Mood Disorders and Dysfunction of the Hypothalamic-Pituitary-Adrenal Axis in Multiple Sclerosis

Association With Cerebral Inflammation

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Objective: To investigate the association between affective and neuroendocrine abnormalities, commonly observed in multiple sclerosis, with inflammatory disease activity.

Design: Cross-sectional design. Twenty-three patients with definite relapsing-remitting multiple sclerosis and age- and sex-matched control subjects were investigated. Depression and anxiety were assessed using structured interviews, self-report measures, and Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised criteria. Neurologic impairment was assessed by the Kurtzke Expanded Disability Status Scale and function of hypothalamic-adrenal-pituitary axis was analyzed using a corticotropin-releasing hormone stimulation test after dexamethasone suppression. Inflammatory disease activity was evaluated first by routine and experimental laboratory tests, and second by magnetic resonance assessment of gadolinium uptake of multiple sclerotic plaques.

Setting: University hospital, a major provider of acute neurologic care.

Results: Compared with controls, patients with multiple sclerosis had higher scores on depression and anxiety scales and exhibited a failure of suppression of cortisol release after dexamethasone pretreatment. Both affective symptoms and neuroendocrine abnormalities were correlated with cerebrospinal fluid white blood cell counts and presence of gadolinium-enhancing lesions on magnetic resonance images; however, no association with the degree of neurologic impairment was observed.

Conclusion: Affective and neuroendocrine disorders were related to inflammatory disease activity but not to degree of disability, supporting the hypothesis that these symptoms are causally associated with brain injury.

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CONCOMITANT with the de-emphasis of euphoria, depression is now considered as the chief psychiatric implication of multiple sclerosis (MS). The lifetime rate of depression has been estimated between 27% and 54%.

Minor depressive symptoms and anxiety are even more common. The pathophysiological basis for a possible relationship between depression and MS is unknown.

Abnormal negative feedback control of the hypothalamic-pituitary-adrenal (HPA) system is the most consistent biologic laboratory sign in major depression. Dysregulation of the HPA axis is considered to be involved in the pathophysiology of mood disorders, as it coincides with depressive episodes and partially reverses after recovery. Interestingly, a similar dysfunction of the HPA axis has been observed in MS, as evidenced by altered feedback sensitivity of the central nervous system to glucocorticoids in response to the dexamethasone suppression test, the increased basal plasma and urinary concentrations of cortisol, or the observation of an increased number of corticotropin-releasing hormone (CRH) neurons in the hypothalamic paraventricular nucleus as well as larger adrenal glands in postmortem studies.

A major drawback of the dexamethasone suppression test, which relies solely on a single determination of these rapidly fluctuating hormones, is its low sensitivity, which ranges between 20% and 50%. In depression research, the combined dexamethasone-CRH test is, therefore, considered the best neuroendocrine tool now available for identifying HPA-axis abnormalities. In healthy individuals, pretreatment with dexamethasone suppresses a substantial CRH-induced release of corticotropin and cortisol. However, if the same protocol is applied to depressed patients, the
PATIENTS AND METHODS

PATIENTS AND CONTROL SUBJECTS

Twenty-three patients (16 women, 7 men) aged between 35 and 62 years (median, 35 years) with clinically definite relapsing-remitting MS were initially suspected neurologic or inflammatory diseases that had, however, been excluded after extensive evaluation.

A bidirectional interaction between the immune and the neuroendocrine systems has been demonstrated. Pluripotent monocyte- and lymphocyte-derived proinflammatory cytokines, ie, tumor necrosis factor α (TNF-α), interleukin (IL) 1β, or IL-6, interfere with the regulation of the HPA axis at all levels, eg, by triggering hypothalamic CRH release, stimulating production of pituitary hormones, or inducing adrenal glucocorticoid release. Interestingly, detection of the same cytokines in MS plaques, cerebrospinal fluid (CSF), or serum represents one of the major findings in recent MS research. The plasma and CSF levels in patients with MS are thought to mirror the effector phase of the immune attack together with levels of the soluble form of the intercellular adhesion molecule 1 (sICAM-1), which is responsible for adhesion to endothelium and transendothelial migration of lymphocytes and monocytes into the afflicted brain tissues, and of myelin basic protein (MBP), which is released in the context of the consecutive inflammatory myelin breakdown.

