Psychological Medicine, 1998, **28**, 285–293. Printed in the United Kingdom

Naloxone-mediated activation of the hypothalamic–pituitary–adrenal axis in chronic fatigue syndrome

L. V. SCOTT, F. BURNETT, S. MEDBAK AND T. G. DINAN[1]

From the Departments of Psychological Medicine and Chemical Endocrinology, St Bartholomew's and the Royal London School of Medicine, London

ABSTRACT

Background. Opioidergic pathways have an inhibitory regulatory influence on the hypothalamic–pituitary–adrenal axis (HPA) in man. Previous studies have suggested impairment of pituitary–adrenal activation in chronic fatigue syndrome (CFS). We, therefore, decided to investigate the extent of opioid inhibition of HPA activity in CFS as a possible explanation for the reputed HPA hypofunctioning in patients with CFS.

Method. Thirteen patients with CFS, diagnosed according to CDC criteria, were compared with thirteen healthy subjects. Adrenocorticotropin (ACTH) and cortisol (CORT) responses were measured following the administration of the opiate antagonist naloxone.

Results. Baseline ACTH and cortisol levels did not differ between the two groups. The release of ACTH (but not cortisol) was significantly blunted in the CFS subjects compared with controls.

Conclusions. Naloxone mediated activation of the HPA is attenuated in CFS. Excessive opioid inhibition of the HPA is thus an unlikely explanation for the HPA dysregulation in this disorder.

INTRODUCTION

Chronic fatigue syndrome (CFS) has provoked controversy in recent years with considerable debate surrounding its definition, aetiology, pathophysiology and treatment. An operational definition, introduced by the Centres for Disease Control (CDC) and Prevention (Fukuda *et al.* 1994), has facilitated research and improved diagnostic uniformity in this area. CFS is defined as persistent or relapsing fatigue of 6 months duration, which results in functioning at less than 50% of pre-morbid level, and which is associated with any four of the following eight symptoms being concurrently present over this period: recurrent sore throats, enlarged and tender lymph glands, unrefreshing sleep, arthralgia, myalgia, post-exertional malaise, neuropsychological complaints and recurrent headaches.

[1] Address for correspondence: Professor T. G. Dinan, Department of Psychological Medicine, St Bartholomew's Hospital, West Smithfield, London EC1A 7BE.

Much of the early work focused on a viral aetiology in this condition, but these studies were not found to be consistent or readily replicable (Shorter, 1993). Attention has shifted to a neuroendocrine basis of this disorder, fuelled by the opinion that the fatigue may be of central and not peripheral origin (Wessely & Powell, 1989).

The hypothalamic–pituitary–adrenal (HPA) axis is the core endocrine stress axis in man. Corticotropin releasing hormone (CRH) is released from the hypothalamus into the portal vasculature at the median eminence and brings about ACTH release from the corticotropes of the anterior pituitary; ACTH in turn stimulating the adrenal gland to produce cortisol (Michelson *et al.* 1995). Demitrack and colleagues (1991) have recently conducted a dynamic assessment of the HPA axis in a cohort of CFS patients diagnosed according to CDC criteria. They found a number of abnormalities including basal cortisol levels that were significantly lower in

CFS subjects, than healthy controls, as were 24 h urinary free-cortisol values. Evening ACTH levels were higher in the CFS cohort. Following administration of corticotropin releasing hormone (CRH) the release of ACTH, but not cortisol (CORT), was blunted. Using a series of tests that employed graded doses of ACTH to stimulate CORT, low doses (0·003 μg/kg) produced robust CORT responses in CFS subjects, whereas at higher doses (1 μg/kg) the CFS cohort had attenuated cortisol release compared to the control subjects. The former was suggestive of a supersensitivity of the adrenocortical receptors due to low circulating ACTH while the latter implied a reduced overall secretory reserve again possibly reflecting low levels of neurotrophic ACTH and/or an atrophied adrenal gland. The inclusive explanation given by Demitrack and colleagues for these findings was that of a mild secondary (central) adrenal insufficiency (a primary adrenal deficit, as in Addison's disease, is associated with an exaggerated CRH/ACTH output), due either to a deficiency of CRH or some other central stimulus of the pituitary–adrenal axis.

The HPA is under the control of several classic neurotransmitters, of which, the indoleamine, serotonin, has been the most widely studied to date in CFS. There is now a significant volume of evidence to link serotonin (5HT) with ACTH release mechanisms (Dinan, 1996). Liposits *et al*. (1987) demonstrated a direct synaptic connection between serotonergic nerve terminals and CRH-containing neurones in the paraventricular hypothalamic nucleus and moreover, 5HT directly stimulates CRH release in hypothalamic explants (Tsagarakis *et al*. 1989). The 5HT precursors L-tryptophan stimulate ACTH release (Heninger *et al*. 1984; Grossman & Costa, 1993) and D-fenfluramine also promotes cortisol production (O'Keane & Dinan, 1991). It is suggested that the positive regulatory action occurs predominantly through the 5HT1a receptor (Lesch *et al*. 1989). Dinan and colleagues (1997), using ipsapirone, the 5HT1a agonist, found evidence for significant blunting of 5-HT mediated ACTH release in CFS compared to healthy subjects. Other studies which implicate serotonergic mechanisms are those of serotonergic mediated prolactin release, of which some (Bakheit *et al*. 1992; Cleare *et al*. 1995; Sharpe *et al*. 1996) but not all (Yatham *et al*. 1995) point to a dysregulation of serotonergic activity in CFS.

