total tryptophan were associated with increased PRL responses to mCPP (Cowen *et al*. 1996). The current data suggest that this relationship may not hold between lowered plasma-free tryptophan and mCPP-induced PRL release. Another, perhaps more likely possibility, is that patients with CFS have complex abnormalities in the regulation of the hypothalamic 5-HT receptors that control PRL release.

We thank David Laver, John Odontiadis and Alison Reed for technical assistance and Rena Hockney and Gabriella Pearson for nursing care. The study was supported by the Linbury Trust.

REFERENCES

Albinsson, A., Palazidou, E., Stephenson, J. & Andersson, G. (1994). Involvement of the 5-HT_3 receptor in the 5-HT receptor-mediated stimulation of prolactin release. *European Journal of Pharmacology* **251**, 157–161.

Bakheit, A. M., Behan, P. O., Dinan, T. G., Gray, C. E. & O'Keane, V. (1992). Possible upregulation of hypothalamic 5-HT receptors in patients with post-viral fatigue syndrome. *British Medical Journal* **304**, 1010–1012.

Castell, L. M., Yamamoto, T., Phoenix, J. & Newsholme, E. A. (1999). The role of tryptophan in fatigue in different conditions of stress. In *Tryptophan, Serotonin and Melatonin* (ed. G. Huether, W. Kochen, T. Simat and H. Steinhart), pp. 697–704. Kluwer Academic Press, New York.

Chaouloff, F., Kennett, G. A., Serrurrier, B., Merino, D. & Curzon, G. (1986). Amino acid analysis demonstrates that increased plasma free tryptophan causes the increase of brain tryptophan during exercise in the rat. *Journal of Neurochemistry* **46**, 1647–1650.

Claiborne, N., Krause, T. M., Heilman, A. E. & Leung, P. (1999). Measuring quality of life in back patients. Comparison of Health Status Questionnaire 2·0 and Quality of Life Inventory. *Social Work in Health* **28**, 77–94.

Cleare, A. J., Bearn, J., Allain, T., McGregor, A., Wessely, S., Murray, R. M. & O'Keane, V. O. (1995). Contrasting neuroendocrine responses in depression and chronic fatigue syndrome. *Journal of Affective Disorders* **35**, 283–289.

Cowen, P. J. (1993). Serotonin receptor subtypes in depression: evidence from studies in neuroendocrine regulation. *Clinical Neuropharmacology* **16** (suppl 3), S6–S18.

Cowen, P. J., Clifford, E. M., Walsh, A. E. S., Williams, C. & Fairburn, C. G. (1996). Moderate dieting causes 5-HT_{2C} receptor supersensitivity. *Psychological Medicine* **26**, 1155–1159.

Curzon, G. (1988). Feeding, stress, exercise and the supply of amino acids to the brain. In *Amino Acid Availability and Brain Function in Health and Disease* (ed. G. Huether), pp. 39–49. Springer-Verlag: Berlin.

Demitrack, M. A., Dale, J. K., Straus, S. E., Laue, L., Listwak, S. J., Kruesi, M. J. P., Chrousos, G. P. & Gold, P. W. (1991). Evidence for impaired activation of the hypothalamic–pituitary–adrenal axis in patients with chronic fatigue syndrome. *Journal of Clinical Endocrinology and Metabolism* **73**, 1224–1234.

Dinan, T. G., Majeed, T., Lavelle, E., Scott, L. V., Berti, C. & Behan, P. (1997). Blunted serotonin-mediated activation of the hypothalamic–pituitary–adrenal axis in chronic fatigue syndrome. *Psychoneuroendocrinology* **22**, 261–267.

Franklin, M. (1992). Determination of *m*-chlorophenylpiperazine in plasma by high-performance liquid chromatography with coulometric detection. *Journal of Liquid Chromatography* **15**, 1553–1563.

Furst, P., Pollack, T. A., Graser, H., Godel, H. & Stehle, P. (1990). Appraisal of four pre-column derivatization methods for the high-performance liquid chromatographic determination of free amino acids in biological materials. *Journal of Chromatography* **499**, 557–569.

Gijsman, H. J., Van Gerven, H. M. A., Tielman, M. C., Schoemaker, R. C., Pieters, M. C. M., Ferrari, M. D., Cohen, A. F. & Van-Kempen, G. M. J. (1998). Pharmacokinetic and pharmacodynamic profile of oral and intravenous *meta*-chlorophenylipiperazine in healthy volunteers. *Journal of Clinical Psychopharmacology* **18**, 289–295.

Goodall, E. M., Cowen, P. J., Franklin, M. & Silverstone, T. (1993). Ritanserin attenuates anorectic, endocrine and thermic responses to D-fenfluramine in human volunteers. *Psychopharmacology* **112**, 461–466.

Kahn, R. S. & Wetzler, S. (1991). m-Chlorophenylpiperazine as a probe of serotonin function. *Biological Psychiatry* **30**, 1139–1166.

Katsuda, Y., Walsh, A. E. S., Ware, C. J., Cowen, P. J. & Sharpley, A. L. (1993). *meta*-Chlorophenylpiperazine decreases slow-wave sleep in humans. *Biological Psychiatry* **33**, 49–51.

Meltzer, H. Y., Gudelsky, G. A., Lowy, M. T., Nash, J. F. & Koenig, J. I. (1991). Neuroendocrine effects of buspirone: mediation by dopaminergic and serotonergic mechanisms. In *Buspirone: Mechanisms and Clinical Aspects* (ed. G. Tunnicliff, A. S. Eison and O. P. Taylor), pp. 177–192. Academic Press: San Diego, CA.

Morriss, R., Sharpe, M., Sharpley, A. L., Cowen, P. J., Hawton, K. & Morris, J. (1993). Abnormalities of sleep in patients with the chronic fatigue syndrome. *British Medical Journal* **306**, 1161–1164.

Park, S. B. G. & Cowen, P. J. (1997). Effect of pindolol on the prolactin response to D-fenfluramine. *Psychopharmacology* **118**, 471–474.

Rechtschaffen, A. & Kales, A. (1968). *A Manual of Standardized Terminology, Techniques and Scoring System or Sleep Stages of Human Subjects*. Los Angeles Brain Information Service, Brain Research Institute, University of California: Los Angeles.

Seibyl, J. P., Krystal, J. H., Price, L. H., Woods, S. W., D'Amico, C., Heninger, G. R. & Charney, D. S. (1991). Effects of ritanserin on the behavioural, neuroendocrine and cardiovascular responses to meta-chlorophenylpiperazine in healthy human subjects. *Psychiatry Research* **38**, 227–236.

Sharpe, M., Clements, A., Hawton, K., Young, A. H., Sargent, P. & Cowen, P. J. (1996). Increased prolactin response to buspirone in chronic fatigue syndrome. *Journal of Affective Disorders* **41**, 71–76.

Sharpe, M., Hawton, K., Clements, A. & Cowen, P. J. (1997). Increased brain serotonin function in men with chronic fatigue syndrome. *British Medical Journal* **315**, 164–165.

Sharpley, A. L., Elliott, J. M., Attenburrow, M. J. & Cowen, P. J. (1994). Slow wave sleep in humans: role of 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors. *Neuropharmacology* **33**, 467–471.

Yatham, L. N., Morehouse, R. L., Chisholm, T., Haase, D. A., MacDonald, D. D. & Marrie, T. J. (1995). Neuroendocrine assessment of serotonin (5-HT) function in chronic fatigue syndrome. *Canadian Journal of Psychiatry* **40**, 3–96.