At present, it is controversial whether mood disorders are psychological reactions to disability, whether they are causally related to brain abnormalities, or both. Moreover, the pathophysiological significance of the HPA-axis dysregulation in MS is unknown. Because MS is an inflammatory disease, we investigated whether affective and neuroendocrine disorders in patients with MS are associated with indicators of central nervous system inflammation.

Depression and Anxiety

Patients were evaluated by semistructured psychiatric interviews, ie, the 21-item Hamilton Rating Scale for Depression and the 14-item Hamilton Rating Scale for Anxiety. At the same time, patients completed the Zung Self-Reporting Depression Scale, consisting of 20 items for assessment of depressive symptoms, and the Zung Self-Reporting Anxiety Scale, a 20-item self-report scale for quantification of symptoms of anxiety. Major depression was diagnosed using Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised (DSM-III-R) criteria. All neuropsychological and psychiatric investigations were blinded from laboratory results.

Regulation of HPA Axis

Dexamethasone-CRH Suppression Test. For the CRH test, lyophilized human CRH (CorticobissR, Bissendorf Peptide GmbH, Wedemark, Germany) was used as described previously. Briefly, dexamethasone-CRH tests were performed with an intravenous bolus injection of CRH (100 µg) after a single dexamethasone treatment (1.5 mg by mouth) at 11 PM the day before. A cannula was inserted between 12 and 2 PM and blood samples were collected at 2:30, 2:45, and 3 PM. At 3 PM, immediately after blood sampling, human CRH was administered and blood was serially obtained for measurement of plasma corticotropin and cortisol at 15, 30, 45, 60, 75, and 90 minutes. Between sampling, the tubing was kept patent by saline infusion.

Determination of Concentrations of Cortisol and Corticotropin. Venous blood samples for quantification of corticotropin and cortisol were collected in prechilled ethylenediaminetetraacetic acid (EDTA) tubes from a venous catheter, centrifuged (4°C, 3000 rpm), stored at −80°C, and thawed shortly before use. All samples from an individual patient were measured in duplicate in a single assay to eliminate interassay variations. An immunoluminometric 2-step assay (LUMItestR ACTH, Hennig, Berlin, Germany) was used for the determination of intact human corticotropin in plasma. Two monoclonal antibodies, one luminescent labeled and the other unlabeled, were used to quantify neurologic impairment.

continued on next page
immobilized on the inner surface of the tube, recognize different binding sites on corticotropin to form a sandwich-type complex bound to the tube. The luminescence signal is directly proportional to the corticotropin concentration. The limit of detection was 0.6 pg/mL. The measured intra-assay coefficients of variation were 4.8% for 28 pg/mL and 5.2% for 210 pg/mL. The interassay coefficients of variation were 9.8% for 28 pg/mL and 8.6% for 210 pg/mL. Using the Enzymun-TestR Cortisol (Boehringer, Mannheim, Germany), serum cortisol and cortisol-peroxidase conjugate compete in the first incubation step for a limited quantity of specific antibodies coated onto the wall of the tubes. The amount of antibody-cortisol-peroxidase complex, detected by the formation of a colored complex, is a measure of the cortisol content of the sample. Within-run and between-run imprecisions (coefficients of variation) were less than 8% and 12%, respectively. The detection limit was 30 nmol/L.

Intracerebral Inflammation

Routine Laboratory Indicators of Inflammatory Activity. Standard examination of CSF included determination of CSF white blood cell count, considered as standard indicator of acute central nervous system inflammation, albumin quotients, IgG index, and oligoclonal bands.

Experimental Laboratory Indicators of Inflammatory Activity. Serum and CSF levels of IL-1β, IL-6, and TNF-α (R&D Systems, Minneapolis, Minn) and sICAM-1 (Bender MedSystems, Vienna, Austria) were quantified with sandwich immunoassays. Briefly, a monoclonal antibody specific for these antigens was coated onto a 96-well microtiter plate. In a single-step reaction, samples were then incubated in the microtiter plate together with a second horseradish peroxidase-linked monoclonal antibody specific for a different epitope of these antigens. After washing, the bound enzyme-antibody conjugate was measured enzymatically with tetramethylbenzidine as the substrate. Adsorbance was measured at 450 nm on a spectrophotometer (MR 4100, Dynatech, Burlington, Mass) using 630 nm as the reference wavelength. A standard curve was established using recombinant antigens. The lower limits of detection of IL-1β, TNF-α, and IL-6 were 0.3, 0.7, and 4.4 pg/mL, respectively. The lower limit of detection of sICAM-1 was 1.6 ng/mL. The quantification of CSF and serum levels of MBP was performed using a competitive double-antibody radioimmunoassay (Diagnostic Systems Laboratories, Webster, Tex), containing human basic protein (whole molecule) as antigen and rabbit anti–human MBP as antiserum. Goat anti-rabbit γ-globulin was used as MBP precipitation reagent. Human MBP (iodine 125; 2 µCi per vial) was included in the assay. A standard curve was constructed from 7 MBP standards and unknown values were determined from the standard curve and expressed as nanograms per milliliter. The lowest detection limit was 0.26 ng/mL.

