Hypothalamic-pituitary-adrenal axis activity in patients with rheumatoid arthritis


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Abstract

Objective

To study the hypothalamic-pituitary-adrenal (HPA) axis in patients with rheumatoid arthritis (RA).

Methods

Fifty patients with RA participated in 3 groups: recent onset active RA (n = 20), longstanding active RA (n = 20) and long-standing RA in remission (n = 10), and were compared with 20 healthy controls. The activity of the HPA-axis was assessed under basal conditions and in response to stress (insulin tolerance test, ITT). In addition, patients with recent onset RA underwent a corticotropin releasing hormone (CRH) test and a dexamethasone suppression test. Plasma levels of interleukin (IL)-1β, tumor necrosis factor-α (TNF-α) and IL-6 were also measured.

Results

Basal plasma, salivary and urinary cortisol levels and plasma adrenocorticotropic hormone (ACTH) levels were not different between patients with RA and healthy controls. During the ITT, cortisol levels were consistently lower in RA patients than in healthy controls. ACTH levels during the ITT were not different between patients with RA and healthy controls. ACTH and cortisol responses to CRH were assessed only in patients with recent onset RA and were found to be within normal limits. Basal circulating plasma IL-6 levels were significantly higher in patients with active RA than in the other groups.

Conclusion

Under the standardized conditions of the ITT, patients with RA have decreased plasma cortisol levels compared to healthy controls, despite elevated levels of IL-6. The defect is probably located at the adrenal level and may be of pathogenetic significance for the development of chronic arthritis.

Key words

Rheumatoid arthritis, hypothalamic-pituitary-adrenal axis, HPA-axis, ACTH, cortisol.

Introduction
During inflammatory processes the release of proinflammatory cytokines like interleukin (IL)-1β, tumor necrosis factor-α (TNF-α) and IL-6 stimulates the hypothalamic-pituitary-adrenal axis (HPA-axis) to produce larger amounts of glucocorticoids. The increased adrenal glucocorticoid production suppresses inflammatory responses. Defects in glucocorticoid production might cause an acute arthritis to develop into chronic arthritis and such defects have been suggested to contribute to the pathogenesis of rheumatoid arthritis (RA). The possible existence of a defective HPA-axis in RA has been studied extensively. Three recent reviews agree that previous, mostly small, studies have failed to show significantly decreased basal cortisol levels in patients with untreated RA, but indicate that there may be an inability to mount adequate cortisol responses in relation to the level of inflammation (1-3).
To further explore the possible role of abnormal HPA-axis responses in the pathogenesis of RA, we studied HPA-axis activity in a group of 50 patients with RA. Antirheumatic drug therapy was discontinued prior to the study in all patients. Results were compared with those obtained in healthy controls. The activity of the HPA-axis was assessed by measuring plasma ACTH and cortisol levels under basal conditions and during the insulin tolerance test (ITT), a standardized form of hypoglycaemia induced stress. In addition, we performed a corticotropin-releasing hormone (CRH) test and a dexamethasone suppression test in patients with recent-onset RA. In order to assess whether abnormal reactions of the HPA-axis might be related to abnormal cytokine levels, we also measured the plasma concentrations of the cytokines IL-1β, IL-6 and TNF-α.

Patients and methods
Patients
Four different groups of subjects were included: healthy controls (n = 20), patients with recent onset (<1 year) active RA (n = 20), patients with longstanding (>5 years) active RA (n = 20) and patients with longstanding (>5 years) RA in remission (n = 10).
All patients with RA fulfilled the revised criteria for RA of the American College of Rheumatology (4) and were IgM rheumatoid factor positive. Disease activity was defined according to well accepted criteria (5). RA patients with active disease had a Disease Activity Score (DAS) ≥ 3.5, and patients with RA in remission a DAS ≤ 1.5.
None of the patients had ever been treated with oral or intravenous glucocorticoids, and none of them had been treated with intramuscular or intra-articular corticosteroids in the year previous to the study. The patients with recent onset RA had not been treated with a disease modifying antirheumatic drug (DMARD), and treatment with nonsteroidal antiinflammatory drugs (NSAID) was stopped in all patients one week prior to the study. In patients with longstanding RA the use of a DMARD was discontinued two weeks before the study.
The healthy controls were age and sex matched to the patients with active, recent onset RA.
All participants were aged between 18 and 65 years and were excluded if they had any condition or medication that is known to influence the activity of the HPA-axis. This also excluded patients using oral contraceptives (6). Other exclusion criteria employed were anaemia (Hb ≤ 6.5 mmol/l), renal or hepatic disorders, and contra-indications for undergoing the stress of an ITT.
All participants voluntarily signed an informed consent form. The study protocol was approved by the hospital’s review board for experiments on human subjects.