The opioidergic system, unlike the stimulatory serotonergic system above, is known to exert a predominantly inhibitory influence on HPA activity in man (Stubbs *et al*. 1978; Taylor *et al*. 1983). Naloxone, an opiate receptor antagonist, acting it is thought through the kappa (κ) and delta (δ) receptors (Delitala *et al*. 1983; Grossman *et al*. 1986), blocks the inhibitory influence of endogenous opioids and produces a rise in circulating ACTH and CORT in normal subjects (Volavka *et al*. 1979, Al-Damulji *et al*. 1990; Torpy *et al*. 1993). Although the adrenergic system has been implicated in modulating opioidergic activity, the ACTH stimulatory effect of naloxone is thought primarily to be due to endogenous CRH release (Jackson *et al*. 1995; Hockings *et al*. 1995); the cortisol response to naloxone is abolished in pigs by pituitary stalk transection (Estienne *et al*. 1988) and furthermore, prior administration of CRH antiserum in rats causes loss of naloxone induced ACTH release (Nikolaris *et al*. 1987). However, recent evidence in man (Delitala *et al*. 1994) indicates that naloxone induced ACTH release can occur via a CRH-independent mechanism possibly via vasopressin (VP), its core HPA regulatory companion (Antoni, 1993). Thus, although Jackson *et al*. (1995) propose that the naloxone test is a useful test of estimating hypothalamic CRH reserve, Inder *et al*. (1995*a*) caution against this, stating that until the precise mechanism of naloxone-induced ACTH is defined, the response to naloxone should be regarded as simply a marker of the extent of central opioid inhibitory tone of the HPA axis (Inder *et al*. 1995*a*).

The naloxone test has been used in a clinical setting in the diagnosis of suspected central adrenal insufficiency (Blevins *et al*. 1994) as a test of pituitary ACTH reserves and in the diagnosis of Cushing's syndrome (Gaitan *et al*. 1993; Torpy *et al*. 1993). Naloxone has been used also, to study opioid tone in a number of psychiatric conditions including alcoholism (Inder *et al*. 1995*b*) and post-traumatic stress disorder (Hockings *et al*. 1993), as well as major depression, in which consistent hyperactivity of the HPA axis, probably related to CRH overdrive, has been demonstrated (Gold *et al*. 1991). Decreased opioidergic tone has been suggested

as a pathophysiological explanation for this HPA dysregulation, but studies have failed to support this theory (Judd *et al.* 1981; Extien *et al.* 1982; Zis *et al.* 1989).

In this study we wish to evaluate opioid control of the HPA in CFS patients. We hypothesize that the decreased forward drive of the HPA in CFS might be due to the excessive inhibitory influence of an enhanced opioid tone.

METHOD

Subjects

Thirteen patients (5 male and 8 female) fulfilling the CDC criteria for CFS, together with 13 age and sex matched healthy comparison subjects, were recruited. All were Caucasians. None of the CFS subjects was clinically depressed at the time of study according to DSM-III-R criteria (APA, 1987). The control group had a full physical and psychiatric interview. None had a past or current history of chronic fatigue, psychiatric illness, neurological, endocrine, cardiovascular, renal or hepatic disease. None had a history of excessive alcohol consumption or illicit drug use. No subject in the CFS group had been on any medication known to affect the HPA (including antidepressants) in the 6 weeks preceding their participation in the study. All female participants were studied in the early follicular phase of the menstrual cycle and none was taking an anovulant form of contraceptive.

Mean ($\pm$S.E.M.) age of patients was $36{\cdot}2 \pm 3{\cdot}1$ years (range 21–52 years) compared with healthy subjects: $31{\cdot}0 \pm 2{\cdot}2$ years (range 21–47 years). The mean $\pm$S.E.M. weight of the healthy and CFS groups were $72{\cdot}2 \pm 2{\cdot}1$ kg and $65{\cdot}0 \pm 2{\cdot}8$ kg respectively. Neither age nor weight differences between the two groups was significant. No participants had a BMI outside the normal range. The mean duration of illness for the CFS patients was 4 years and 9 months, range (18 months to 10 years).

Ethics committee approval was obtained and all participants in the study gave written, informed consent.

Procedure

Naloxone stimulation tests were performed in the afternoon when basal secretory activity for ACTH and cortisol is at an intermediate stage between the high morning levels and low levels during the late evening. Subjects were fasted after a light meal taken not later than 08.30 h. At 12.30 a forearm venous cannula was inserted and subjects were allowed to relax for 30 min. The cannula was kept patent by flushing with heparinized saline. Samples for basal ACTH and cortisol levels were taken (time 0). A standard dose of naloxone 0·125 mg/kg was then administered intravenously over a period of 1 min. Further blood for ACTH and cortisol estimation was drawn at $+15$, $+30$, $+45$, $+60$, $+90$ and $+120$ min. The samples was immediately centrifuged and stored at -80 °C until analysis. Blood pressure and heart rate were measured at 15 min intervals throughout.

ACTH was measured using a commercially available two-site immunoradiometric assay. This is a non-extraction assay supplied by the Nichols Institute, San Juan Capistrano, CA (Raff & Findling, 1989). The sensitivity of the assay is 5 ng/ml. Intra- and inter-assay coefficients of variation were 3% and 6% respectively. The reliable lower limit of detection was 4·4 pmol/l (10 ng/l). Cortisol was measured by an automated system using an enzyme immunoassay method (Immuno-I, Bayer Diagnostics, Newbury, England) (Dash *et al.* 1975). The sensitivity of the method is 10 nmol/l and has a between batch variation of < 5% over the range 50 to 1600 nmol/l.

