

Hypothalamo-Pituitary-Adrenal Axis Dysfunction in Chronic Fatigue Syndrome, and the Effects of Low-Dose Hydrocortisone Therapy

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These neuroendocrine studies were part of a series of studies testing the hypotheses that 1) there may be reduced activity of the hypothalamic-pituitary-adrenal axis in chronic fatigue syndrome and 2) low-dose augmentation with hydrocortisone therapy would improve the core symptoms. We measured ACTH and cortisol responses to human CRH, the insulin stress test, and D-fenfluramine in 37 medication-free patients with CDC-defined chronic fatigue syndrome but no comorbid psychiatric disorders and 28 healthy controls. We also measured 24-h urinary free cortisol in both groups. All patients (n = 37) had a pituitary challenge test (human CRH) and a hypothalamic challenge test [either the insulin stress test (n = 16) or D-fenfluramine (n = 21)]. Baseline cortisol concentrations were significantly raised in the chronic fatigue syndrome group for the human CRH test only. Baseline ACTH concentrations did not differ between groups for any test. ACTH responses to human CRH, the insulin stress test, and D-fenfluramine were similar for patient and control groups. Cortisol responses to the insulin stress test did not differ between groups, but there was a trend for cortisol responses both to human CRH and D-fenfluramine to be lower in the chronic fatigue syndrome group. These differences were significant when ACTH responses were controlled. Urinary free cortisol levels were lower in the chronic fatigue syndrome group compared with the healthy group. These results indicate that ACTH responses to pituitary and hypothalamic challenges are

intact in chronic fatigue syndrome and do not support previous findings of reduced central responses in hypothalamic-pituitary-adrenal axis function or the hypothesis of abnormal CRH secretion in chronic fatigue syndrome. These data further suggest that the hypocortisolism found in chronic fatigue syndrome may be secondary to reduced adrenal gland output.

Thirty-two patients were treated with a low-dose hydrocortisone regime in a double-blind, placebo-controlled cross-over design, with 28 days on each treatment. They underwent repeated 24-h urinary free cortisol collections, a human CRH test, and an insulin stress test after both active and placebo arms of treatment. Looking at all subjects, 24-h urinary free cortisol was higher after active compared with placebo treatments, but 0900-h cortisol levels and the ACTH and cortisol responses to human CRH and the insulin stress test did not differ. However, a differential effect was seen in those patients who responded to active treatment (defined as a reduction in fatigue score to the median population level or less). In this group, there was a significant increase in the cortisol response to human CRH, which reversed the previously observed blunted responses seen in these patients. We conclude that the improvement in fatigue seen in some patients with chronic fatigue syndrome during hydrocortisone treatment is accompanied by a reversal of the blunted cortisol responses to human CRH. (*J Clin Endocrinol Metab* 86: 3545–3554, 2001)

CHRONIC FATIGUE SYNDROME (CFS) is an operationally defined condition probably representing the end of a continuum (1). Current definitions require the presence of prolonged fatigue with marked disability, together with several somatic symptoms, in the absence of organic illness or severe psychiatric disorder that would explain the fatigue (2). There seems little doubt that CFS is a heterogeneous and multifactorial illness best understood from a biopsychosocial perspective (3).

Within this paradigm, there are several reasons for implicating reduced function of the hypothalamic-pituitary-adrenal (HPA) axis in the etiology of CFS. There are similarities

between the onset, course, and clinical syndromes of CFS and glucocorticoid deficiency states. Patients with CFS usually report the onset of their syndrome following a significant stressor, most frequently a viral infection, and the course of the syndrome remits and relapses with the occurrence of physical and psychological stressors (4). Addison's disease can give rise to vague symptoms of chronic malaise before the onset of biochemical abnormalities, which can culminate in an Addisonian crisis at a time of acute viral infection (5). Finally, the clinical syndromes of CFS and Addison's disease share many common features: one of the principal clinical features of Addison's is fatigue, the core feature of CFS. Other common symptoms include arthralgia, myalgia, adenopathy, exacerbation of allergic responses, intermittent fever, postexertional fatigue, and depressed mood. Furthermore, these symptoms can also be experienced by those withdrawing from hypercortisolaemic states (6).

It is, therefore, of great interest that abnormal function of

Abbreviations: AUC, Area under the curve; CGI, Clinical Global Impression; CI, confidence interval; CFS, chronic fatigue syndrome; CV, coefficient(s) of variation; hCRH, human CRH; HPA, hypothalamic-pituitary-adrenal; IST, insulin stress test; UFC, urinary free cortisol; WSAS, Work and Social Adjustment Scale.

the HPA axis in CFS has been demonstrated in several centers. Common to many of the studies is the finding of reduced adrenal output of cortisol. Thus, reduced basal morning (7, 8) and evening (9) plasma cortisol levels, reduced 24-h urinary free cortisol (UFC) output (9, 10), and reduced salivary cortisol levels (11) have all been reported. Some studies have failed to replicate this data, including one study failing to find either lowered salivary cortisol or 24-h UFC levels (12), and one finding increased salivary cortisol, although numbers were small and some patients had high depression scores (13).

The underlying HPA axis disturbance that might cause this reduction in cortisol output remains unclear, however (14). In view of this uncertainty, we wished to define further the dynamics of the HPA axis in CFS patients who would not have perturbations of their HPA axes as a consequence of major depressive disorder. To this end, we looked at ACTH and cortisol responses to several challenge agents in a group of patients with CFS without comorbid psychiatric disorder, and compared these responses to those of a healthy control group. The challenges used were human CRH (hCRH), insulin-induced hypoglycemia [the insulin stress test (IST)], and the 5-hydroxytryptamine (5-HT)-releasing drug *D*-fenfluramine. Unstimulated output from the adrenal glands was assessed using 24-h UFC measurement.

We have hypothesized previously that treating patients with a low dose of hydrocortisone sufficient to replace this presumed deficiency might be beneficial. We felt that whether low cortisol output is a primary disturbance in the illness, or secondary to effects of illness such as sleep disturbance or inactivity, it is plausible to suggest that low cortisol levels could be contributing to the maintenance of a chronically fatigued state. We tested this using a placebo-controlled double-blind cross-over study comparing two doses of hydrocortisone with placebo (15). Results showed that, overall, both doses of hydrocortisone resulted in a significant reduction in mean self-reported fatigue scores compared with placebo. About one third of patients on active treatment showed a clinically meaningful drop in fatigue to a level at or below the normal population mean, compared with less than 10% on placebo.

Thus, here we also describe the effect of this replacement strategy on the HPA axis in CFS. We hypothesized that if a reduction in adrenal output of cortisol was the primary abnormality of the HPA axis in CFS, then cortisol replacement would be associated with a normalization of the other HPA axis abnormalities in CFS. Furthermore, given that only a proportion of patients respond to cortisol replacement, we hypothesized that it may only be in these patients that significant changes occur.