Neuroradiological Indicators of Inflammatory Activity. All patients with MS underwent cranial magnetic resonance imaging at entry. T1-weighted, proton-density, and T2-weighted images, as well as images enhanced with gadolinium–pentetic acid (1 mol/L; Schering, Berlin, Germany), were obtained on a 1.5-T superconducting unit (Siemens, Erlanger, Germany). Dependent on the results of the clinical examination, some patients received additional spinal gadolinium–pentetic acid-enhanced magnetic resonance imaging.

STATISTICAL ANALYSIS

The mean plasma hormone concentrations over the time points between 2:30 and 3 PM after dexamethasone treatment, but before CRH application are reported as basal values. As a derivative parameter of the net change of hormonal concentrations, \( \Delta_{\text{max cortisol}} \) (maximum concentration of cortisol after stimulation minus basal concentrations) was calculated, which has been shown to be useful in multiple studies. Response curves of all patients exhibited their peak values within the time window selected in this study. For statistical analysis, the Mann-Whitney U test and Spearman linear correlation were used. Results are reported as means±SEM.

RESULTS

DEPRESSION AND ANXIETY SYMPTOMS

Although only 4 of 24 patients fulfilled the DSM-III-R criteria for major depression, most of the patients with MS had mild to moderate depressive symptoms. Thus, these patients suffered from significantly more depressive symptoms than control subjects (Table 1). Scores for the MS group on the Hamilton Rating Scale for Depression ranged between 1 (no depressive symptoms) and 38 (multiple severe depressive symptoms) and revealed a significantly increased overall group mean compared with the control group (Table 1). Similarly, scores on the Self-Reporting Depression Scale (range, 22-53) were significantly increased in the MS group.

Compared with control subjects, scores on the Hamilton Rating Scale for Anxiety (range, 2-32) and on the Self-Reporting Anxiety Scale (range, 23-55) were significantly increased in patients with MS (Table 1), indicating a considerably elevated anxiety level in these patients.

REGULATION OF HPA AXIS

Compared with control subjects, patients with MS exhibited a marked failure of suppression of CRH-induced corticotropin and cortisol response after dexamethasone administration (Figure 1). The \( \Delta_{\text{max cortisol}} \) level was significantly increased (205.77±35.75 nmol/L vs 71.24±23.59 nmol/L; \( P < .05 \)) in the MS group. The mean latencies of reaching peak concentrations of corticotropin and cortisol were 56.59±4.41 minutes and

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69.00±3.67 minutes, respectively, and did not significantly differ from the corresponding values in healthy volunteers.

ASSOCIATION BETWEEN HPA-AXIS REGULATION AND MOOD DISORDERS

In patients with MS, the Δmaxcortisol significantly correlated with scores on the Hamilton Rating Scale for Depression (r=0.56, P<.05), Hamilton Rating Scale for Anxiety (r=0.60, P<.05), and the Self-Reporting Anxiety Scale (r=0.59, P<.05) but not with scores on the Self-Reporting Depression Scale (r=0.26, P not significant).

ASSOCIATION OF AFFECTIVE AND NEUROENDOCRINE ABNORMALITIES TO DISEASE SEVERITY

In patients with MS, ratings on the EDSS varied between 1 and 7.5 with a mean disability level of 3.5. Scores for depression (Hamilton Rating Scale for Depression, r=0.008; Self-Reporting Depression Scale, r=0.085) and anxiety (Hamilton Rating Scale for Anxiety, r=0.31; Self-Reporting Anxiety Scale, r=0.38) were not significantly correlated with scores on the EDSS. Moreover, Δmaxcortisol did not significantly correlate with EDSS results (r=-0.08).

