Hypothalamo–pituitary–adrenal axis dysregulation in patients with rheumatoid arthritis after the dexamethasone/corticotrophin releasing factor test

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Abstract

A defective hypothalamo–pituitary–adrenal axis response to inflammatory cytokines may contribute to the pathophysiology of rheumatoid arthritis (RA). The purpose of this study was to define further the mechanisms responsible for this dysregulation. Six normal individuals and seven patients with active RA were recruited and given an oral dose of dexamethasone at 2300 h the evening before the study. The next day, an i.v. catheter was fitted at 1300 h. Blood samples were collected between 1400 h and 1700 h before and after infusion (at 1500 h) of corticotrophin releasing factor (CRF). Plasma was separated and stored at -20°C before radioimmunoassay for ACTH, cortisol and dihydroepiandrosterone (DHEA). Before the CRF challenge, ACTH and cortisol were significantly increased and DHEA significantly decreased in the

patients with RA compared with the controls. Neither ACTH nor DHEA was significantly altered after CRF infusion. Control individuals did not mount a cortisol response to infusion of CRF. Similarly, four of the patients with RA did not respond to CRF. However, in contrast to the controls, three of the patients mounted an immediate and sustained cortisol response after receiving CRF. These data reveal that three of the seven patients with RA were able to escape from dexamethasone suppression and mount a cortisol response to CRF challenge. This suggests that there may be a subpopulation of patients with RA who have impaired glucocorticoid feedback. The implications of this alteration for disease progression remain to be determined.

Journal of Endocrinology (2003) **178**, 55–60

Introduction

Rheumatoid arthritis (RA) is an immune-mediated inflammatory condition. It is characterised by joint pain, swelling and stiffness through inflammation in the synovial joint lining, with increasing joint impairment and disability as a result of gradual erosion of the bone and cartilage of the joints. The pathogenesis or progression of the disease is influenced by a combination of genetic and environmental factors, although the precise mechanisms remain obscure. In recent years, attention has focused on the interactions of neuroendocrine and immune components of the inflammatory response in regulating the onset and severity of RA. Both basic and clinical research have identified the hypothalamo–pituitary–adrenal (HPA) axis as being of potential importance in regulating disease activity.

At the hypothalamic level, activity of the HPA axis is co-ordinated by corticotrophin releasing factor (CRF) neurones located in the parvocellular cells of the paraventricular nucleus (PVN). Under normal conditions in

man and in rodents, approximately 50% of these CRF neurones also contain arginine vasopressin (AVP) (Erkut *et al.* 1995, Harbuz & Lightman 1997). After a variety of challenges there is an increase in the number of CRF cells co-expressing AVP (Erkut *et al.* 1995, Harbuz & Lightman 1997). Axons from these parvocellular neurones terminate in the external zone of the median eminence, where CRF and AVP are released into the hypophysial portal blood and carried to the corticotrophs of the anterior pituitary. CRF is currently the only factor shown to increase the levels of mRNA for pro-opiomelanocortin (POMC), the adrenocorticotrophic hormone (ACTH) precursor. CRF and AVP exert a synergistic action on the release of ACTH into the general circulation. ACTH causes the synthesis and release of cortisol from the adrenal cortex, and cortisol exerts a negative feedback action at the pituitary, hypothalamus and other central areas, to maintain homeostasis (Harbuz & Lightman 1997).

The HPA axis is activated in response to stress and also in response to acute immune challenge – for example, from lipopolysaccharide (LPS) or cytokines such as tumour

necrosis factor- α (TNF α), interleukin (IL)-1 and IL-6 (Harbuz & Lightman 1997). After such acute challenges, there is an activation at all levels of the axis, resulting in the release of glucocorticoids from the adrenal cortex; in man it is cortisol that is released. These glucocorticoids exert a negative feedback action at the level of the pituitary, hypothalamus and other higher brain centres to regulate further activation. In addition, glucocorticoids have a potent immunosuppressive effect, preventing further immune stimulation of the HPA axis and overactivity of the immune system. This anti-inflammatory action is crucial in chronic inflammatory disease conditions. Blocking glucocorticoid synthesis with metyrapone results in a sudden flare in disease activity in patients with RA (Saldanha *et al.* 1986).

The integrity of the HPA axis in humans with RA is less clear, with some workers reporting major alterations whereas others believe that dysfunction may be more subtle (Harbuz & Jessop 1999). However, AVP is believed to have an important role in glucocorticoid regulation in RA (Chikanza *et al.* 2000). Other hormones are also abnormally regulated in RA. In particular, dehydroepiandrosterone (DHEA) and the sulphated form, DHEA-S, have been reported to be decreased in both RA and systemic lupus erythematosus (Hedman *et al.* 1989, Masi *et al.* 1999, 2000, Dessein *et al.* 2001). These adrenal steroids are the most abundant among those found in the human circulation. DHEA has been reported to inhibit secretion of proinflammatory cytokines such as IL-6 and TNF α *in vitro* (Rook *et al.* 1994, Di Santo *et al.* 1996, Straub *et al.* 1998). The naturally occurring decrease in androgen secretion associated with ageing and the increased incidence of autoimmune disease in aged individuals make DHEA a suitable target for investigation.

The dexamethasone/CRF test has been used extensively to assess HPA axis dysfunction in a variety of psychiatric disorders (Von Bardeleben & Holsboer 1991, Steckler *et al.* 1999). Recently this test, which eliminates cortisol from the feedback control loop, has been extended to patients with multiple sclerosis (Kumpfel *et al.* 1999). In the present study we have determined concentrations of DHEA, cortisol and ACTH after the dexamethasone/CRF test in control individuals and in patients with active RA.