Materials and Methods

Subjects

A total of 37 patients with CFS were recruited into the study from referrals to CFS clinics at King's College School of Medicine and Dentistry and Addenbrooke's NHS trust (Cambridge, UK). All patients had undergone thorough medical screening to exclude a detectable organic cause for their fatigue, including physical examination and relevant investigation, with a minimum of urinalysis, full blood count, urea and electrolytes, thyroid function tests, liver function tests, 0900-h cortisol,

and erythrocyte sedimentation rate. All patients were interviewed using a semistructured interview for CFS and psychological disorder (16) by a psychiatrist (A.J.C., E.H., V.O., or G.S.M.). Included subjects had to fulfill both international consensus criteria for CFS (2, 17) and be free from comorbid psychiatric disorder as defined in The Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (18). Patients with an illness duration of more than 100 months, or aged less than 18 or more than 65 yr, were excluded. All were drug free for a minimum of 2 months before endocrine testing, with the exception of three patients using oral contraception or hormone replacement therapy. Female patients were tested during d 1–7 of their menstrual cycle or hormone replacement cycle; all postmenopausal females were taking hormone replacement therapy. Other exclusion criteria were the presence of a medical contraindication for receiving hydrocortisone or an inability to attend hospital for screening, testing or follow-up visits.

Twenty-eight control subjects were recruited from hospital staff and students. Only those without significant medical histories and with no current medical or psychiatric disorder were recruited.

The hospital ethics committees approved all procedures. All patients and controls gave written informed consent.

Procedures

Baseline testing. Subjects underwent two challenge tests. All 37 patients underwent a hCRH test, whereas those tested at the King's site ($n = 16$) had an IST and those at the Addenbrooke's site ($n = 21$) had a *D*-fenfluramine challenge test. Healthy controls also had a maximum of two challenge tests. In total, 28 controls underwent a hCRH test, together with 18 who underwent a *D*-fenfluramine test at Addenbrooke's and 16 who underwent an IST assessment at King's. A single 24-h urine collection for UFC measurement was obtained before the first test in all patients and controls.

Medication. Patients were randomized to receive either hydrocortisone treatment first or placebo first, for 28 days, before crossing over to the other treatment. None of the staff or patients had access to the codes during the course of the study. The first 16 patients were allocated a 5-mg replacement dose of hydrocortisone, and the remainder 10 mg. Drug preparations were identical opaque white capsules. Patients were instructed to take one tablet each morning at 0900 h with breakfast for 28 days. The investigator dispensed the medication at each visit; compliance was assessed by counting returned tablets and directly questioning patients. A total of 32 patients entered and completed this therapy, as reported previously (15).

Follow-up testing. All 32 patients who completed treatment received repeat hCRH tests, and an additional 16 patients underwent follow-up ISTs. Repeat testing was undertaken on d 24 (hCRH) and d 28 (IST) of each treatment period; on these days subjects were asked to omit the capsule until after the test. All tests in females were performed on d 1–7 of their menstrual cycle on each occasion. In one patient the menstrual cycle was approximately 34 d long, and her medication was extended by 6 d for each treatment period to ensure tests were carried out during the same menstrual phase.

Endocrine test protocols. All tests were carried out in the hospital programmed investigation unit. Challenge tests were separated by intervals of at least 2 d. Subjects presented to the laboratory on the morning of testing following an overnight fast. Testing commenced at 0900 h with the insertion of an iv cannula in a forearm vein. The cannula was kept patent throughout by the use of heparinized saline (1 ml bolus of 10 U heparin after each sample was drawn). Following a 15-min interval, blood samples for plasma ACTH and serum cortisol were taken at –30 and 0 min (–60 and 0 min for the *D*-fenfluramine challenge). Active drug was then administered, and the protocols for each test differed as described: 1) hCRH test: 1 μ g/kg body weight hCRH (Shire Laboratories, Inc., Andover, UK) was given as a bolus dose. Additional samples were drawn at 15, 30, 60, and 90 min for plasma ACTH and serum cortisol; 2) *D*-fenfluramine challenge test: subjects took 30 mg *D*-fenfluramine orally in capsular form and had samples for plasma ACTH and serum cortisol drawn at hourly intervals for 5 h thereafter (*i.e.* at +1, +2, +3, +4, and +5 h); 3) IST: subjects underwent a standard IST using 0.15 U/kg body weight insulin (19). Before testing, an electrocardiogram was obtained to exclude significant cardiac abnormality. The insulin was given

in a bolus via the cannula. Additional samples were drawn at 30, 60, 90, and 120 min for plasma ACTH and serum cortisol. In addition, blood glucose was monitored at 0, 30, 45, 60, 90, and 120 min using an immediate Glucostix monitor (Bayer PLC, Newbury, UK). More accurate analysis was carried out on stored samples in the laboratory. Patients were given 75 g oral glucose if they showed prolonged hypoglycemia on Glucostix testing or symptomatology. Two patients and three controls received glucose during baseline testing for this reason and were also given glucose on subsequent follow-up testing. Patients were given a full meal with a glucose drink after the end of the test.

After cannulation, subjects remained relaxed and semirecumbent throughout the procedure. For each test, plasma for ACTH was taken in an EDTA tube onto ice, immediately spun using a refrigerated centrifuge at 4 C, separated, and frozen at -20 C until assay. Similarly, serum for cortisol was allowed to clot before separation and then frozen at -20 C until assay.

Clinical variables

Patients were assessed using the following questionnaires: the Chalder Fatigue Scale (1, 20), the Clinical Global Impression (CGI) Scale (21), the Work and Social Adjustment Scale (WSAS) (22), and the 12-item General Health Questionnaire (23). The Chalder Fatigue Scale measures subjective fatigue, the WSAS assesses the degree of disability, the General Health Questionnaire measures psychological symptoms, and the CGI is an overall objective assessment of morbidity. Patients were assessed before endocrine testing at baseline and on d 28 of each treatment period. The main outcome measure was chosen as the fatigue scale of Chalder and co-workers (1, 20).

Hormone assays

ACTH was measured using a RIA (Diagnostics Systems Laboratories, Inc., Webster, TX). Sensitivity of the assay was 0.8 pmol/liter. Interassay coefficients of variation (CV) were 9.6% at 35 pg/ml, 4.0% at 72 pg/ml, and 7.3% at 160 pg/ml. Intra-assay CV were 6.9% at 35 pg/ml, 5.9% at 71 pg/ml, and 5.3% at 152 pg/ml. Cortisol was measured using a solid-phase RIA from Diagnostic Products (Los Angeles, CA). Intra-assay CV were 4.8% at 85 nmol/liter, 4.7% at 273 nmol/liter, and 3.0% at 551 nmol/liter. Interassay CV were 5.2% at 91 nmol/liter, 4.0% at 579 nmol/liter, and 6.4% at 993 nmol/liter. Sensitivity was 5.5 nmol/liter.