Disease activity
Disease activity was assessed with a composite disease activity score (DAS), which includes the erythrocyte sedimentation rate (ESR), the Ritchie articular index, the number of swollen joints and a visual analogue scale for general well-being (5).

HPA-axis activity
Basal values. Blood was drawn at 0900 h (fasting) and at 1600 h for determination of plasma total cortisol, free corti-
sol, cortisol binding globulin (CBG; only in healthy controls and patients with recent onset RA) and ACTH concentrations. Urine was collected for determination of total cortisol excretion in 24 hours, and on the same day salivary samples were taken every 4 hours for a period of 24 hours starting at 0800 h, for determination of the circadian cortisol rhythm. Salivary samples were collected by means of an absorbent swab, taken in the mouth for 5 minutes. It was then placed in a tube and kept in the refrigerator, and the next day all samples of 24 hours were brought to the laboratory, where they were centrifuged and the saliva samples frozen, until they were analysed. Corticotropin releasing hormone (CRH) test. After an overnight fast, subjects were placed in a supine position. At 0830 h a catheter was inserted in an antecubital vein and kept patent by saline solution. After a 30 minute rest 100 µg human CRH (CRH®, 100 µg/ml corticorelin trifluoracetate, Ferring BV, Hoofddorp, the Netherlands) was administered at 0900 h as a bolus injection. Blood samples for ACTH and cortisol assays were collected at 0, 20, 30, 60, 120 and 180 minutes. Dexamethasone suppression test. Two tablets of 0.5 mg dexamethasone (Centrafarm services BV, Etten-Leur, the Netherlands) were taken orally at 2300 h, and the next morning, after an overnight fast, blood was drawn at 0900 h for determination of ACTH and cortisol. Insuline tolerance test (ITT). Subjects were fasting and in a supine position. At 0830 h a catheter was inserted in an antecubital vein and kept patent by saline solution. After a 30 minutes rest insulin (Actrapid®, Novo-Nordisk, Bagsvaerd, Denmark) was administered at 0900 h as a bolus injection at a dose of 0.1 units/kg body weight. Blood samples were collected at 0, 20, 30, 45, 60, 90, 120 and 180 minutes. Cytokines. Plasma levels of IL-1β, IL-6 and TNF-α were measured in all participants at 0900 h (fasting) and at 1600 h.

Laboratory assays

ACTH. ACTH in plasma was measured by an immunoradiometric assay (IRMA) based on two polyclonal antibodies (EuroDiagnostics, Arnhem, The Netherlands). The catching antibody is directed against the C-terminal part of the ACTH molecule and coupled via a sheep anti-rabbit antibody to a polystyrene tube. The detecting antibody is directed against the N-terminal part of ACTH and radioiodinated. Standard curves were prepared by spiking ACTH-free plasma with ACTH-(1-39) (MRC 74/555). The assay was performed as follows. 200 µl of sample or standard (0-220 pmol/l) were added to the coated tubes and subsequently iodinated ACTH antiserum (250,000 dpm/200µl) was added. The mixture was incubated for 24 hours at room temperature. The supernatant was decanted and the tubes washed two times with 0.9% NaCl. Radioactivity in the tubes was counted using an automatic gamma-counter (1470 Wizard TM Wallac, Turku, Finland). The sensitivity of the assay was 0.5 pmol/l and the within- and between-assay coefficients of variation were 4.4% and 7.2% respectively. All samples were measured in duplo. The IRMA specifically detects ACTH-(1-39). Crossreactivity with ACTH-(1-24), CLIP and β-endorphin was < 0.1%. Normal range at 8:00 a.m.: 1.3-9.2 pmol/l.

Plasma cortisol. Plasma cortisol levels were measured by radioimmunoassay (RIA) using an antiserum raised in rabbit against a cortisol-21-hemisuccinate-bovine serum albumin conjugate. The sensitivity of the assay was 0.02 µmol/l. The within- and between-assay coefficients of variation were 4.5% and 6.6% at 021 µmol/l. Normal range at 0800 h 0.19 – 0.55 µmol/l and at 1600 h 0.06 – 0.38 µmol/l.