ACTH and cortisol responses were measured as the maximum level post-naloxone relative to baseline (δ ACTH and δ cortisol). Two-tailed Student's t tests were used to compare means. An area under the curve (AUC) analysis was performed for ACTH response. Where appropriate Pearson product-moment correlation coefficients were employed as were chi-square tests. The statistical package Statgraphics (v2.7) was used to conduct the statistical analysis (SGCS, 1987).

RESULTS

Baseline ACTH levels did not differ significantly between the two groups: chronic fatigue $17{\cdot}4 \pm 3{\cdot}1$ ng/ml, healthy controls $23{\cdot}0 \pm 4{\cdot}3$ ng/ml ($t = 1{\cdot}07$, df = 24, NS). Neither did baseline cortisol levels differ significantly: CFS $311{\cdot}5 \pm 36{\cdot}6$ nmol/l, healthy subjects $265{\cdot}2 \pm 30{\cdot}8$ nmol/l ($t = -0{\cdot}9$, df = 24, NS).

The mean $\pm$S.E.M. δ ACTH values for both

https://doi.org/10.1017/S0033291797006260 Published online by Cambridge University Press

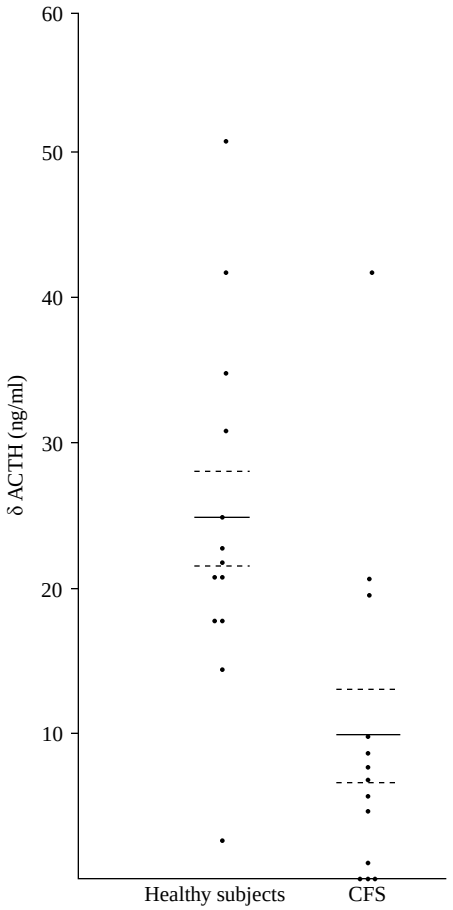

FIG. 1. δ-ACTH responses in chronic fatigue ($N = 13$, CFS) and in healthy subjects ($N = 13$). (Mean (−)±S.E.M. (− − −).)

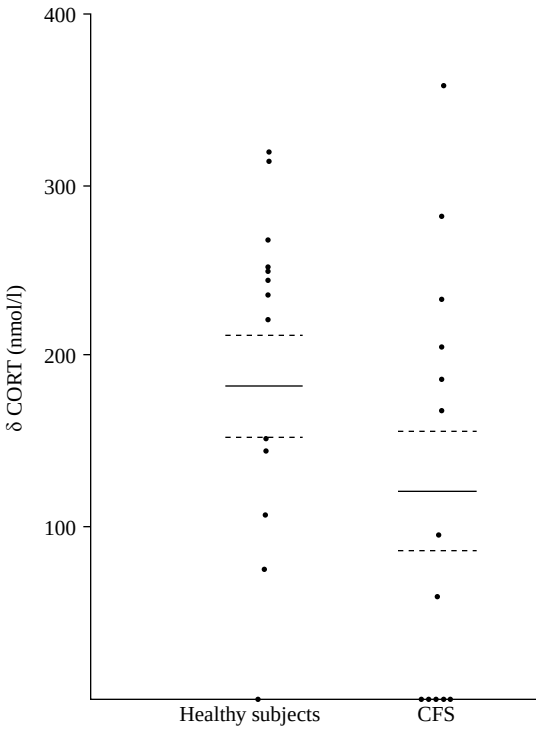

FIG. 2. δ-cortisol responses in chronic fatigue ($N = 13$, CFS) and in healthy subjects ($N = 13$). (Mean (−)±S.E.M. (− − −).)

groups were: CFS 10·2±3·2 ng/ml, healthy subjects 25·0±3·4 ng/ml. Overall, the chronic fatigue subjects released less ACTH than normal subjects ($t = 3{\cdot}2$, df = 24, $P < 0{\cdot}005$) (Fig. 1). An AUC analysis similarly found significantly lower ACTH responses in the chronic fatigue group compared with controls ($t = 3{\cdot}2$, df = 24, $P < 0{\cdot}005$).

With regard to cortisol responses to naloxone challenge, one of the healthy control group failed to show any increase from baseline. In the CFS group, five subjects showed no increase in cortisol from baseline and two further subjects had only minimally elevated levels. The mean δ-CORT response for the CFS subjects was 123·8±34·8 nmol/l and for the control group was 181·1±30·4 nmol/l (Fig. 2). This difference fails to reach significance ($t = -1{\cdot}2$, df = 24, $P = 0{\cdot}22$).