Analysis

The hormone responses were calculated either as the peak-minus baseline (or Δ response) or as the integrated area under the curve (AUC) values on the baseline (0 min) corrected response, using the trapezoidal model. Statistical comparisons were made using independent or paired *t* tests or one-way ANOVA. For the cortisol responses to CRH, we calculated an ANOVA using both the raw values, and using the ACTH response as a covariate to correct for pituitary responses. The criterion chosen for a response to treatment was the self-rated fatigue scale; a value of 12 or less after treatment (equal to the median population score; Ref. 1) was determined to be a response (15). Means are given \pm SD or with 95% confidence intervals (CIs) in parentheses.

Results

Baseline testing

Description of subjects. The CFS test group was composed of 12 males and 25 females. The mean duration of illness was 42.9 (95% CI, 31.7–54.1) months, whereas 23 of the 37 attributed the onset of their symptoms to a viral illness. The control group included 14 males and 14 females. Mean ages in the two groups did not differ [33.8 (95% CI 30.3–37.3) yr in the CFS group and 32.4 (95% CI 28.5–36.3) yr in the healthy group, $P = 0.6$]. The body mass index in the CFS group was 23.3 (95% CI 22.1–24.2) kg/m² and in the control group was 24.4 (95% CI 23.0–25.8) kg/m², $P = 0.23$). Endocrine results are summarized in Tables 1–3.

hCRH challenge. See Table 1 for results. Baseline cortisol concentrations were significantly raised in the CFS compared with the healthy group (508 \pm 216 nmol/liter *vs.* 402 \pm 225 nmol/liter, $P = 0.047$). Baseline ACTH concentrations did not differ between groups. ACTH responses to hCRH, as measured by the AUC, were similar between CFS and healthy groups (34 \pm 28 pg/ml·h *vs.* 31 \pm 32 pg/ml·h, $P = 0.67$) (see Fig. 1). There was a trend for AUC cortisol values to be reduced in CFS compared with control subjects (206 \pm 213 nmol/liter·h *vs.* 313 \pm 257 nmol/liter·h, $P = 0.069$) (see Fig. 1). Given that ACTH responses to hCRH were similar between groups, the CFS group had a proportionately smaller release of cortisol: covarying for ACTH release indicated a significantly reduced release of cortisol: $F = 6.07$, $df (1,63)$, $P = 0.016$.

D-Fenfluramine challenge. See Table 2 for results. There were no differences in baseline concentrations of either cortisol or ACTH between groups. ACTH responses to challenge, as measured by Δ values, were similar in the two groups (24 \pm 45 pg/ml *vs.* 22 \pm 53 pg/ml, $P = 0.9$) (see Fig. 2). There was a trend for Δ cortisol responses to be reduced in CFS subjects (122 \pm 151 nmol/liter *vs.* 219 \pm 177 nmol/liter, $P = 0.077$) (see Fig. 2). When ACTH responses were covaried for, cortisol responses to D-fenfluramine were significantly reduced in the CFS patients [$F = 4.95$, $df (1, 31)$, $P = 0.033$].

IST assessment. See Table 3 for results. There were no differences between groups in the hypoglycaemic response to insulin. In patients, basal glucose was 3.9 \pm 0.43 mmol/liter, falling to a nadir of 0.91 \pm 0.30 mmol/liter, whereas in controls glucose fell from 4.0 \pm 0.53 mmol/liter to 1.0 \pm 0.29 mmol/liter ($P = 0.3$). All subjects attained adequate hypoglycemia, defined as a blood glucose value of 2.2 mmol/liter or less. Baseline concentrations of ACTH and cortisol were similar between test and control groups. There were no significant differences between either ACTH or cortisol responses to IST between test and control groups (see Fig. 3).

UFC. The CFS group had significantly lower UFC output than the control group. The mean UFC values for the control and CFS groups were 169.1 \pm 15.6 nmol/d and 122.3 \pm 11.7 nmol/d, respectively ($F = 5.73$, $df (1, 23)$, $P = 0.025$).

Clinical variables and endocrine responses. The mean fatigue score in the group was 24.8 \pm 0.83. Most participants had a CGI score of 4 (group mean, 4.03 \pm 0.11): this represents significant clinician-rated morbidity ("markedly ill"), the maximum score being 6. The mean WSAS disability score was 24.7 \pm 1.23 (maximum, 40). There was no statistical relationship between any of the clinical measures and any of the endocrine responses.

Effect of gender. We looked separately at male and female patients in comparison to their gender-matched controls. No significant differences were apparent in either of these two smaller subgroups. The observed differences were noted to be in the same direction as in the group as a whole.

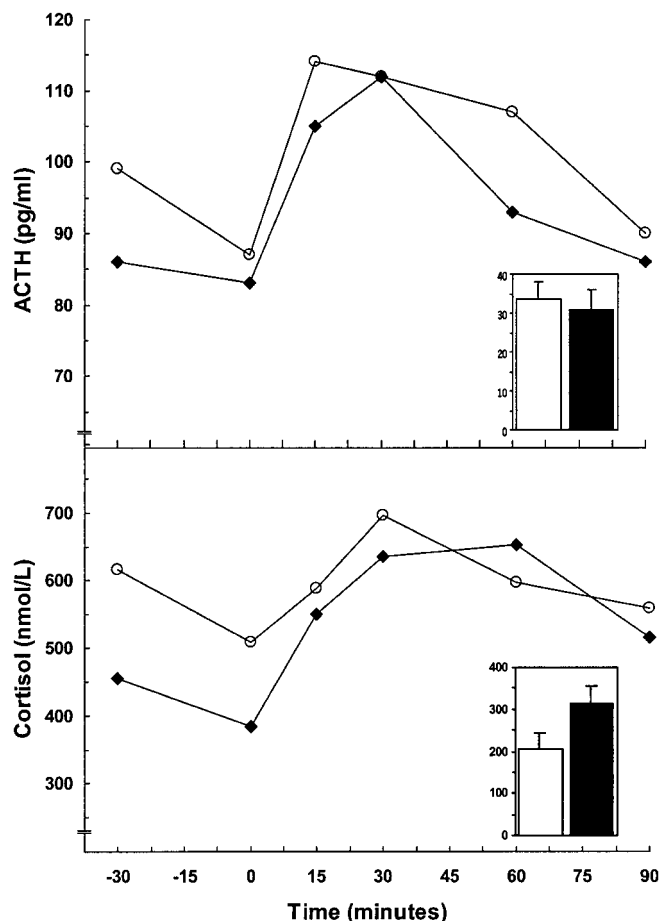


FIG. 1. Mean plasma ACTH and serum cortisol concentrations in CFS patients (circles) and healthy control subjects (diamonds) at baseline and following iv administration of hCRH in a dose of 1 μ g/kg body weight. Inset, The mean integrated AUC values and SEM (ACTH units pg/ml-h; cortisol units nmol/liter-h) in patients (□) and controls (■).