Plasma CBG. CBG was measured by means of a radioimmunoassay kit manufactured by RADIM (Angleur-Liege, Belgium). Within- and between-assay precision were 3.7% and 7.0% at a level of 0.50 µmol/l.

Plasma free cortisol. The plasma free cortisol fraction was measured by indirect equilibrium dialysis by a method described earlier for androstenedione (7). The within-assay coefficient of variation (cv) was 8.5% and the between-assay cv of duplicate means was 5.5% at an average free cortisol fraction of 5.0%.

Salivary and urine cortisol. Salivary cortisol as well as 24-hour urinary free cortisol (i.e. extractable by organic solvents) were measured by radioimmunoassay after previous extraction and paper chromatography (7).

Statistical analysis

The first analysis considered all groups separately. All 3 patient groups were compared with the healthy controls. Subsequently, the 2 groups of patients with longstanding RA were also compared with the group of patients with recent onset RA and with each other. In the absence of differences between the 3 RA groups (p > 0.20 for all comparisons), all 50 patients with RA were finally considered as one group and compared with the healthy controls. Responses of ACTH and cortisol to hypoglycemia were integrated over time as area under the response curve (AUC) from 0 to 180 minutes. The AUCs were calculated with the trapezoid method from the individual hormone levels at the various time points and expressed as square millimeters (mm²). In these calculations 1 minute equals 1 mm (both curves), 1 pmol/l equals 1 mm (ACTH) and 1 µmol/l equals 1 mm (cortisol). The maximal rise in ACTH and cortisol after insulin administration was calculated as the maximal difference in hormone levels compared to the value at 0 minutes. An AUC was also calculated with the
the trapezoid method from the cortisol levels in salivary samples over 24 hours. Again AUC was expressed as square millimeters (mm²): 1 hour equals 1 mm and 1 nmol/l equals 1 mm.

Comparisons between groups were made with the unpaired t-test or with the Mann-Whitney test if data were not normally distributed. P-values are based on 2 tailed tests and considered significant at the 0.05 level. Due to the exploratory nature of the study, no adjustments to probability values were made for multiple comparisons.

Results
The characteristics of the patients in each of the 4 groups are shown in Table I. The 20 patients with recent onset RA and the 20 healthy controls were matched for age and sex and included 3 men and 17 women. The groups of patients with RA in remission included more men than women. There were no significant differences in age between the groups. The high DAS in patients with recent onset RA and longstanding active RA reflects active disease, in contrast to the low DAS in longstanding RA in remission.

Basal values
Basal plasma levels of ACTH and cortisol in the 4 groups are shown in Table II. No significant differences were present between the healthy controls on the one hand and any of the RA groups on the other. The 3 RA groups also did not differ from each other (p > 0.20 for all comparisons). When all RA patients were analysed in one group, again no significant differences in plasma ACTH, total and free cortisol levels were found between the combined RA group and the healthy controls.

CBG was measured in healthy controls and patients with recent onset RA. No significant differences were present between the 2 groups. At 9:00 a.m. the mean (SD) CBG was 0.83 (0.10) µmol/l and 0.83 (0.11) µmol/l respectively (p > 0.20). At 4:00 p.m. these values were 0.83 (0.15) and 0.84 (0.17) µmol/l respectively (p > 0.20). The urinary cortisol excretion in the 3 patient groups and in the combined RA group, did not significantly differ from the healthy controls (Table II).

Salivary cortisol levels (Fig. 1) showed a normal circadian cortisol rhythm in all groups with a somewhat higher peak at 0800 h in patients with recent onset RA than in healthy controls, but this difference was not statistically different: 10.9 (7.4) versus 8.9 (4.0) nmol/l (p > 0.20). The analysis of the AUC’s for salivary cortisol also showed no significant differences between the healthy controls and the other groups (p > 0.15; data not shown).

The mean (SD) plasma levels of IL-6 in healthy controls were 8 (2) pg/ml at 0900 h and 8 (3) pg/ml at 1600 h. In patients with recent active RA these values were 28 (8) pg/ml (p = 0.004 versus healthy controls) and 17 (16) pg/ml (p = 0.04) respectively. In patients with active longstanding RA these values were 37 (35) pg/ml (p = 0.002 versus healthy controls) and 15 (13) pg/ml (p = 0.05) respectively. In patients with RA in remission, the values were not significantly different from those in healthy controls. Basal values of TNF-α and IL-1β were similar in RA patients and healthy controls and no significant differences were present between the groups (data not shown).

