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Multiple Sclerosis Is Associated with Alterations in Hypothalamic-Pituitary-Adrenal Axis Function

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ABSTRACT

In the LEW/N rat model, a decreased hypothalamic-pituitary-adrenal (HPA) axis response to inflammatory and immune mediators confers susceptibility to the development of a variety of inflammatory and immune diseases, including experimental allergic encephalomyelitis. In humans with optic neuritis, early intervention with steroids is associated with a decrease in the number of patients who go on to develop multiple sclerosis (MS). The current study was designed to determine whether patients with MS show evidence of a hypoactive HPA axis. Thirteen patients with MS were studied at baseline and with provocative tests of HPA axis function [ovine CRH, arginine vasopressin (AVP), and ACTH stimulation]. Compared to matched controls, patients with MS had significantly higher plasma cortisol levels at baseline. Despite this hypercortisolism and in contrast to patients with depression who had similar elevations in plasma cortisol levels, patients with MS showed normal, rather than blunted, plasma ACTH responses to ovine CRH, suggesting that the pathophysiology of hypercortisolism in MS is different from that in depression. Patients with MS also showed blunted ACTH responses to AVP stimulation and normal cortisol responses to high and low dose ACTH stimulation. Taken together, these findings are compatible with data from studies of experimental animals exposed to chronic inflammatory stress, which showed mild increased activation of the HPA axis with increased relative activity of AVP in the regulation of the pituitary-adrenal axis. These data do not support a role for hypocortisolism in MS once the disease is established. (*J Clin Endocrinol Metab* **79:** 848–853, 1994)

RECENT work with animal models suggests that the responses of the hypothalamic-pituitary-adrenal (HPA) axis to inflammatory mediators influence susceptibility to inflammatory and immune illnesses (1). The HPA axis is part of a negative feedback loop in which inflammatory cytokines acutely and chronically stimulate the hypothalamus to activate pituitary-adrenal function, with resultant glucocorticoid-mediated restraint of the immune response. In the LEW/N rat model, susceptibility to inflammatory illness depends on the animal's inability to adequately increase the secretion of CRH after exposure to an inflammatory mediator and can be blocked by the administration of glucocorticoids (1). By contrast, the F344/N strain, although closely related to the LEW/N rat genetically, has a much more robust CRH response to inflammatory triggers and is markedly resistant to the development of inflammatory disease (1). This resistance can be reversed by coadministration of the glucocorticoid antagonist RU 486 (2). After administration of myelin basic protein, LEW/N rats develop experimental allergic encephalomyelitis (EAE), an animal model of multiple sclerosis (MS). EAE can be prevented by the administration of corticosterone, whereas EAE-resistant strains of rats can be made susceptible by adrenalectomy (3). Recovery from EAE depends on glucocorticoid secretion, although resistance to reinduction of subsequent episodes is glucocorticoid independent (4).

Despite the growing body of animal data pointing to the importance of HPA axis function in such illnesses and a recent report that early intervention with steroids in optic neuritis is associated with a decrease in the number of patients who go on to develop full-blown MS (5), HPA axis activity in human inflammatory and immune illness has not been well characterized. We report here a study of HPA axis activity in patients with MS. Our aim was to determine whether hypoactivity of the HPA axis could have contributed to the initial susceptibility to MS or to continuation of the ongoing process. To examine the HPA axis, we measured morning and evening basal plasma ACTH and cortisol levels on multiple occasions in a group of patients with MS and in healthy controls. We also measured the plasma ACTH and cortisol responses to ovine CRH (oCRH) and synthetic arginine vasopressin (AVP), both potent stimuli of anterior pituitary ACTH release, as well as cortisol responses to maximal and submaximal stimulatory doses of ACTH (measures of adrenal cortical activity) and 24-h urinary free cortisol as an index of total daily cortisol production.