TABLE 1. Mean baseline and AUC ACTH and cortisol values for the corticotropin-releasing hormone (CRF) challenge test in CFS patients and healthy controls

	Basal ACTH (pg/ml)	Basal cortisol (nmol/liter)	AUC ACTH responses (pg/ml-h)	AUC cortisol responses (nmol/liter-h)
CFS group (n = 37)	88 ± 36.8	508 ± 216	34 ± 28	206 ± 213
Control group (n = 28)	81 ± 45.4	402 ± 225	31 ± 32	313 ± 257
Results	P = 0.49	P = 0.047	P = 0.67	P = 0.069

Follow-up testing

Description of subjects. The mean age of the 32 subjects followed up was 35.3 yr (95% CI, 31.2-39.5). Twenty were female; 9 (28%) gave a past psychiatric history, whereas 19 (59%) dated the onset of their illness to an infection. The mean length of illness was 36 months (95% CI, 28-45), and mean baseline fatigue score was 25.1 points (95% CI, 23.7-26.5). All 32 subjects completed the treatment trial; nine were categorized as treatment responders. Thirty-one of 32 attended for all tests reported here, except 1 subject (a

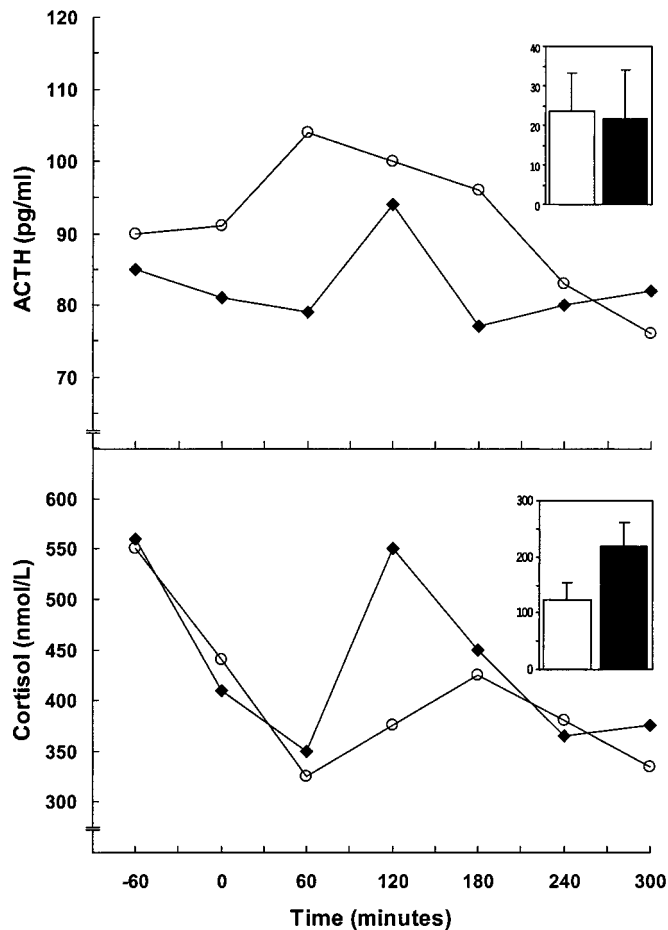


FIG. 2. Mean plasma ACTH and serum cortisol concentrations in CFS patients (circles) and healthy control subjects (diamonds) at baseline and following oral administration of D-fenfluramine in a dose of 30 mg. Inset, The mean Δ values and SEM in patients (□) and controls (■).

TABLE 2. Mean baseline and Δ and cortisol values in the d-fenfluramine challenge test in CFS patients and healthy controls

	Basal ACTH (pg/ml)	Basal cortisol (nmol/liter)	Δ ACTH responses (pg/ml)	Δ cortisol responses (nmol/liter)
CFS group (n = 21)	87 ± 43	439 ± 169	24 ± 45	122 ± 151
Control group (n = 18)	81 ± 51	430 ± 191	22 ± 53	219 ± 177
Results	P = 0.7	P = 0.87	P = 0.9	P = 0.077

treatment-responder) who did not undergo testing after the active treatment phase.

Effects of hydrocortisone on endocrine variables

hCRH test. The effect of hydrocortisone on the response to the hCRH test is shown in Table 4. Paired t tests between active and placebo, and between pretreatment and active treatment conditions, revealed no significant overall effects of the hydrocortisone on either AUC or basal ACTH or AUC cortisol. Basal cortisol was lower in the active treatment group than baseline (t = 2.99, P = 0.005) but not significantly

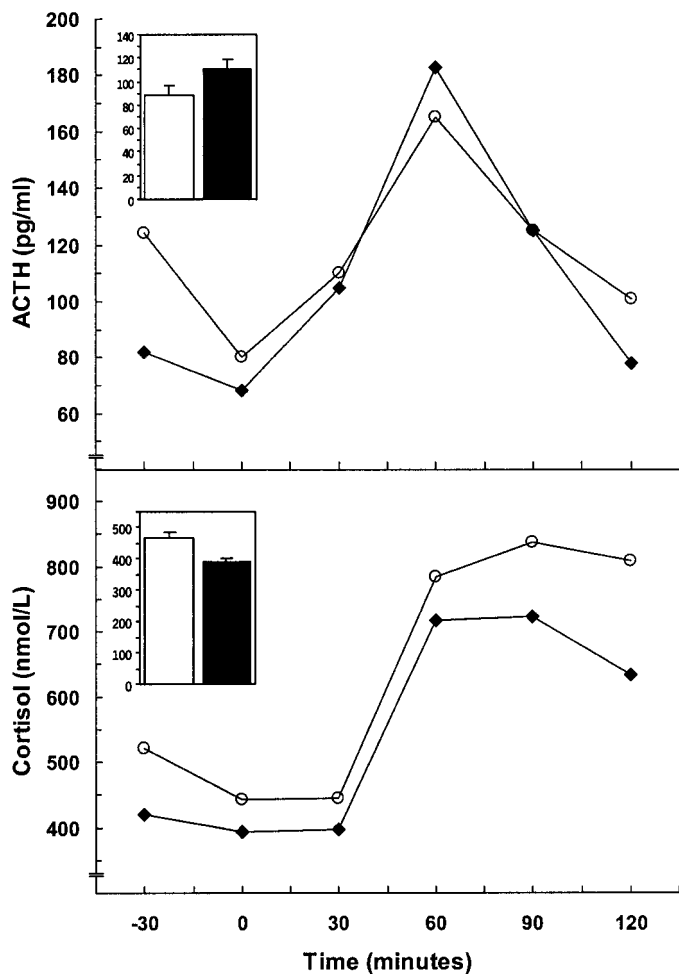


FIG. 3. Mean plasma ACTH and serum cortisol concentrations during insulin-induced hypoglycemia in CFS patients (circles) and healthy control subjects (diamonds). Inset, The mean integrated AUC values and SEM (ACTH units pg/ml·h; cortisol units nmol/liter·h) in patients (□) and controls (■).