ASSOCIATION OF AFFECTIVE AND NEUROENDOCRINE ABNORMALITIES TO CEREBRAL INFLAMMATION

Routine CSF Analysis

As expected, the mean CSF cell counts (Table 2) were significantly increased in patients with MS compared with controls. Compared with the subpopulation with lower cell counts (≤5×10⁶/L), the subpopulation with higher cell counts (>5×10⁶/L) had significantly increased scores on the Hamilton Rating Scale for Depression (P<.05), the Hamilton Rating Scale for Anxiety (P<.01), and the Self-Reporting Anxiety Scale (P<.05) but not the Self-Reporting Depression Scale (P=.13) (Figure 2).

Cerebrospinal fluid cell counts significantly correlated with Δmaxcortisol in patients with MS (Figure 3).

Table 1. Scores on Scales for Assessment of Depression and Anxiety in Multiple Sclerosis (MS) and Healthy Condition

<table>
<thead>
<tr>
<th>Scale</th>
<th>MS Group</th>
<th>Control</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamilton Rating Scale for Depression</td>
<td>9.65±1.97</td>
<td>1.80±0.38</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Self-Reporting Depression Scale</td>
<td>32.14±2.03</td>
<td>25.93±0.89</td>
<td>.05</td>
</tr>
<tr>
<td>Hamilton Rating Scale for Anxiety</td>
<td>11.63±2.12</td>
<td>2.93±0.97</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Self-Reporting Anxiety Scale</td>
<td>31.61±2.21</td>
<td>25.53±1.00</td>
<td>.05</td>
</tr>
</tbody>
</table>

Table 2. Routine and Experimental Laboratory Indicators for Acute Inflammatory Activity

<table>
<thead>
<tr>
<th>Indicator</th>
<th>MS Group</th>
<th>Control Group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell count, ×10⁶/L</td>
<td>5.87±1.08</td>
<td>1.10±0.20</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>IL-1β, pg/mL</td>
<td>0.34±0.23</td>
<td>0.34±0.15</td>
<td>.55</td>
</tr>
<tr>
<td>CSF</td>
<td>1.47±0.64</td>
<td>1.81±0.70</td>
<td>.13</td>
</tr>
<tr>
<td>Serum</td>
<td>3.16±0.35</td>
<td>2.00±1.44</td>
<td>.61</td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
<td>1.26±0.35</td>
<td>1.27±0.23</td>
<td>.47</td>
</tr>
<tr>
<td>CSF</td>
<td>0.21±0.03</td>
<td>0.17±0.07</td>
<td>.31</td>
</tr>
<tr>
<td>Serum</td>
<td>2.55±0.49</td>
<td>2.06±0.40</td>
<td>.37</td>
</tr>
<tr>
<td>TNF-α, pg/mL</td>
<td>1.82±0.14</td>
<td>2.58±0.48</td>
<td>.66</td>
</tr>
<tr>
<td>CSF</td>
<td>324.89±22.43</td>
<td>321.17±16.10</td>
<td>.76</td>
</tr>
<tr>
<td>Serum</td>
<td>9.69±2.12</td>
<td>8.26±0.67</td>
<td>.79</td>
</tr>
<tr>
<td>MBP, ng/mL</td>
<td>4.13±1.05</td>
<td>0.89±0.18</td>
<td>&lt;.005</td>
</tr>
<tr>
<td>CSF</td>
<td>6.92±2.12</td>
<td>8.26±0.67</td>
<td>.79</td>
</tr>
<tr>
<td>Serum</td>
<td>6.92±2.12</td>
<td>8.26±0.67</td>
<td>.79</td>
</tr>
</tbody>
</table>

*MS indicates multiple sclerosis; IL, interleukin; CSF, cerebrospinal fluid; TNF, tumor necrosis factor; sICAM-1, soluble form of intercellular adhesion molecule 1; and MBP, myelin basic protein.
Experimental Laboratory Indicators of Cerebral Inflammation

Surprisingly, neither concentrations of inflammatory cytokines (IL-1β, IL-6, TNF-α) nor those of sICAM-1 were significantly increased in CSF or serum of patients with MS. Only CSF concentrations of MBP were significantly elevated in patients with this disease (Table 2). None of these experimental markers correlated significantly with scores on scales for affective disorders or with Δmax₅₅₅₆cortisol (data not shown).

Neuroradiological Indicators of Inflammatory Activity

Whereas all patients with MS revealed typical MS lesions on magnetic resonance images, 8 of them exhibited gadolinium-enhancing plaques. These patients had significantly increased scores on scales for assessment of depression and anxiety (Figure 4).