A relationship was established between baseline ACTH values and the ACTH response to naloxone in the healthy volunteers ($r = 0{\cdot}58$, df = 11, $P < 0{\cdot}05$) but not in the chronic fatigue subjects ($r = 0{\cdot}33$, df = 11, $P = 29$), or between baseline cortisol values and cortisol response in either the chronic fatigue ($r = -0{\cdot}48$, df = 11, $P = 0{\cdot}09$) or control subjects ($r = -0{\cdot}49$, df = 11, $P = 0{\cdot}09$). Similarly, no relationship was found between illness duration, age or sex and ACTH response. There was a significant correlation between δ-ACTH and δ-cortisol responses in the healthy subjects ($r = 0{\cdot}69$, df = 11, $P < 0{\cdot}01$) and also in the CFS patients ($r = 0{\cdot}85$, df = 11, $P < 0{\cdot}001$).

The ratio of CORT to ACTH responses over time are shown in Fig. 3.

Overall, the test was well tolerated. Only one subject reported an episode of nausea and vomiting 6 h after the test. Blood pressure and pulse remained stable in all subjects throughout the test.

https://doi.org/10.1017/S0033291797006260 Published online by Cambridge University Press

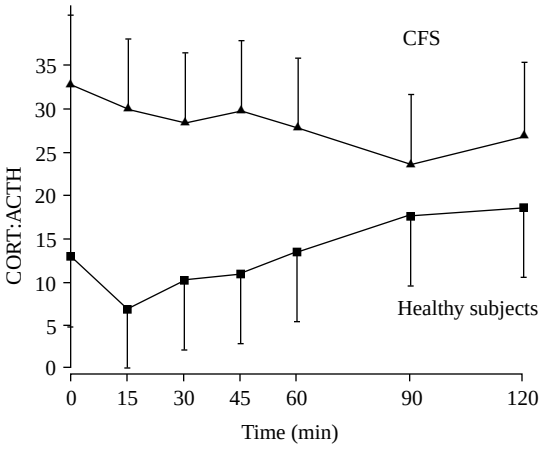

FIG. 3. Ratio of cortisol to ACTH output in chronic fatigue (▲, CFS) and in healthy subjects (■) following 125 μg/kg naloxone administered at time 0 min. (Mean ± S.E.M. shown.)

DISCUSSION

The opioid system has a pronounced inhibitory influence on HPA activity. The pituitary–adrenal responses following the removal of this inhibition by naloxone, the opioid antagonist, will thus provide an indication of central opioid tone. In the case of excessive inhibitory opioid tone an exaggerated rebound release of ACTH/cortisol to naloxone stimulation would be seen. In this study we demonstrated an attentuation of the ACTH response to the opioid antagonist naloxone in a group of patients with CFS. Blunted ACTH responses to the agonistic effects of CRH and ipsapirone in CFS subjects have been previously demonstrated (Demitrack *et al.* 1991; Dinan *et al.* 1997). Our study effectively negates the possibility that the poor response to these HPA activators is the result of the restraining effect of increased inhibitory opioidergic activity, and furthermore, it suggests that central opioid tone may be decreased. Examination of β-endorphin, met-enkephalin and dynorphins, the endogenous opioid receptor ligands, which have not to date been explored in CFS, may help clarify this issue. The endogenous opioids are intrinsically involved in pain modulation, with pain prone individuals being reported to have lower than normal concentrations of bioassayable opiates in the CSF (Terenius & Wahlstrom, 1978). A reduction in endogenous opioid tone may explain the pain symptoms, such as myalgia and headaches, experienced by patients with chronic fatigue syndrome.

In this study only one of 13 CFS subjects produced an ACTH rise greater than the mean of the control group. There was considerable variability in the ACTH response in control subjects (see Fig. 1). This failure of response, and variability of response to naloxone, in normal subjects, has been previously reported (Inder *et al.* 1995*a*), limiting the use of the naloxone test in a diagnostic setting. Although it has been suggested that up to 20 mg of naloxone is needed for maximal response of ACTH and CORT, most recent investigators have used naloxone at doses of 125 μg/kg (Gaitan *et al.* 1993; Torpy *et al.* 1993). It is, nevertheless, possible that the lower dosage employed by us increased the non-responders in both the CFS and volunteer group.

Despite the blunted ACTH response to naloxone in CFS the cortisol response, as measured by maximum increase from baseline, did not differ significantly from that of healthy subjects. It is not possible to conclude, however, that this reflects an increased sensitivity of adrenocortical receptors, as five of 13 CFS subjects failed to produce any increase in circulating cortisol. That the δ-ACTH/δ-CORT responses were highly correlated in the CFS group further undermines this likelihood. On examination of the CORT:ACTH ratio curves in the two groups a differing response pattern emerges (see Fig. 3). It is apparent that at baseline, the CORT:ACTH value is higher in the CFS group, that is, the amount of cortisol released per unit of ACTH is larger in CFS subjects than in the control group. However, in response to naloxone infusion in the healthy subjects there is a trend towards an increasing cortisol to ACTH output reflecting activation of the axis, the fall at +15 min indicating the initial surge in ACTH output to inhibition by naloxone in this group. In the CFS subjects this pattern is not seen. There is in fact a downward trend with a decline in the CORT:ACTH ratio, that is, the increase in ACTH in CFS, though significantly lower than in healthy subjects is unable to stimulate the adrenal gland to produce further increments in cortisol output. If the administration of naloxone is ecologically valid as an activator of the HPA axis, this response in CFS

subjects suggests an impaired ability to mount a stress response and moreover may suggest a reduced adrenal secretory reserve. This inability to increase cortisol levels appropriately to stressors may explain the reduced exercise capacity and exacerbation of symptoms with intercurrent infections in CFS patients.