TABLE 3. Mean baseline and AUC ACTH and cortisol values in the IST challenge test in CFS patients and healthy controls

	Basal ACTH (pg/ml)	Basal cortisol (nmol/liter)	AUC ACTH responses (pg/ml·h)	AUC cortisol responses (nmol/liter·h)
CFS group (n = 16)	80 ± 39	443 ± 211	84 ± 79	465 ± 324
Control group (n = 16)	68 ± 32	393 ± 226	110 ± 71	390 ± 185
Results	P = 0.34	P = 0.52	P = 0.41	P = 0.42

different from placebo ($t = -1.90, P = 0.07$). Analyzing the two treatment doses of hydrocortisone separately made no difference to these findings.

IST. All subjects became hypoglycemic after insulin in every test, as defined by achieving a plasma glucose of under 2.2 mmol/liter. The mean glucose nadir was 0.91 ± 0.30 pretreatment, 1.02 ± 0.32 after active treatment, and 0.97 ± 0.43 after placebo treatment. Table 4 shows the cortisol and AUC values pretreatment and after active or placebo treatments. Paired t tests showed that neither AUC cortisol nor

AUC or basal ACTH was altered in the whole group by hydrocortisone treatment. Basal cortisol was lower after active treatment than at baseline ($t = 2.23, P = 0.04$) but no different from placebo ($t = -1.07, P = 0.31$). Analyzing the two treatment doses of hydrocortisone separately made no difference to these findings.

UFC. Table 4 shows the effect of active or placebo treatment on the 24-h UFC output. Active treatment resulted in significantly higher output than both pretreatment measurements ($t = 2.52, P = 0.019$) and placebo treatment ($t = 3.30, P = 0.003$).

Relation of treatment response to endocrine variables

hCRH test. In those patients who did not respond to the treatment, there was little effect of the treatment on the cortisol response. In contrast, those patients who did respond to active treatment showed a marked enhancement of the cortisol response (see Fig. 4). The integrated AUC cortisol after active treatment was significantly larger than that after placebo treatment (333 ± 255 nmol/liter·h v 179 ± 194 nmol/liter·h, paired $t = 3.22, P = 0.015$) in these treatment-responders. Comparable data for the nonresponders were 242 ± 150 nmol/liter·h after active and 246 ± 196 nmol/liter·h after placebo treatments (paired $t = -0.11, P = 0.92$). Looking at this another way, the mean increase in the AUC cortisol after active treatment compared with placebo (Δ AUC cortisol) was significantly higher in responders (154 ± 135 nmol/liter·h) than nonresponders (-4 ± 194 nmol/liter·h; $t = -2.12, P = 0.04$). Only 1 of 8 (12.5%) of the responders had a higher AUC cortisol after placebo than active treatment compared with 10 of 23 (43%) of nonresponders.

These changes in AUC in treatment-responders were not accounted for by changes in the baseline cortisol level. Thus, the 0-min cortisol value was 540 nmol/liter after placebo treatment and 475 nmol/liter after active treatment, a non-significant difference (paired $t = -1.58, P = 0.16$). In non-responders, 0-min cortisol values were 411 nmol/liter after placebo and 394 nmol/liter after active treatments (paired $t = -0.59, P = 0.56$). The data from matched healthy controls given for comparison in Fig. 4 show that in treatment-responders, the CRH/cortisol response increased on active treatment to a level comparable with controls, whereas on placebo it remained unchanged.

Treatment response made no difference to the effect of active and placebo treatments on the AUC ACTH response. This is represented in Fig. 5. AUC ACTH mean values for nonresponders were: active 16.5 ± 32.4 pg/ml·h and placebo 23.0 ± 31.6 pg/ml·h, $t = -0.66, P = 0.51$. AUC ACTH mean values for responders were: active 27.2 ± 27 pg/ml·h and placebo 17.8 ± 28 pg/ml·h, $t = 0.79, P = 0.46$. We calculated Δ AUC ACTH values as the mean increase after active compared with placebo treatment: values were 9.8 ± 35.2 pg/ml·h in responders and -5.3 ± 47.7 pg/ml·h in nonresponders, not significantly different ($t = -0.81, P = 0.42$). However, the Δ AUC ACTH values did reduce the significance of the comparison of Δ AUC cortisol between responders and nonresponders when entered as a covariate ($F_{1,26} = 2.19, P = 0.13$). Thus, part of the differential effect of hydrocortisone on the cortisol response to CRH in treatment re-

TABLE 4. Basal and AUC values for ACTH and cortisol for CRF test, IST, and 24-h UFC in all patients (n = 32) before treatment, after active treatment, and after placebo treatment

	Pretreatment	Active treatment	Placebo treatment
hCRH test			
Basal ACTH (pg/ml)	90 ± 38	92 ± 39	93 ± 42
AUC ACTH (pg/ml·h)	28 ± 32	19 ± 31	22 ± 30
Basal cortisol (nmol/liter)	497 ± 200	410 ± 159 ^a	442 ± 195
AUC cortisol (nmol/liter·h)	193 ± 232	263 ± 180	230 ± 190
IST test			
Basal ACTH (pg/ml)	80 ± 39	86 ± 32	78 ± 37
AUC ACTH (pg/ml·h)	84 ± 79	115 ± 80	83 ± 65
Basal cortisol (nmol/liter)	442 ± 211	343 ± 93 ^a	420 ± 296
AUC cortisol (nmol/liter·h)	465 ± 324	541 ± 171	498 ± 299
24-h UFC (nmol/d)	105 ± 51	146 ± 93 ^{a,b}	100 ± 51