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Materials and Methods

Subjects

Thirteen patients with MS (five males and eight females; mean age ± SEM, 43.2 ± 1.9 yr) were recruited through notices in the local MS society newsletter. Patients were medication free for at least 2 weeks before the study (except for two postmenopausal women who were

being treated with replacement estrogen), had not been treated with steroids for more than 2 weeks during the preceding year, and received a complete medical history, physical, and screening laboratory examination to rule out the presence of concurrent medical illness. All patients were seen by a neurologist (L.S.) to confirm the diagnosis of MS using Poser criteria (6), and severity of illness was rated using the expanded disability status score (EDSS) (7). Ten patients had a course characterized as remitting-relapsing, whereas three had a course that was chronic-progressive. The clinical severity of illness in the group of patients studied was generally mild as determined by the EDSS, with scores between 1.5–6.5 (mean, 2.2 ± 1.6). No patient was in a clinical exacerbation at the time of study. Controls were age- and sex-matched healthy volunteers recruited through the NIH normal volunteer program. Patients and controls were also screened with a structured psychiatric interview Schedule for Affective Disorders and Schizophrenia Lifetime Version Revised [SADS-LA (R)] as well as psychometric instruments (Hamilton depression rating scale) to assess the presence or absence of depressive illness, which is commonly associated with HPA axis activation. All studies were approved by the NIMH investigational review board, and all subjects gave informed consent before beginning the study.

Procedures

24-h urinary free cortisol. Two (4 patients and 3 controls) or 3 (8 patients and 9 controls) complete 24-h urine collections were obtained from 12 patients and 12 healthy controls (results for 1 patient were unavailable due to sample loss). Total creatinine excretion was quantitated to assess completeness of collection, and 24-h urinary free cortisol was determined by the NIH clinical pathology service.

Baseline cortisol and ACTH. Baselines were determined before oCRH stimulation as the mean of samples taken at 15-min intervals between 1800–2000 h before the administration of oCRH. The cortisol baseline before ACTH stimulation was determined by taking the mean of the 1800 h samples before the two tests. Baselines before AVP infusion were determined as the means of samples taken at 15-min intervals for 1 h before the administration of AVP.

ACTH stimulation. Thirteen patients with MS and 13 healthy controls received, on different days, stimulation with 0.1 μg/kg (lowest maximal stimulatory dose) and 0.003 (submaximal stimulatory dose) μg/kg synthetic ACTH-(1–24) at 1800 h, as previously described (8). Serum was sampled 0, 10, 30, and 60 min after administration of ACTH and assayed for total cortisol by the NIH clinical laboratories.

oCRH stimulation. Thirteen patients with MS and 13 healthy controls received 1 μg/kg oCRH at 2000 h as an iv bolus. Blood was sampled 5, 15, 30, 60, 90, and 120 min after the injection, stored on ice, spun down within 2 h of collection, immediately frozen over dry ice, and stored at −70 C until assayed.

AVP stimulation. One hour after insertion of an iv catheter at 0730 h, 10 patients with MS and 10 healthy controls had blood drawn at 15-min intervals over 1 h for determination of baseline ACTH and cortisol, followed at 0930 h by an infusion of AVP at a dose of 1 mIU/kg·min for 60 min (3 patients chose not to participate in this portion of the study because of concern about possible side-effects). This dose and time were chosen on the basis of a pilot study performed at the NIMH to determine optimal dose- and time-response relationships (Demitrak, M., unpublished data). Blood was sampled during the infusion and for 1 h afterward, stored on ice, and spun down within 3 h of collection, after which the plasma was frozen over dry ice and stored at −70 C until assayed.

Assays

Assays for cortisol for the ACTH stimulation tests were performed on fresh serum by the NIH clinical laboratories using a fluorescence polarization kit (Abbott Laboratories, North Chicago, IL). Urinary free cortisol was measured by a specific RIA after extraction with dichloromethane (Smith Kline Bioscience, St. Louis, MO). Assays for cortisol for the oCRH and AVP stimulation tests were performed on previously frozen plasma by commercially available RIA kits (Diagnostic Products Corp., Los Angeles, CA). Assays for ACTH were performed on previously frozen plasma by commercially available immunoradiometric assay (Nichols Institute, San Juan Capistrano, CA). AVP was assayed as previously described (9). Interleukin-1β (IL-1β) and IL-6 were assayed using commercially available specific enzyme-linked immunosorbent assays [IL-1β: Cistron Biotechnology (Pine Brook, NJ); IL-6: R & D Systems (Minneapolis, MN)].