This subpopulation also revealed a pronounced resistance of cortisol release to dexamethasone suppression (Figure 5) and a significantly increased Δmax₅₅₅₆cortisol value (294.04±60.56 nmol/L vs 149.97±36.36 nmol/L; P<.05) compared with the subpopulation of patients without gadolinium-uptaking lesions.

Depressive and anxiety symptoms in patients with MS have often been interpreted as psychological reactions to physical disability.1,26 To our knowledge, this is the first study comparing the affective, neuroendocrine, and inflammatory abnormalities in patients with MS to show that affective symptoms and dysfunction of HPA system are associated with laboratory (cell counts) and
neuroradiological (gadolinium enhancement of MS plaques)\textsuperscript{36} indicators of cerebral inflammation but not with the degree of physical disability. Therefore, our results support the hypothesis that affective and neuroendocrinological disorders in MS are pathogenetically linked to cerebral inflammation. Such a causal relation between mood disorders and brain pathology would also be in accordance with the higher rate of depression in MS compared with other severely disabling diseases,\textsuperscript{37,38} the frequent occurrence of depression in patients with MS even before the onset of neurologic symptoms,\textsuperscript{39} and the interesting correlation between affective disorders and the CD4/CD8 ratio or CD8\textsuperscript{+} cell counts, as presumed indicators of chronic immunoactivation.\textsuperscript{37} In contrast, the lack of correlation between affective symptoms and extent of chronic immunoactivation observed in this study is not consistent with earlier interpretations that mood disorders merely represent emotional reactions to disability.\textsuperscript{1,26}

This clinical study, however, does not demonstrate such causal relationships or exclude the role of numerous other factors, eg, psychosocial variables or personality, in the pathogenesis of mood disorders and HPA dysfunction.

In depression research, affective disorders have been linked to neuroendocrine abnormalities. Here, the use of the dexamethasone-CRH test with assessment of the hormonal response kinetics revealed a profound abnormality of the HPA-axis function in patients with MS. The cortisol release after CRH stimulation exceeded by far that of control subjects, indicating a failure of feedback suppression of the HPA axis. The extent of cortisol release was related to severity of mood disorders. The physiologic basis for this insensitivity to glucocorticoid feedback inhibition in MS is unclear. In major depression, alterations at supratuitary sites, particularly decreases in the number and/or function of glucocorticoid receptors in the hippocampus or hypothalamus, have been implicated in causing the glucocorticoid negative feedback resistance contributing to the depressive symptoms.\textsuperscript{6,12} In this study, dysregulation of the HPA axis in patients with MS was associated with measures of central nervous system inflammation (eg, cell counts and presence of active, gadolinium-enhancing brain plaques), but not with the degree of neurologic disability. Therefore, inflammation itself could be responsible for the neuroendocrine changes in these patients. The neuroendocrine and the immune systems are part of one integrated system of defense, which is the object of the rapidly developing area of psychoneuroimmunology.\textsuperscript{14,15} In this investigation, however, proinflammatory cytokines, which have been discussed to mediate the cross talk between the immune and the neuroendocrine systems,\textsuperscript{16,17} were not related to neuropsychiatric or neuroendocrine variables. Moreover, the unexpected failure to observe increased levels of these experimental indicators of inflammation in MS casts considerable doubt on their presumed clinical utility. It cannot be excluded that actions of such cytokines are locally confined on neurons regulating the HPA axis, nor can it be excluded that CSF and blood concentrations in patients with MS may mirror such relationships.

Alternatively, preclinical studies demonstrate that the HPA system itself modulates inflammation and immunity. Compared with other strains, Lewis rats, which have defective CRH neurons, exhibit a diminished HPA response capacity to inflammatory stressors.\textsuperscript{40} After administration of MBP, Lewis rats develop experimental allergic encephalomyelitis. Whereas normal function of the HPA axis was essential for the benign disease course, adrenalectomy or glucocorticoid receptor blockade led to a lethal outcome that could be prevented by glucocorticoid substitution.\textsuperscript{51,52}

In conclusion, this study showed that affective and neuroendocrine disorders are associated with indicators of cerebral inflammation but not with neurologic impairment.

These data are in accordance with the hypothesis that affective disorders in patients with MS are causally related to inflammatory brain injury. Constituting a correlative triad with intracerebral inflammation and affective symptoms, dysfunction of the HPA system should be investigated as a potential link between cerebral inflammation and mood disorders in patients with MS.

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