CRH-tests and dexamethasone suppression tests
After stimulation with CRH all 20 patients with recent onset active RA showed an increase of ACTH levels followed by an increase in cortisol levels in the normal range of our laboratory (data not shown). After suppression
with dexamethasone all patients had adequately low levels of cortisol (< 0.06 µmol/l).

Insulin tolerance tests
In response to insulin injection, plasma glucose levels decreased in all groups with a nadir at 30 minutes. The test was considered adequate if a glucose level < 2.0 mmol/l was reached. If not, the test was repeated with a 50% higher dose of insulin. This occurred in two patients, which both reached a glucose level < 2.0 mmol/l when the test was repeated. There were no significant differences between the groups in the hypoglycemia nadir that was reached during the ITT.

During the ITT no significant differences were found in plasma ACTH levels between the group of healthy controls and any of the 3 patient groups, and the AUC’s for ACTH during the ITT were not different between the groups (p > 0.20, data not shown). The 3 groups with RA patients also did not significantly differ from each other (p > 0.20 for all comparisons). Figure 2a shows the ACTH curves for the healthy controls and the combined RA group. No significant differences were found between the 2 groups at any point in time.

In the 3 RA groups, plasma cortisol levels during the ITT were not different from each other (p > 0.20 for all comparisons) and consistently lower than in healthy controls. Figure 2b shows the cortisol curves for the combined RA group and the healthy controls only. Plasma cortisol levels were significantly lower in the RA patients at all points in time (p-values varying between 0.002 and 0.05). Figure 3 shows the AUC for cortisol during the ITT for all groups. The patients with active, recent onset RA showed a trend towards a lower AUC (p = 0.07 versus healthy controls). For both groups with longstanding RA the difference with the healthy controls was statistically significant (p = 0.01 for both comparisons). When all patients with RA were considered as one group, the AUC for cortisol was also significantly lower than in the healthy controls: 103 (18) mm² versus 89 (17) mm² (p = 0.005).

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Fig. 1. Circadian levels of cortisol in salivary samples during 24 hours. No significant differences were found between the different groups compared to healthy volunteers at any time point.

Fig. 2. Plasma levels (mean and SEM) of ACTH (A) and cortisol (B) during insulin tolerance tests of healthy controls (n = 20) and all RA patients (n = 50). Insulin bolus injections were administered at 0900 h. Asterisks indicate a significant difference between the 2 groups (p < 0.05, **p < 0.01 and ***p < 0.005).
The maximal rise of cortisol during the test was not different between healthy controls, recent onset RA, longstanding active RA and longstanding RA in remission. The mean (SD) values were 0.31 (0.14), 0.31 (0.15), 0.29 (0.15) and 0.30 (0.18) µmol/l respectively (p > 0.20 for all comparisons).

**Discussion**

For some time it has been hypothesized that patients with RA may have a defective activity of the HPA-axis. Three recent reviews discuss this topic extensively. Abnormalities, if any, could reside in the hypothalamus, the pituitary or the adrenal gland (1-3). We have studied a large group of RA patients and tested the activity of the HPA-axis by several methods. Basal ACTH and cortisol levels were measured and the response of the HPA axis to insulin induced hypoglycaemia, a standardized form of stress, was also assessed.

The results of our study are compatible with the hypothesis of alterations in adrenal activity in RA. During the ITT, lower plasma cortisol levels were consistently found in all 3 groups of RA patients when compared to healthy controls, both before and after hypoglycaemia. In the group of recent-onset RA a trend towards a lower AUC for cortisol was observed (p = 0.07). In both groups of patients with longstanding RA the difference in AUC for cortisol compared to healthy controls was statistically significant (p = 0.01). Between the 3 RA groups no differences were found and when the combined group of all RA patients (n = 50) was compared with the healthy controls, the AUC for cortisol was also significantly lower in healthy controls (p = 0.005). The longstanding RA groups had a male overrepresentation and one might argue that this influenced our results. We therefore performed an additional analysis that was limited to the female participants. Again, the AUC for cortisol was significantly lower (p = 0.01) in female RA patients (n = 28) than in female healthy controls (n = 17). The differences in total plasma cortisol levels between RA patients and healthy controls are not due to differences in CBG levels because the mean CBG levels were similar in RA patients and controls.