Statistical analysis

Mean baseline ACTH values and cortisol values and urinary free cortisol were compared between patient and control groups using unpaired two-tailed t tests. Responses to ACTH stimulation, oCRH infusion, and AVP infusion were compared between groups by unpaired two-tailed t test comparison of mean area under the curve of the response (calculated using the trapezoidal approximation method) after subtracting the baseline area. Values are reported as mean ± SEM. Secondary analyses to determine the effect of depression and illness type (chronic progressive or remitting/relapsing) were performed using group t tests. The effects of illness severity (assessed by EDSS score) on HPA axis measures were examined using linear regressions.

Results

Urinary free cortisol

Mean 24-h urinary free cortisol in patients was significantly higher than that in controls (MS, 212.0 ± 19.8 nmol/day; controls, 153.8 ± 10.9 nmol/day; $P < 0.02$). Results are summarized in Fig. 1.

Baseline ACTH and cortisol

Baseline plasma cortisol was significantly higher in the evening in patients than in controls both before ACTH

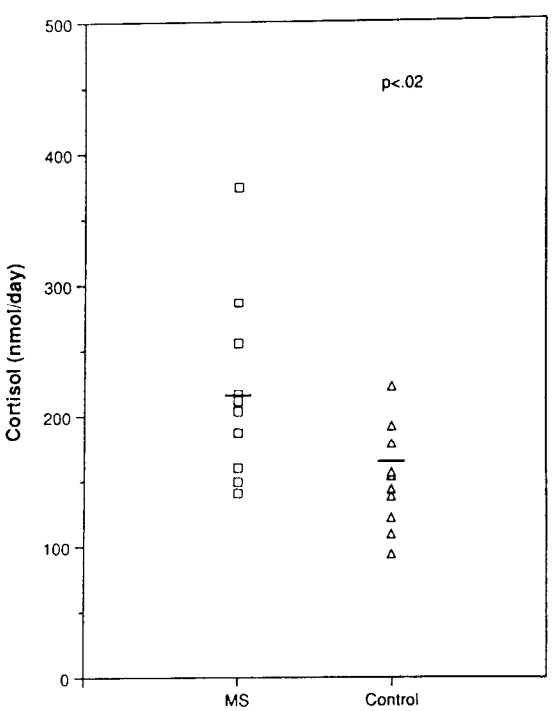
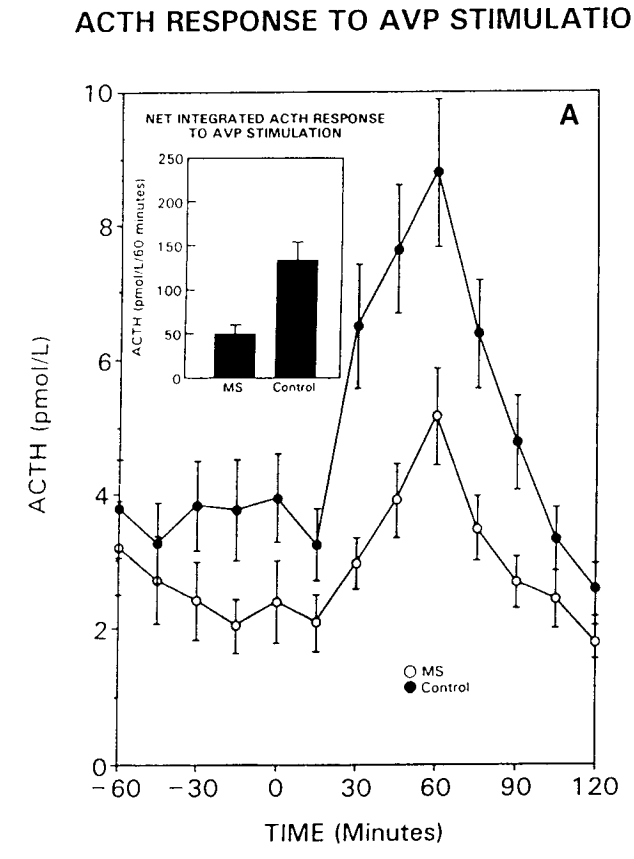
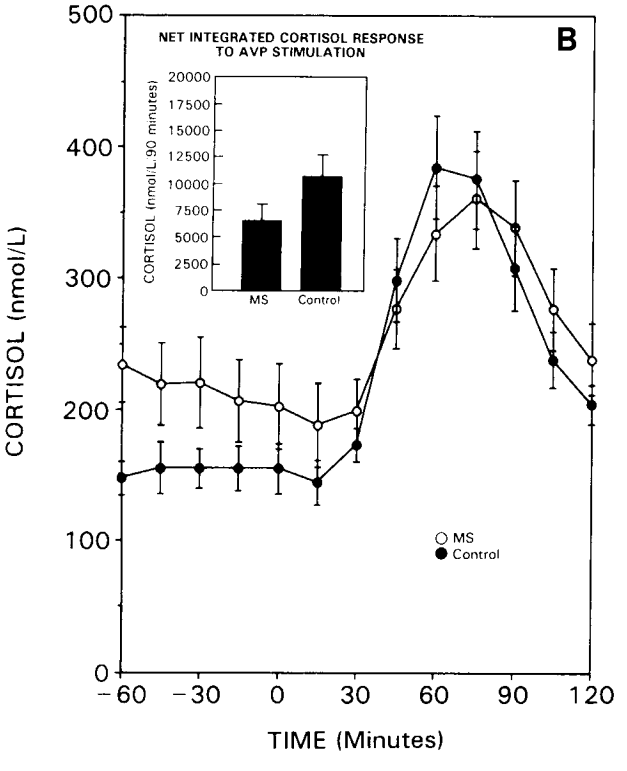