Our study found no difference in baseline cortisol and ACTH levels between the CFS and control subjects. Other studies have, similarly, not demonstrated a reduction in basal cortisol levels (Bearn *et al*. 1995; Dinan *et al*. 1997). In fact, the basal cortisol levels in the CFS subjects in this study were non-significantly higher than the healthy volunteer group. However, it has been demonstrated that basal cortisol levels do not influence naloxone-induced ACTH response (Grossman *et al*. 1982; Inder *et al*. 1995*a*), reducing the relevance of the marginally higher basal cortisol levels in CFS compared to healthy subjects. Nonetheless, it is possible that a negative feedback effect of cortisol at the level of the hypothalamus, or the pituitary, contributes to the attenuated response seen. In depressive illness the blunted ACTH response to exogenous CRH is explained by the restraining influence of high cortisol levels at a pituitary level (Holsboer *et al*. 1986). In this study no subject had a co-morbid depressive disorder. Demitrack demonstrated elevated ACTH levels, while we and others (Bearn *et al*. 1995) did not find any difference between patients and comparison subjects in terms of baseline ACTH. This may be due to test time differences between groups – Demitrack *et al*. (1991) sampled in the evening when levels are usually low. We sampled in the early afternoon when ACTH levels have not yet reached their nadir.

Although the literature is neither extensive nor methodologically consistent a number of recent studies have linked alterations in opioid tone and HPA dysregulation in psychiatric disorders. In a group of recently abstinent alcoholics with normal basal HPA axis hormone levels, Inder *et al*. (1995*b*) demonstrated a blunted ACTH response to naloxone and CRH. They proposed that reduced levels of central endogenous opioid peptides may explain the attenuated ACTH response, but seemed to favour the possibility of a reduced pituitary responsiveness to CRH. This was considered to reflect a direct pituitary effect of chronic ethanol exposure or a reduction in hypothalamic–hypophyseal vasopressin levels, with which CRH normally acts in a synergistic fashion in bringing about ACTH release (DeBold *et al*. 1984; Lamberts *et al*. 1984).

In post-traumatic stress disorder, following naloxone administration an enhanced ACTH response to CRH was observed (Hockings *et al*. 1993). This was compatible with the observation that low cortisol levels are a feature of chronic PTSD (Yehuda *et al*. 1990). Both findings were posited to reflect enhanced central inhibitory opioid activity. Although direct comparisons are difficult due to methodological differences, it is interesting that in CFS, in which low basal cortisol (Demitrack *et al*. 1991) and low UFC levels (Demitrack *et al*. 1991; Scott & Dinan, 1997) have been demonstrated, that the diametrically opposite naloxone mediated ACTH response is observed. It has been posited that the hyperactivity of the HPA axis in depression may be due to a diminution in opioidergic inhibitory influence. Both Judd *et al*. (1981) and Extein *et al*. (1982) reported no differences in cortisol responses to 20 mg naloxone between depressed subjects and healthy volunteers. These patients were not uniformly of the melancholic subtype, pituitary ACTH responses were not measured, and furthermore, those in the first study were not drug-naive. A further more methodologically sound study again failed to find any differences between depressives and control subjects (Zis *et al*. 1989). Recent unpublished work by our group did, however, find a blunted cortisol release in subjects with major depression using a test protocol identical to that employed in this study. Thus, a review by Zis & Garland (1991) of opioid peptides and depression in which it was concluded that there was no evidence to support the hypothesis that the hypercortisolaemia present in depression was associated with a decreased endogenous opioidergic tone cannot be taken as a definitive statement and further exploration of this interaction is required.

Exercise is a potent stimulus of the HPA axis (Wittert *et al*. 1991), and there is evidence that highly trained male athletes have increased central opioid tone (Inder *et al*. 1995*c*). The corollary of this is that sedentary individuals may have a reduction in opioid tone. We did not

objectively measure levels of activity in our CFS subjects. This is a limitation of our study. There is no study, to our knowledge, relating reduced activity to a lowering in opioid tone, although interestingly, obesity has been linked with such an effect (Satta *et al*. 1992). None of the control of CFS subjects in this study were obese.

A possible explanation for the blunting of ACTH release seen in CFS is that of central adrenergic dysregulation. Naloxone-induced ACTH release in humans is blocked by thymoxamine, the α1-antagonist (Grossman & Besser, 1992) and increased by methylphenidate the α2-adrenergic agonist (Joyce & Donald, 1987). These studies support the concept of central α1-adrenergic mediated pathways, which stimulate ACTH secretion and which are modulated by tonic inhibitory effects of endogenous opioids. Naloxone, in blocking the central opioidergic pathways, causes a disinhibition of the excitatory α1-noradrenergic pathways that project from the locus coeruleus to the parvocellular neurons of the hypothalamic paraventricular nuclei (Swanson *et al*. 1983). This increase in central α1-noradrenergic activity stimulates the secretion of hypothalamic CRH (Grossman & Besser, 1982; Nikolaris *et al*. 1987). Co-administration of naloxone with agents that increase noradrenergic transmission, such as desipramine the noradrenaline reuptake inhibitor (Torpy *et al*. 1995), leads to a synergistic ACTH/cortisol response (Al-Damluji *et al*. 1990). The blunting of ACTH release following naloxone in CFS patients may thus reflect an abnormality of the noradrenergic system, either centrally at the locus coeruleus or at the hypothalamus where there is an intrinsic association of the noradrenergic and CRH fibres (Swanson *et al*. 1983). Other than an examination of CSF monoamines (Demitrack *et al*. 1989), the levels of which were normal in CFS, no neuroendocrine challenge tests of noradrenergic functioning in this condition have to the authors knowledge been conducted, and they warrant exploration.