The initial cortisol levels before hypoglycaemia were significantly different between patients with RA and healthy controls and may reflect basal hormonal activity better than the other basal assessments. During the ITT the first blood samplings were done 30 minutes after injection of an intravenous catheter during which period all subjects were at rest in a supine position. In contrast, the other blood samplings were done under conditions that were not standardized and which probably varied more between patients. This may explain why no significant differences were found in basal cortisol levels outside the context of the ITT.

Patients with RA showed normal ACTH responses to hypoglycaemia. The hypothesis in the literature of a defective hypothalamic function in RA is supported by a study, in which a defective cortisol response to major surgery in combination with normal ACTH and cortisol responses in the CRH test, was found in 10 RA patients (8). In a similar study, these results were not reproduced (9). Dekkers et al. used daily life stressors to study the activity of the HPA-axis in 29 patients with RA and found a trend towards a smaller ACTH response than in healthy controls (10). Most patients in their study however, used NSAIDs which have been associated with lower ACTH levels (11). The ITT has so far not been used to compare ACTH responses between patients with RA and healthy controls. CRH tests have been done more widely. In most studies, ACTH responses to CRH have been normal suggesting no pituitary defects (8, 12-16). In a study by Cutolo et al., the peak ACTH value but not the AUC for the ACTH response curve after CRH was lower in RA patients than in controls (17). In contrast in another study, Hall et al. found significantly elevated unstimulated ACTH levels in untreated RA patients (11). In our study both basal ACTH levels and ACTH levels during the ITT were not significantly different compared to healthy controls. We conclude that the decreased adrenal activity in RA is probably not due to decreased pituitary ACTH secretion.

Three other studies have found subtle changes in stimulated cortisol levels. First, Gudbjörnsson et al. showed that patients with untreated RA (n = 18) had a decreased cortisol response especially in the later phases after CRH administration (12). Other authors have found no significant differences in cortisol responses after CRH in RA patients when compared to healthy controls (8, 12-16). In our study, the CRH test was performed only in the patients with recent-onset RA and plasma ACTH and cortisol levels were found to be within the normal range of our laboratory. Second, Gutiérrez et al. studied 10 patients with active RA with an ITT. Although the integrated cortisol response to hypoglycaemia was not different from the control group, the early rise in cortisol was significantly lower in the RA patients than in the normal controls (18). Finally, Dekkers et al. found a significantly decreased cortisol response after daily life stressors in RA patients treated with NSAIDs (10). Other factors may also influence HPA-axis activity and must be taken into account, making interpretation of study...
The changes in the adrenal responsivity in patients with active RA has been shown to influence HPA-axis activity in patients with untreated RA and normal levels in patients with long-standing RA in remission. Decreased plasma cortisol levels were found during the ITT both in RA in remission and in active RA. Apparently, elevated plasma IL-6 levels failed to stimulate the HPA-axis in patients with active RA. This may have contributed to the development of chronic arthritis since glucocorticoids inhibit inflammatory responses (3). In other chronic inflammatory diseases, such as Crohn’s disease, decreased activity of the HPA-axis has also been suggested to exist (3). In the aforementioned study by Guðbjörnsson et al., treatment with an NSAID has been suggested to influence the HPA-axis response, especially at the hypothalamic or pituitary level (12). In our study all patients with RA had discontinued their antirheumatic drugs. NSAIDs were stopped one week and DMARDs two weeks before the tests. The results therefore imply that patients with RA without anti-rheumatic treatment.

We conclude that HPA-axis responsiveness is reduced in untreated patients with RA when compared to healthy controls and that the defect may be located at the adrenal level. This phenomenon may contribute to the pathogenesis of the disease. However, the observation that elevated plasma IL-6 levels failed to stimulate the HPA-axis in patients with active RA, also suggests a possible defect at the hypothalamic level. A single defect at only one of the levels of HPA-axis function can not fully explain our findings, demonstrating the complexity of this system in which hormones and cytokines influence each other and are themselves influenced by inflammatory status and the use of medication. Further research is needed to elucidate the underlying mechanisms and the influence of anti-inflammatory drug therapy on HPA-axis responsivity.

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References