FIG. 1. Twenty-four-hour urinary free cortisol.





stimulation (MS, 167.5 ± 16.1 nmol/L; controls, 99.6 ± 9.0 nmol/L; $P < 0.002$) and before oCRH infusion (MS, 112.9 ± 12.8 nmol/L; controls, 75.7 ± 9.2 nmol/L; $P < 0.03$). In the morning there was a trend toward higher plasma cortisol which did not reach statistical significance (MS, 216.4 ± 29.9 nmol/L; controls, 152.2 ± 15.2 nmol/L; $P < 0.08$). Differences in baseline plasma ACTH in the morning (MS, 10.7 ± 2.3 pmol/L; controls, 14.6 ± 2.3 pmol/L; P = NS) and evening (MS, 4.6 ± 0.7 pmol/L; controls, 6.1 ± 1.0 pmol/L; P = NS) were not statistically significant. The ratios of evening and morning plasma ACTH to cortisol were significantly lower in patients than in controls [evening: MS, 1.37 ± 0.19; controls, 2.97 ± 0.46 ($P < 0.004$); morning: MS, 1.62 ± 0.30; controls, 2.96 ± 0.48 ($P < 0.03$)].

AVP stimulation

The mean time-integrated plasma ACTH response to AVP was significantly lower in patients with MS than in controls (MS, 50.5 ± 9.9 pmol/L·60 min; controls, 134.3 ± 20.2 pmol/L·60 min; $P < 0.02$). Although integrated cortisol responses to AVP infusion were higher in controls than in patients, this difference was not statistically significant (MS, 6,503 ± 1,557.9 nmol/L·90 min; controls, 10,608 ± 2,044.2 nmol/L·90 min; P = NS). There was no difference between mean basal or peak (60 min) AVP levels in patients (basal, 0.5 ± 0.05 pmol/L; peak, 101.1 ± 8.83 pmol/L) and controls (basal, 0.4 ± 0.02 pmol/L; peak, 102.5 ± 22.19 pmol/L). Analysis of ACTH and cortisol responses was unchanged when peak ACTH and cortisol responses above baseline were substituted for integrated responses. The basal plasma cortisol concentration did not correlate with peak plasma ACTH or cortisol responses to AVP (peak responses were used for this measure because basal cortisol is contained in the net area under the curve). Results are summarized in Fig. 2, a (ACTH) and b (cortisol).