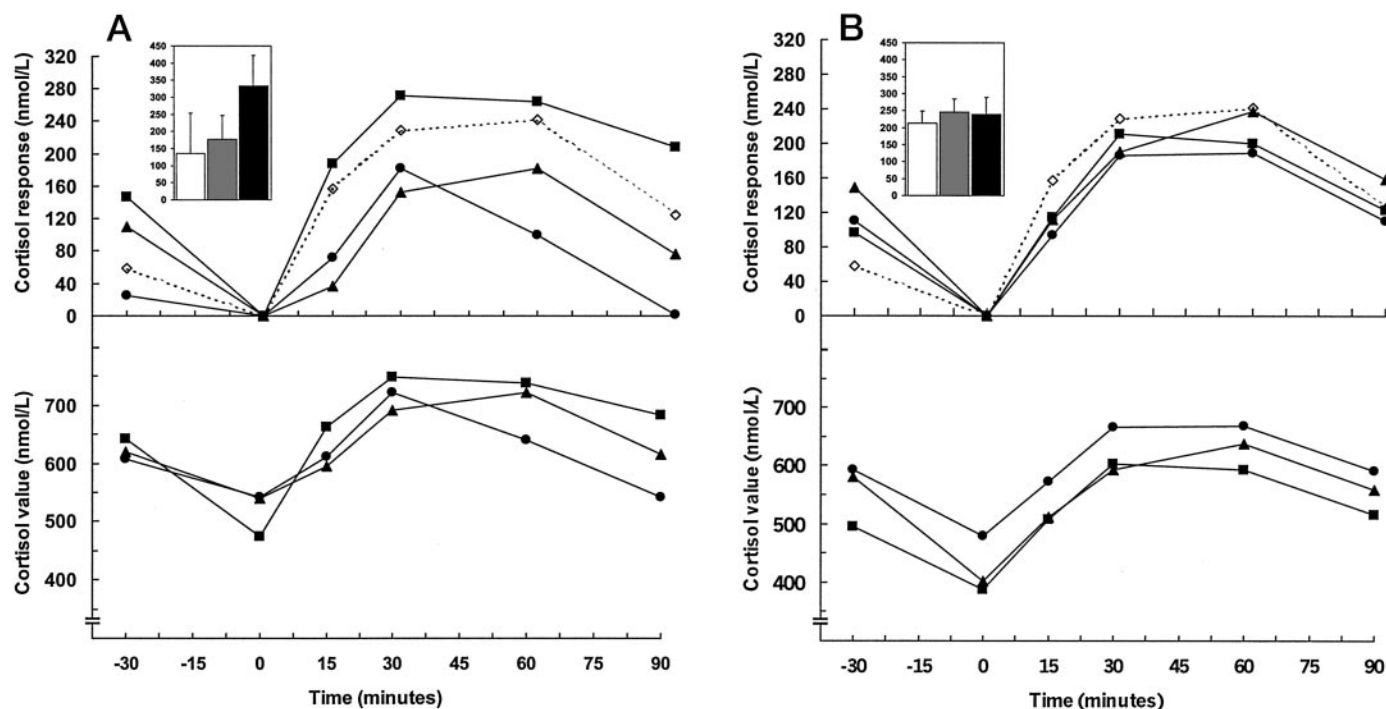
^a *P* < 0.05 vs. pretreatment (see text).^b *P* < 0.05 vs. placebo treatment (see text).

FIG. 4. The *bottom panels* show the raw cortisol values following administration of 1 μ g/kg body weight hCRH, and the *top panels* show the response measured as the change from baseline (0 min). The *lines* represent patients with CFS before treatment (circles), after active treatment with hydrocortisone (squares), and after placebo treatment (triangles). The *dotted line* and *open diamonds* show the control subjects for comparison. A, Patients who responded to the active treatment (responders, n = 8). B, Those who did not respond to active treatment (nonresponders, n = 23). The mean integrated AUC values together with SEM are shown in the *insets*, with *bars* representing baseline (white), placebo (gray), and active (black) phases of treatment. The AUC was significantly higher after active treatment than placebo treatment in responders, but not in nonresponders (see text).

sponders and nonresponders could have been explained by changes in the ACTH responses.

UFC. UFC values at baseline, after placebo treatment, and after active treatment did not differ between responders and nonresponders.

IST. Only three responders had IST tests during active and placebo treatment; we, therefore, decided that the group was too small for meaningful analysis.

Prediction of treatment response. No pretreatment endocrine variables were significantly different between subsequent treatment responders or nonresponders (Table 5).

Discussion

Baseline study

These results demonstrate similar ACTH responses to hCRH, IST, and D-fenfluramine challenges in CFS patients and a healthy control group. There were nonsignificant trends for cortisol responses to hCRH and D-fenfluramine challenge to be reduced in the CFS group, which were significant when ACTH responses were controlled for. Twenty-four-hour UFC was reduced in the test group.

This is the first study in patients with CFS using a pituitary and hypothalamic challenge in the same patient group: hCRH acts directly on the pituitary, whereas the accepted