The attenuated ACTH response to naloxone seen in CFS may be indicative of a reduction in the secretagogue/s through which naloxone has been described to act in bringing about pituitary–adrenal activation. CSF CRH levels have not been shown to differ between CFS patients and controls (Demitrack *et al*. 1991). This conclusion was based on a single sample taken in the morning at the nadir of the normal circadian rhythm for CRH release (Garrick *et al*. 1987). Furthermore, CSF CRH may not reflect accurately, levels of CRH at the median eminence. Studies of the circadian release of CRH (also ACTH and cortisol) in CFS are necessary to clarify this possibility. A downregulation/subsensitivity of the anterior pituitary corticotrope would also explain the blunted ACTH release seen, as indeed could an abnormality of the pituitary corticotrope itself, with a reduced synthesizing and releasing capacity for ACTH. Of importance also is a study by Bakheit and colleagues (1993) who demonstrated low levels of AVP in CFS/post-viral fatigue syndrome subjects. The blunted ACTH response to naloxone may thus also reflect a reduction in hypothalamic–hypophyseal vasopressin levels as in the above mentioned study by Inder and colleague (1995*b*).

Conclusion

The opioid system is one of the primary inhibitory regulators of HPA activity. This study demonstrates that abnormalities of pituitary–adrenal activation in CFS are not readily explicable by excessive central opioidergic tone. Future studies may include those that help uncover the relative contribution of alterations in central adrenergic activity, and of hypothalamic CRH and VP levels, to the attenuated naloxone-mediated ACTH release in CFS subjects as revealed in this study.

Dr L. Scott is supported by a Linbury Trust project grant.

REFERENCES

Al-Damluji, S., Bouloux, P., White, A. & Besser, G. M. (1990). The role of α-2 adrenoceptors in the control of ACTH secretion: interaction with the opioid system. *Neuroendocrinology* **51**, 76–81.

American Psychiatric Association (1987). *Diagnostic and Statistical Manual*, *3rd edn* (*revised*). American Psychiatric Association: Washington.

Antoni, F. (1993). Vasopressinergic control of pituitary adrenocorticotropin secretion comes of age. *Frontiers in Neuroendocrinology* **14**, 76–122.

Bakheit, A., Behan, P., Dinan, T. G., Gray, C. E. & O'Keane, V. (1992). Possible upregulation of the 5HT1a receptor in patients with post-viral fatigue syndrome. *British Medical Journal* **304**, 1010–1012.

Bearn, J., Allain, T., Coskeran, P., Munro, N., Butler, J., McGregor, A. & Wessely, S. (1995). Neuroendocrine responses to D-fenfluramine and insulin-induced hypoglycaemia in chronic fatigue syndrome. *Biological Psychiatry* **37**, 245–252.

Blevins, S. S., Dobs, A. S. & Wand, G. S. (1994). Naloxone-induced activation of the hypothalamic–pituitary–adrenal axis in suspected central adrenal insufficiency. *American Journal of Medical Sciences* **308**, 167–170.

Cleare, A. J., Bearn, J., Allain, T., McGregor, A., Wessely, R. & O'Keane, V. (1995). Contrasting neuroendocrine responses in depression and chronic fatigue syndrome. *Journal of Affective Disorders* **34**, 283–889.

DeBold, C., Sheldon, W. & DeCherney, G. (1983). Arginine vasopressin potentiates adrenocorticotropin release induced by ovine corticotropin-releasing factor. *Journal of Clinical Investigation* **73**, 533–538.

Delitala, G., Grossman, A. & Besser, M. (1983). Differential effects of opiate peptides and alkaloids on anterior pituitary hormone secretion. *Neuroendocrinology* **37**, 275–279.

Delitala, G., Trainer, P. J., Oliva, O., Fanciulli, G. & Grossman, A. B. (1994). Opioid peptide and α-adreno-receptor pathways in the regulation of pituitary–adrenal axis in man. *Journal of Endocrinology* **141**, 163–168.

Demitrack, M. A., Gold, P. W., Dale, J. K., Krahn, D. D., Kling, M. A. & Straus, S. E. (1989). Plasma and cerebrospinal fluid metabolism in patients with chronic fatigue syndrome. *Biological Psychiatry*, **32**, 1065–1077.

Demitrack, M. A., Dale, J. K., 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 chronic fatigue syndrome. *Journal of Clinical Endocrinology and Metabolism* **73**, 1224–1234.

Dash, R. J., England, B. J., Midgley, A. R. & Niswender, G. D. (1975). A specific non-chromatographic radioimmunoassay for human cortisol. *Steroids* **26**, 647.

Dinan, T. G. (1996). Serotonin and the regulation of the hypothalamic–pituitary–adrenal axis function. *Life Sciences* **58**, 1683–1694.

Dinan, T. G., Majeed, T., Lavelle, E., Scott, L., Berti, C. & Behan, P. (1997). Serotonin-mediated activation of the hypothalamic–pituitary–adrenal axis in chronic fatigue syndrome. *Psychoneuroendocrinology* **22**, 261–268.

Estienne, M. J., Kesner, J. S., Barb, C. R., Kraeling, M. J. & Rampecek, G. B. (1988). On the site of action of naloxone-stimulated cortisol secretion in gilts. *Life Sciences* **43**, 161–166.

Extien, I., Pottash, A. L. C. & Gold, M. S. (1982). A possible receptor of dysfunction in some depressive disorders. *Annals of New York Academy of Sciences* **398**, 113–119.

Fukuda, K., Straus, S. E., Hickie, I., Sharpe, M., Dobbins, J. G., Komaroff, A. & the International Chronic Fatigue Syndrome Study Group (1994). The chronic fatigue syndrome: a comprehensive approach to its definition and study. *Annals of Internal Medicine* **12**, 953–959.