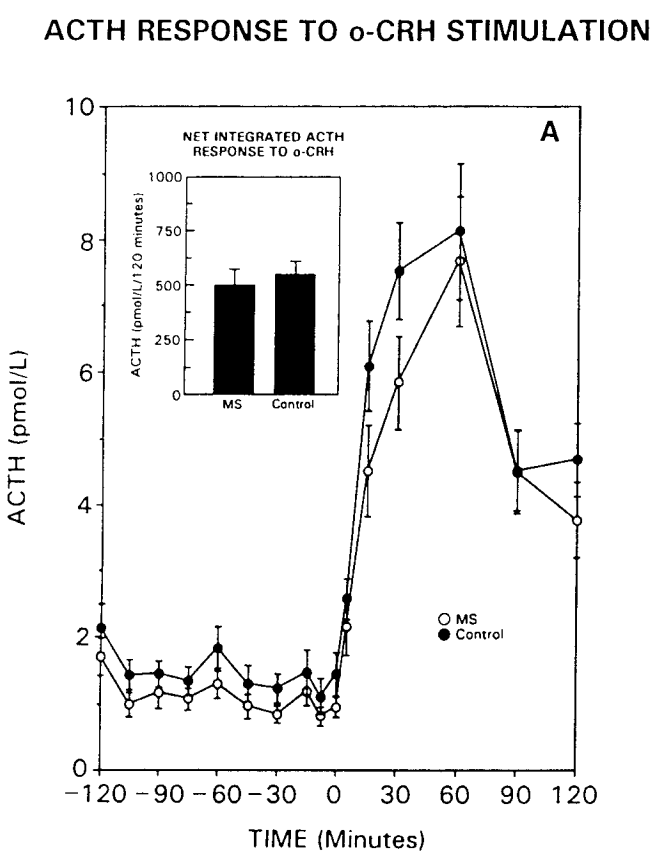
CRH stimulation

Integrated plasma ACTH and cortisol responses to oCRH and the ratios of plasma ACTH to cortisol released during oCRH stimulation were similar in patients and controls. There was no correlation between the basal plasma cortisol concentration and either integrated plasma ACTH or cortisol responses to oCRH in patients or controls. Results are summarized in Fig. 3, a (ACTH) and b (cortisol).

ACTH stimulation

Plasma cortisol responses to both submaximal (patients, 5,651 ± 920.1 nmol/L·60 min; controls, 6,530 ± 715.4 nmol/L·60 min) and maximal (patients, 28,346 ± 1,894.3 nmol/L·60 min; controls, 26,233 ± 1,301.3 nmol/L·60 min) stimulatory doses of ACTH were similar in patients and controls.

FIG. 2. a, ACTH response and net integrated ACTH response (*inset*) to AVP infusion. b, Cortisol response and net integrated cortisol response (*inset*) to AVP infusion.



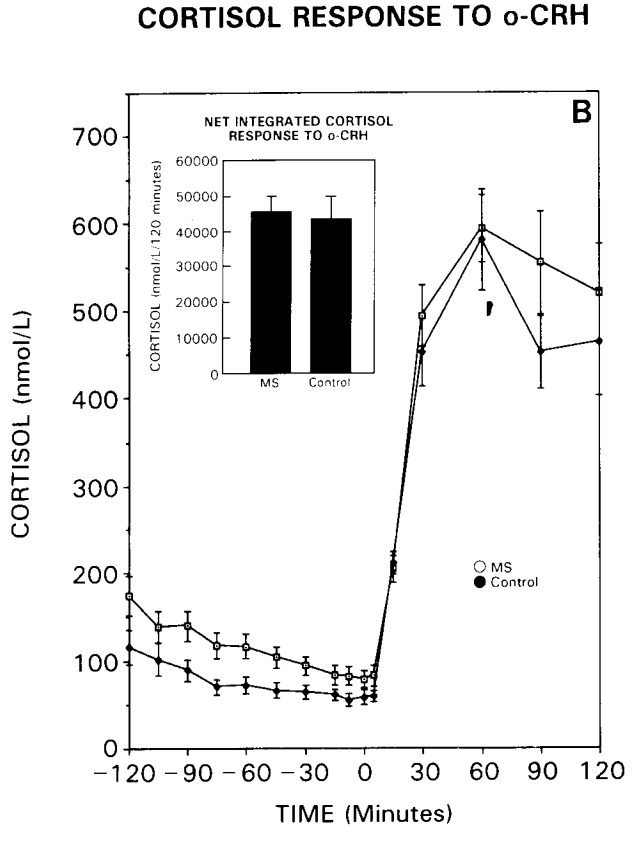


FIG. 3. a, ACTH response and net integrated ACTH response (*inset*) to oCRH infusion. b, Cortisol response and net integrated cortisol response (*inset*) to oCRH infusion.