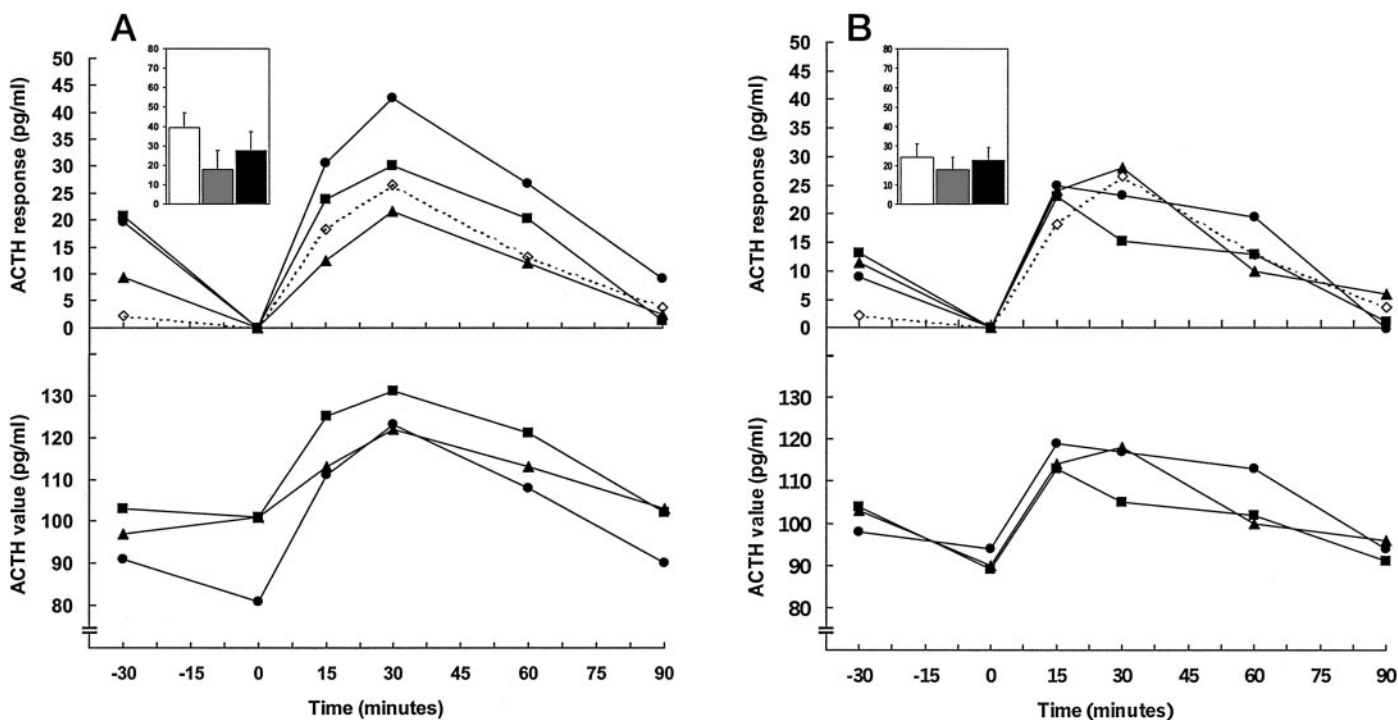


FIG. 5. The *bottom panels* show the raw ACTH values following administration of 1 $\mu\text{g}/\text{kg}$ body weight hCRH, and the *top panels* show the response measured as the change from baseline (0 min). The *lines* represent patients with CFS before treatment (*circles*), after active treatment with hydrocortisone (*squares*), and after placebo treatment (*triangles*). The *dotted line* and *open diamonds* show the control subjects for comparison. A, Patients who responded to the active treatment (responders, $n = 8$). B, Those who did not respond to active treatment (nonresponders, $n = 23$). The mean integrated AUC values together with SEM are shown in the *insets*, with *bars* representing baseline (*white*), placebo (*gray*), and active (*black*) phases of treatment. There were no differences in the integrated AUC values when responses after active and placebo treatment were compared, both in responders and nonresponders (see text).

TABLE 5. Endocrine responses to the hCRH test and UFC values before treatment, grouped into those who subsequently responded or did not respond to hydrocortisone treatment

	Responders ($n = 9$)	Nonresponders ($n = 23$)
hCRH test		
Basal ACTH (pg/ml)	91 ± 28	94 ± 41
AUC ACTH (pg/ml·h)	39 ± 23	24 ± 35
Basal cortisol (nmol/liter)	541 ± 255	482 ± 188
AUC cortisol (nmol/liter·h)	136 ± 353	214 ± 182
24-h UFC (nmol/d)	120 ± 53	99 ± 54

Values are shown for the basal and AUC values for ACTH and cortisol. No values are significantly different.

mechanism of action of the IST and D-fenfluramine challenges is via the release of hypothalamic-releasing hormones (24, 25). D-Fenfluramine is a centrally-acting, serotonin-releasing drug, thought to bring about the release of CRH via stimulation of either 5-HT_{2A} or 5-HT_{2C} receptor subtypes (26). The findings of similar ACTH response to all challenges, both pituitary and hypothalamic, in test and control groups suggests that central control of the HPA axis is intact in CFS and that CRH secretion is, therefore, not abnormal. Thus, this study does not support previous findings of reduced ACTH responses to CRH (9, 27, 28) in CFS and, by extrapolation, the hypothesis of reduced central response of the HPA axis in CFS secondary to abnormal CRH secretion.

Several methodological differences between previous studies and the current one may account for the different

findings. First, it should be noted that the previous studies used ovine CRH, which has a longer half-life, different protein-binding characteristics and a more sustained ACTH response than the human CRH we used in this study (29). Our choice of hCRH was based on a desire to use the more naturalistic challenge hormone, but this could contribute to the different results between studies. Perhaps more importantly, our study specifically excluded depressed patients unlike the previous ones. Lastly, the time of challenge testing differed between studies. This study conducted challenge tests in the morning whereas the two other studies performed endocrine testing in the afternoon. It may be that there is a centrally mediated variation in the diurnal secretion of ACTH in CFS that is only apparent in the afternoon, with relatively intact morning secretion. Were this the case, one would expect reduced basal secretion of ACTH in the afternoon, whereas Demitrack *et al.* (9) found the reverse (*i.e.* increased basal ACTH concentrations in the afternoon). It is difficult to reconcile a theory of central hyporesponsivity of the HPA axis with increased basal secretion of ACTH and normal CSF levels of CRH (9). The HPA axis picture of reduced ACTH response to CRH is, however, typical of the findings in major depressive disorder (30, 31). Scott *et al.* (27) excluded subjects with depression, but did still find reduced ACTH responses. The sample size was, however, small. Thirty-seven test subjects underwent the hCRH challenge test in this current study, compared with sample sizes of 19 (9) and 14 (27) in the other studies.

There are two other published studies examining HPA axis responses to *D*-fenfluramine (7, 32), and one that looked at HPA axis responses to *D,L*-fenfluramine (33). One study using the *D*-fenfluramine challenge compared responses between a CFS, a depressed, and a control group found that cortisol responses were significantly higher in the CFS group compared with the depressed group (7). This difference was not significant when the depressed group was removed from the analysis (*i.e.* there was not a significant difference specifically between the CFS and the control group). The *D,L*-fenfluramine study did not find any difference in cortisol responses between test and control groups (33). Both studies were limited by measurement of cortisol only. The other *D*-fenfluramine challenge study measured both ACTH and cortisol responses and found relatively increased ACTH, but normal cortisol responses in the CFS group ($n = 9$) (32).

There is one other published study examining ACTH and cortisol responses to serotonin-activating challenges. This study used the 5-HT_{1A} receptor partial agonist ipsapirone and found blunted ACTH, but normal cortisol, responses in CFS patients (34). This may indicate a hypothalamic serotonergic abnormality specific for the 5-HT_{1A} receptor subtype that is masked by the less specific serotonin-stimulating properties of *D*-fenfluramine. Alternatively, undetected depression may have accounted for this finding, because ACTH responses to ipsapirone have been found to be blunted in depression (35). Interpretation of these data are further complicated by the possibility that central serotonin function may be abnormal in those with CFS. Thus, PRL responses both to the 5-HT_{1A} partial agonist buspirone (36, 37) and to *D*-fenfluramine (7, 38) are increased in CFS compared with healthy controls. The finding of increased CSF levels of the serotonin metabolite 5-hydroxyindoleacetic acid lends further support to this hypothesis (39). Were central serotonin function increased in CFS, this would tend to minimize reductions in any HPA axis responses using serotonin agonists.

We found no differences in either ACTH or cortisol responses to the IST. One other study has used this challenge in patients with CFS (32) and found increased ACTH, but normal cortisol, responses in the test group. This finding of increased ACTH responses to challenge was on a small sample and seems to be an isolated one in the literature.