Gaitan, D., Loosen, P. T. & Burns, D. (1993). Plasma ACTH response to naloxone in major depressive disorder (MDD), chronic alcoholism (ALC), Cushings disease (CD) and normal subjects (NL). *Program of the Endocrine Society USA* (abstr) No. 752C, 238.

Garrick, N. A., Hill, J. L., Szele, F. G., Tomai, T. P. & Gold, P. W. (1987). Corticotropin-releasing factor: a marked circadian rhythm in primate cerebrospinal fluid peaks in the evening and is inversely related to the cortisol circadian rhythm. *Endocrinology* **121**, 1329–1334.

Gold, P. W., Licinio, J., Wong, M. & Chrousos, G. P. (1991). Corticotropin releasing hormone in the pathophysiology of melancholic and atypical depression and in the mechanism of action of antidepressant drugs. *Annals of New York Academy of Sciences* **77**, 716–729.

Grossman, A. & Besser, G. M. (1982). Opiates control ACTH release through a noradrenergic mechanism. *Clinical Endocrinology* **17**, 287–290.

Grossman, A., Gaillard, R. C., McCartney, P., Rees, L. H. & Besser, G. M. (1982). Opiate modulation of the pituitary-adrenal axis: effects of stress and circadian rhythm. *Clinical Endocrinology* **17**, 279–286.

Grossman, A. & Costa, A. (1993). The regulation of hypothalamic CRH: impact of in vitro studies on the central control of the stress response. *Functional Neurology* **8**, 325–334.

Grossman, A., Moult, P. J. A., Cunnah, D. & Besser, G. M. (1986). Different opioid mechanisms are involved in the modulation of gonadotrophin and ACTH release in man. *Neuroendocrinology* **42**, 357–360.

Heninger, G. R., Charney, D. S. & Sternberg, D. E. (1984). Serotonergic function in depression. Prolactin responses to intravenous tryptophan in depressed patients and healthy subjects. *Archives of General Psychiatry* **41**, 398–402.

Hockings, G. I., Grice, J. E., Ward, W. K., Walters, M. M., Jensen, G. R. & Jackson, R. V. (1993). Hypersensitivity of the hypothalamic–pituitary–adrenal axis to naloxone in post-traumatic stress disorder. *Biological Psychiatry* **33**, 585–593.

Hockings, G. I., Grice, J. E., Walters, M. M., Crosbie, G. V., Torpy, D. J. & Jackson, R. V. (1995). A synergistic adrenocorticotropin response to naloxone and vasopressin in normal humans: evidence that naloxone stimulates endogenous corticotropin-releasing hormone. *Neuroendocrinology* **61**, 198–206.

Holsboer, F., Gerken, A., Von Bardeleben, U., Grimm, W., Beyer, H., Muller, O. A. & Stalla, G. K. (1986). Blunted corticotropin and normal cortisol response to human corticotropin-releasing factor (h-CRF) in depression. *New England Journal of Medicine* **311**, 1127.

Inder, W. J., Ellis, M. J., Evans, M. J. & Donald, R. A. (1995*a*). A comparison of the naloxone test with ovine CRH and insulin hypoglycaemia in the evaluation of the hypothalamic–pituitary–adrenal axis. *Clinical Endocrinology* **43**, 425–431.

Inder, W. J., Joyce, P. R., Ellis, M. J., Evans, M. J., Livesey, J. H. & Donald, R. A. (1995*b*). The effects of alcoholism on the hypothalamic–pituitary–adrenal axis: interaction with endogenous opioid peptides. *Clinical Endocrinology* **43**, 283–290.

Inder, W. J., Hellemans, J., Ellis, M. J., Evans, M. J., Livesey, J. H. & Donald, R. A. (1995*c*). Elevated basal adrenocorticotropin and evidence for increased central opioid tone in highly trained male athletes. *Journal of Clinical Endocrinology and Metabolism* **80**, 244–247.

Jackson, R. V., Grice, J. E., Hockings, G. I. & Torpy, D. J. (1995). Naloxone-induced ACTH release: mechanism of action in humans. *Clinical Endocrinology* **43**, 423–424.

Joyce, P. R. & Donald, R. A. (1987). Naloxone augments the hypothalamic–pituitary–adrenal axis response to methylphenidate in normal subjects. *Journal of Psychiatric Research* **21**, 197–200.

Judd, L. L., Janowsky, D. S., Zettner, A., Huey, L. Y. & Takahashi, K. I. (1981). Effects of naloxone hydrochloric on cortisol levels in patients with affective disorder and normal controls. *Psychiatry Research* **4**, 277–283.

Lamberts, S., Verleun, S., Oosteron, R., DeJong, F. & Hackeng, W. (1984). Corticotropin-releasing factor (ovine) and vasopressin exert a synergistic effect on adrenocorticotropin release in man. *Journal of Clinical Endocrinology and Metabolism* **58**, 298–303.

Lesch, K. P., Rupprecht, R., Poten, B., Muller, U., Sohnle, K., Fritze, J. & Schulte, H. M. (1989). Endocrine responses to 5-HT1a receptor activation by ipsapirone in humans. *Biological Psychiatry* **26**, 203–205.

Liposits, Z. C., Phelix, C. & Paull, W. K. (1987). Synaptic interaction of serotonergic axons and corticotropin releasing factor (CRF) synthesizing neurons in the hypothalamic paraventricular nucleus. *Histochemistry* **86**, 541–549.