Inflammatory cytokines

Plasma IL-1β was undetectable in all patients and all but one control subject (23 ng/L). Plasma IL-6 was less than 3.13 ng/L in all but one patient (45 ng/L) and two controls (15 ng/L and 175 ng/L).

Effects of illness severity and depression

None of the above findings correlated to illness severity, as assessed by EDSS. The course of illness (*i.e.* remitting/relapsing or chronic progressive) had no effect on basal cortisol levels or responses to oCRH and ACTH, but patients with chronic-progressive illness (n = 3) had significantly lower ACTH responses to AVP than patients with remitting-relapsing illness (n = 7; chronic-progressive, 15.5 ± 15.5 pmol/L; remitting-relapsing, 65.7 ± 8.5 pmol/L; $P < 0.02$). It is theoretically possible that demyelinating lesions in the central nervous system could interfere with the inhibition of the HPA axis, but of the 11 patients whose magnetic resonance imaging scans were available for review, none provided evidence of a pattern of central nervous system involvement that would contribute to hypercortisolism. Four patients met criteria for major depression, which is often associated with hypercortisolism; however, there was no demonstrable relationship between depressive illness and HPA function in this group of subjects, and depression did not account for elevations in cortisol or blunting of the ACTH response to AVP in the MS patients.

Discussion

Compared with healthy controls, patients with MS had elevations in plasma cortisol at baseline, blunted plasma ACTH responses to AVP, and normal ACTH responses to oCRH. Patients' cortisol responses to AVP, oCRH, and ACTH were similar to those of controls, whereas the ratio of ACTH to cortisol at baseline was significantly lower in both the morning and the evening in patients.

Previous studies of patients with MS have reported basal serum cortisol levels to be either elevated or normal (10–12). Cortisol responses to insulin-induced hypoglycemia have been reported to be blunted (11), adrenal size is increased (13), and up to 50% of patients with MS failed to suppress cortisol appropriately after the administration of dexamethasone (14). These studies, however, were limited by several factors. None attempted to evaluate the entire HPA axis, few examined both ACTH and cortisol levels, and most subjects were studied during clinical exacerbations of their illness (*i.e.* in an acutely stressful setting). The findings of the current study suggest that plasma cortisol elevations in MS are present during periods of clinical stability and do not simply reflect the stress of acutely worsening illness.

The physiological basis for cortisol elevations in patients with MS is uncertain. Although active inflammation in acutely ill patients could produce hypercortisolism via in-

flammatory mediator-induced activation of hypothalamic CRH neurons, none of the patients in this study showed systemic evidence of inflammation, such as elevated erythrocyte sedimentation rate or leukocytosis, and neither IL-1β nor IL-6 levels were elevated in plasma. Whether such mediators are elevated in the cerebrospinal fluid of patients with MS is an unresolved question, as some studies of patients with MS have shown increased levels of inflammatory cytokines, whereas others have not (15–18). It is possible that chronic psychological stress associated with a debilitating illness leads to hypercortisolism; studies of the effects of chronic psychological stress on the HPA axis have produced conflicting data (19, 20). In this context it is important to note that those patients with MS who were also depressed did not differ from the other patients with respect to the various measures of pituitary-adrenal activation, and that the presence or absence of depression does not identify patients with MS who fail to suppress cortisol secretion appropriately after the administration of dexamethasone (14).

With the exception of patients with Cushing's disease, patients suffering from illnesses associated with hypercortisolism of a magnitude similar to that seen in MS (*e.g.* major depression, panic disorder, or anorexia nervosa) show blunted plasma ACTH responses after the administration of oCRH (21, 22). These data have been interpreted to suggest that the pituitary corticotroph in patients with these disorders is intact and appropriately restrained in its response to oCRH by high plasma levels of glucocorticoids, or that persistent increased hypothalamic secretion of CRH down-regulates the corticotroph CRH receptor, blunting the response to exogenous oCRH. By contrast, the pituitary ACTH response to oCRH in patients with MS is not blunted, despite high basal plasma cortisol concentrations. It seems unlikely that this finding is related to a specific defect of the pituitary corticotroph in MS, although we cannot definitively exclude this possibility. These data also suggest that the mechanism of activation of the HPA axis in MS is different from that seen in depression.