We found evidence to support previous findings of impaired adrenal cortical responsiveness in CFS. The trend for cortisol responses to be reduced to hCRH and *D*-fenfluramine challenge was made significant by covarying for hypothalamic-pituitary responses, suggesting an abnormality at the level of the adrenal gland of the HPA axis in the patient group tested in this study. Not all results are consistent with this interpretation. Thus, while we replicated the findings of reduced urinary cortisol output, absolute serum levels of cortisol were not, in fact, lower in the patient group, and on one of the three test days basal values were significantly higher in patients. It is not easy to explain this discrepancy, but it is possible that factors such as increased stress responsivity and sleep disturbance in patients might cause circadian rhythm abnormalities that could underlie this. Thus, basal measures might provide less information than the response to challenge. Further support for the reduced adrenal responsiveness comes from Scott *et al.* (40), who found reduced

cortisol responses to low-dose ACTH challenge (1- μ g test) in patients with CFS. Demitrack *et al.* (9) also found that cortisol responses to low-dose ACTH challenge (0.1 and 1.0 μ g/kg), but not very low doses (0.003 and 0.01 μ g/kg), were reduced in CFS compared with a healthy test group. Another group, however, did not replicate the finding of blunted cortisol responses to the 1- μ g ACTH challenge (41).

A recent study of eight CFS subjects with subnormal responses to the 1- μ g ACTH test examined adrenal gland volume using computerized tomography (42). They found that adrenal glands bilaterally in the CFS group were reduced by greater than 50% compared with a control group. Overall, these findings suggest that the adrenal dysregulation, if present, is subtle and not of sufficient magnitude to be elicited by an indirect challenge (*i.e.* the abnormality is only significant if a direct adrenal challenge is used, rather than a pituitary or hypothalamic challenge).

In conclusion, ACTH responses to hCRH and the hypothalamic challenges IST and *D*-fenfluramine did not differ between CFS subjects and healthy controls. This indicates that central response mechanisms in the HPA axis are intact in CFS. The finding of reduced UFC output, and the finding of reduced adrenal responses to these challenges when pituitary responses are controlled for, together with other findings of reduced adrenal gland output in other studies suggest an alternative hypothesis of adrenal gland dysfunction in CFS. These findings suggest that future studies should focus on adrenal gland, rather than hypothalamic or pituitary, function in CFS.

Follow-up study

We sought to examine the effect of low-dose hydrocortisone replacement therapy on the HPA axis in CFS, and whether any changes in HPA axis function were related to the treatment effect produced. Looking at all patients, we found that 28 d of treatment with low doses of hydrocortisone produced the expected increase in urinary cortisol output, whether or not patients responded to treatment. Thus, a low-dose hydrocortisone supplementation is able to increase the overall cortisol throughput, suggesting that there is not (over 28 d) a compensatory switching off of endogenous cortisol production. Overall, we found there were few major effects of hydrocortisone on the endocrine responses to hCRH challenge or IST. However, this obscured a differential effect related to treatment response. In those who responded to treatment, defined as a fall in fatigue to normal population levels, there was a corresponding normalization of the blunted cortisol response to hCRH challenge.

There has been one previous published study of hydrocortisone treatment in CFS (43). While this study also demonstrated a mild benefit of hydrocortisone on some aspects of CFS, there was also appreciable adrenal suppression, with reduced cortisol response to synthetic ACTH stimulation in those treated with hydrocortisone. However, this study used larger doses of hydrocortisone than ours, ranging from 25–35 mg daily, and treated patients for 3 months rather than 1 month. It is likely that these two factors explain the contrasting findings.

We previously reported that treatment with hydrocorti-

sone resulted in a marked reduction in fatigue in just over one quarter of patients, with no differential response to the 5-mg or 10-mg dose (15). We have now shown that this treatment response was paralleled by a normalization of the cortisol response to hCRH testing. We feel that this adds weight to the suggestion that, whether primary or secondary, HPA axis disturbance may be one reversible factor contributing to fatigue in CFS. However, it is important to recognize that the majority of our sample gained little benefit from hydrocortisone, which also had little effect on their endocrine responses. The sample itself was distilled from a much larger one seen in our clinics, approximately 80% of patients being excluded because of strict inclusion criteria. The generalizability of the findings is, therefore, unknown. Importantly, we could not identify any pretreatment endocrine factors that predicted a subsequent response to hydrocortisone.

Whether hydrocortisone acts directly to normalize the HPA, or whether the HPA axis change is secondary to the clinical improvement is another question. One might expect exogenous hydrocortisone to suppress the HPA axis. However, these doses were very small, and indeed the HPA axis changes in nonresponders were minimal. It is, therefore, possible that clinical improvement, such as increased physical activity, could have mediated the normalization of HPA axis function. One method to test this hypothesis would be to measure HPA axis function before and after a nonpharmacological treatment such as graded exercise or cognitive behavioral therapy, both of which are effective in CFS (44).

Limitations

Cautions in interpreting this study relate to the low number of actual responders to treatment, giving a small sample for the comparison with nonresponders. Clearly, additional studies of patients responding to treatment are needed. For our UFC collections, we did not take a measure such as creatinine that would have allowed a check on the completeness of the collections. Interpretation of urinary cortisol output may also be influenced by physical activity, which can stimulate cortisol production. Thus, one confounding factor interpreting urinary cortisol is the inactivity in CFS patients.

An additional consideration in interpreting the dynamic challenges is the baseline measurement in these studies. The problem of a falling baseline is common to all endocrine procedures undertaken in the early morning. This is influenced by both the diurnal fall in circulating hormone at this time, and residual effects of the stress of cannulation. We attempted to reduce this by using the 0 min value as baseline, thus ignoring the less reliable –30 min measure taken sooner after cannulation. We also note that the baseline falls are similar in patients and controls, and in patients on retesting. We do not, therefore, think this is likely to have made a large difference to the results we found, but raise it as a methodological consideration.

The findings of reduced adrenal responses at baseline were of borderline statistical significance, but were significant when ACTH responses were taken into account. This method was also used by Demitrack *et al.* (9) in their own study, and we include it to allow comparison. Nevertheless, the pulsatility of ACTH and the time lag between ACTH and

cortisol responses means this method is not without problem. Overall, then, the difference between patients and controls is likely to be a subtle one.

Our results are not totally consistent. Clearly, there are a large number of confounding factors that can all exert large effects on HPA axis function, such as sleep disturbance (45), exercise (46), mood (47), and circadian rhythms (48). Each of these confounders may be abnormal in CFS (49–51). In particular, night shift working alters the CRH test results to a pattern very similar to CFS (48). Future studies might concentrate on measuring more precisely these confounding variables to attempt to gain a multidimensional understanding of HPA axis changes in CFS. These could include: measures of sleep and physical activity; comprehensive dimensional psychiatric assessment; separation of patients at different time points of the illness (such as acute, subacute and chronic fatigue of varying durations); prospective cohort study designs; and careful assessment of other factors such as drugs (including the frequent use of herbal and other complementary medicines) that might affect the HPA axis.

Conclusions

In conclusion, this study provides evidence that there may be impaired adrenal cortical function in CFS on some measures and that low-dose hydrocortisone therapy is associated with a reversal of this HPA axis dysfunction in the minority of patients with CFS who gain benefit from treatment.

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