Michelson, D., Licinio, J. & Gold, P. W. (1995). Mediation of the stress response by the hypothalamic–pituitary–adrenal axis. In *Neurobiological and Clinical Consequences of Stress: From Normal Adaptation to PTSD* (ed. M. J. Friedman, D. S. Charney and A. Y. Deutch), pp. 225–238. Lippincott-Raven Publishers: Philadelphia.

Nikolaris, K., Pfeiffer, A., Stalla, K. G. & Herz, A. (1987). The role of CRF in the release of ACTH by opiate agonists and antagonists in rats. *Brain Research* **421**, 373–376.

O'Keane, V. & Dinan, T. G. (1991). Prolactin and cortisol responses to d-fenfluramine in major depression: evidence for diminished responsivity of central serotonergic function. *American Journal of Psychiatry* **148**, 1009–1015.

Raff, H. & Findling, J. W. (1989). A new immunoradiometric assay for corticotropin evaluated in normal subjects and patients with Cushing's syndrome. *Clinical Chemistry* **35**, 595–600.

Satta, M. A., Maussier, M. L., DeRosa, G., Valenza, V., Testa, A., Salenich, I., Napolitano, G. & Monaco, F. (1992). Effects of naloxone on adrenocorticotrophin (ACTH) and cortisol in obese subjects. *Hormonal Metabolic Research* **24**, 44–45.

Scott, L. V. & Dinan, T. G. (1997). Urinary free cortisol levels in chronic fatigue syndrome, in depression and in health. *Journal of Affective Disorders* (in the press).

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

Shorter, E. (1993). Chronic fatigue syndrome in historical perspective. In *Ciba Foundation Symposium 173 Chronic Fatigue Syndrome*. (ed. D. Chadwick, J. Marsh and K. Ackrill), pp. 6–16. Wiley: Chichester. 6–16.

Statistical Graphics Corporation (1987). SGCS: New York.

Stubbs, W. A., Delitala, G. & Jones, A. (1978). Hormonal and metabolic responses to an enkephalin analogue in normal man. *Lancet* ii, 1225–1227.

Swanson, L. W., Sawchencko, P. E., Rivier, J. & Vale, W. W. (1983). Organization of ovine corticotropin-releasing factor like immunoreactive cells and fibers in rat brain: an immunohistochemical study. *Neuroendocrinology* **36**, 165–186.

Taylor, T., Dluhy, R. G. & Williams, G. H. (1983). Beta-endorphin suppresses adrenocorticotrophin and cortisol levels in normal human subjects. *Journal of Clinical Endocrinology and Metabolism*. **57**, 592–596.

Terenius, L. & Wahlstrom, A. (1978). Physiological and clinical relevance of endorphins. In *Centrally Acting Peptides* (ed. J. Hughes), pp. 161–178. Baltimore University Park Press: Maryland.

Torpy, D. J., Jackson, R. V., Grice, G. E., Hockings, G. I., Strakosch, C. R. & Topliss, D. J. (1993). Naloxone stimulation of ACTH secretion during petrosal sinus sampling in Cushing's syndrome. *Clinical and Experimental Pharmacology and Physiology* **20**, 299–302.

Torpy, D. J., Grice, J. E., Hockings, G. I., Crosbie, G. V., Walters, M. M. & Jackson, R. V. (1995). The effect of desipramine on basal and naloxone-stimulated cortisol secretion in humans: interaction of the two drugs acting on noradrenergic control of adrenocorticotropin secretion. *Journal of Clinical Endocrinology and Metabolism* **80**, 802–806.

Tsagarakis, S., Holly, J. M. P., Rees, L. H., Besser, G. M. & Grossman, A. (1989). Morphine directly modulates the release of stimulated corticotropin-releasing factor-41 from the rate hypothalamus in vitro. *Endocrinology* **124**, 2330–2335.

Volavka, J., Cho, D., Mallya, A. & Bauman, A. (1979). Naloxone increases ACTH and cortisol levels in normal man. *New England Journal of Medicine* **300**, 1056–1057.

Wesseley, S. & Powell, R. (1989). Fatigue syndromes: a comparison of chronic 'postviral' fatigue with neuromuscular and affective disorders. *Journal of Neurology*, *Neurosurgery and Psychiatry* **42**, 940–948.

Wittert, G. A., Stewart, D. E., Graves, M. P., Ellis, M. J., Evans, M. J., Wells, J. E., Donald, R. A. & Espiner, R. A. (1991). Plasma corticotropin releasing factor and vasopressin responses to exercise in normal man. *Clinical Endocrinology* (*Oxf*) **35**, 311–317.

Yatham, L. N., Morehouse, R. L., Chisholm, B. T., Haase, D. A., MacDonald, D. D. & Marrie, T. J. (1995). Neuroendocrine assessment of serotonin function in chronic fatigue syndrome. *Canadian Journal of Psychiatry* **40**, 92–96.

Yehuda, R., Southwick, S. M., Nussbaum, G., Wahby, V., Giller, E. L. & Mason, J. (1990). Low urinary cortisol excretion in patients with posttraumatic stress disorder. *Journal of Nervous and Mental Disorders* **187**, 366–369.

Zis, A. P., Grant, B. E. K., Remick, R. A. & Clark, C. M. (1989). Cortisol response to naloxone in depression. *International Congress Series Excerpta Medica* **899**, 66.

Zis, A. P. & Garland, J. E. (1991). Opioid peptides and depression. In *Clinical Endocrinology and Metabolism* (ed. E. Ur), vol. 5, pp. 97–117. Balliérè Tindall: London.