Recent studies of experimental animals provide a potential model for understanding the findings of normal plasma ACTH responses to CRH and elevations in baseline cortisol in patients with MS. In the setting of a chronic inflammatory stimulus, these studies suggest that there is a relative shift from the usual situation in which CRH is the dominant mediator of ACTH secretion to one in which AVP plays a more prominent role (23). As AVP is itself a relatively weak secretogogue of ACTH but strongly potentiates the effects of CRH on ACTH release (24), a relative shift toward mediation of ACTH release by AVP could lead to a robust ACTH response to exogenous oCRH despite elevated circulating glucocorticoid levels (*i.e.* an increase in hypophyseal portal AVP in patients with MS synergizes with synthetic CRH to override glucocorticoid negative feedback at the pituitary).

In contrast to the normal plasma ACTH response to oCRH in patients with MS, ACTH responses to AVP were blunted. These findings are also compatible with a relative shift from CRH to AVP mediation of pituitary-adrenal function, as a relative reduction in CRH in hypophyseal portal blood would attenuate the ACTH response to AVP (less CRH would be available to potentiate AVP than in controls). The blunted ACTH response to AVP in MS could also reflect an impact of relative increased AVP in hypophyseal portal blood to down-regulate pituitary AVP receptors. Although high basal circulating glucocorticoid levels could act to restrain the plasma ACTH response to AVP, the available data suggest that glucocorticoids are less effective in suppressing AVP-induced ACTH release than CRH-induced ACTH release (25).

Unless studies that show activation of inflammatory cytokines in MS can be confirmed, the mechanism by which such activation is induced at the hypothalamus (*i.e.* the physiological effector activating the HPA axis) will remain unknown. We also note that the relatively robust cortisol response to AVP-induced ACTH relative to the blunted ACTH response to AVP in patients with MS could be related to adrenal activation, as there is evidence that AVP has a direct effect on the adrenal to stimulate cortisol secretion through the V1 receptor (26).

Unlike the evening measurements of basal cortisol secretion, the differences between groups in baseline morning cortisol levels were not statistically significant. This could represent a change in diurnal patterns of cortisol secretion, perhaps related to changes in the relative prominence of CRH and AVP as mediators of ACTH release. However, our ability to detect elevations in morning cortisol secretion in patients with MS was limited by the greater variability in morning cortisol levels, and it is possible that such elevations could be demonstrated in a larger study population.

Normal plasma cortisol responses to both maximal and submaximal doses of synthetic ACTH as well as oCRH suggest the patients' adrenal glands were not overtly hypertrophic. However, the decrease in the ratio of ACTH to cortisol, the relatively robust cortisol response to AVP-induced ACTH secretion, and the consistent, though not statistically significant, decrease in baseline ACTH levels do suggest a heightened basal sensitivity in the adrenal cortical response to ACTH and are consistent with data which show that subjects with higher plasma cortisols have lower plasma levels of ACTH (27) and with data showing enlarged adrenal glands in patients with MS (13). We speculate that the normal or diminished ACTH levels consistently observed in these patients may represent a normal pituitary corticotroph caught between overstimulation from above (*i.e.* increased AVP secretion) and mild hyperresponse in the adrenal cortex below.

The results of this study provide evidence that the HPA axis is activated in patients with clinically stable MS, and that the ongoing pathophysiology of the illness cannot be attributed to hypocortisolism. Determining whether the HPA axis is a factor in the pathogenesis of MS would require a prospective study of patients who have not yet developed MS, so the possibility remains open that hyporesponsiveness of the HPA axis contributes to susceptibility to developing MS. Whether the finding of HPA axis activation described here is specific to MS or whether a shift to AVP-driven

hypercortisolism represents a broader phenomenon of chronic activation of the stress response system is a question we hope to address in a future study using other, noninflammatory chronic illnesses as control groups. This study does, however, provide preliminary evidence that the changes in regulation of the HPA axis demonstrated in animals in response to experimentally induced chronic stress also occur in human disease.

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