Participants and Methods

Participants

Seven patients (six women, one man: mean age $51 \pm$ (S.E.M.) 3.5 years; mean disease duration 16 ± 4 years), with seropositive RA who were being admitted to hospital because of a flare of their disease, and six controls (five women, one man: mean age 52 ± 2 years), were recruited to the study. None of the participants had received corticosteroids, either orally or by injection, for at

least 3 months before the study. All the patients were taking non-steroidal anti-inflammatory drugs.

Procedure

Ethics approval for this study was obtained from the Research Ethics Committee of the United Bristol Healthcare NHS Trust. Written informed consent was obtained from all participants in the study. Patients with active, widespread joint inflammation were admitted to the Rheumatology Unit for bed rest. On the day of admission they were asked to complete a Health Assessment Questionnaire (HAQ) and a formal clinical assessment was made of the state of their arthritis (number of tender and swollen joints). In addition to their usual medications, they were given 1.5 mg dexamethasone orally at 2300 h. The next day they remained ambulant and were given breakfast and lunch. At 1300 h they commenced bed rest and an i.v. catheter was fitted. Baseline blood samples (5 ml) were taken at 1400 h and 1430 h. At 1500 h, 100 μ g human CRF was infused through the catheter over a period of 30 s. Further blood samples were withdrawn at 15-min intervals over the next 2 h. Control individuals were given 1.5 mg dexamethasone to be taken at 2300 h and were asked to report for CRF and blood sampling at 1300 h the next day.

Blood samples and radioimmunoassays

Blood samples (5 ml) were placed on ice in EDTA tubes immediately after collection. They were then centrifuged and the plasma separated, aliquoted and frozen at -20°C before measurement of ACTH and cortisol using in-house radioimmunoassays, and DHEA using a radioimmunoassay kit (ICN, Bordon, Tyne and Wear, UK).

Data analysis

Means and 95% confidence intervals (CI) or S.E.M. were calculated for the clinical descriptions and for plasma cortisol, ACTH, DHEA and the cortisol:DHEA ratio. The cortisol, ACTH and DHEA responses to i.v. CRF were inspected for each participant and each was designated a 'responder' or 'non-responder' according to the pattern observed. Statistical analysis was made using the unpaired Student's *t*-test.

Results

Baseline plasma concentrations of ACTH, cortisol and DHEA after oral dexamethasone are shown in Table 1. According to the standard criteria, in the dexamethasone suppression test plasma cortisol concentrations were less than 40 ng/ml in all patients and controls (except one who was excluded as having extremely high cortisol and

Table 1 Plasma cortisol, ACTH and DHEA concentrations in control subjects and patients with RA after dexamethasone but before the CRF test. Values are presented as the mean with 95% CI.

	Mean	95% CI	P
Cortisol (ng/ml)			
Controls	12	7 to 9	0.037
Patients	22	15 to 29	
ACTH (pg/ml)			
Controls	10	9 to 11	<0.0001
Patients	56	50 to 62	
DHEA (ng/ml)			
Controls	625	359 to 891	0.044
Patients	228	122 to 334	

DHEA concentrations: cortisol 143 ng/ml, DHEA 1706 ng/ml). There was a significant increase in plasma concentrations of ACTH ($P<0.0001$) and cortisol ($P=0.037$) in patients with RA compared with controls. In contrast, DHEA concentrations were significantly ($P=0.044$) reduced in the patients with RA.

The cortisol responses to i.v. CRF in the controls and in individual patients with RA are shown in Fig. 1. Control participants did not respond to the CRF challenge, although one did show a short-lived increase in plasma cortisol at 60 and 75 min after receiving CRF. At this late time after CRF infusion, this increase was not considered to be a response to CRF. The data from this patient are included in the mean values presented in Fig. 1. Four of the patients with RA did not respond to CRF and, in contrast to the controls, three of the patients showed an immediate and sustained increase in plasma cortisol.

Individual scores for all the patients for the HAQ, numbers of tender and swollen joints and serum C-reactive protein concentrations are shown in Table 2. They show patients with moderately severe disease and currently active joint inflammation. There was no significant difference between responders and non-responders for any of these parameters measured.

Neither ACTH nor DHEA was significantly altered in response to CRF challenge after dexamethasone pretreatment. Similarly, none of the patients with RA showed any alteration in plasma ACTH or DHEA in response to CRF.

Comparing the groups for baseline cortisol:DHEA ratios before CRF infusion revealed a significant ($P<0.01$) difference between the controls (mean 24.1; 95% CI 15.2 to 33; $n=5$) and patients with RA (mean 8.6; 95% CI 3.2 to 14; $n=7$). The ratio was further reduced when the non-responders were removed from the analysis (mean 5.9; CI 2.2 to 9.6; $n=3$), although this was not significantly different from the group value for all the patients with RA who were tested.

Discussion

This is the first time that the dexamethasone/CRF test has been used in patients with RA. A striking feature of these data is the finding that three of the seven patients with RA were able to escape from dexamethasone suppression and mount a cortisol response to the CRF challenge. In contrast, and as expected, none of the controls was able to generate such a response. In addition, plasma concentrations of cortisol and ACTH were significantly increased in the patients, and DHEA concentrations were significantly decreased. The baseline cortisol:DHEA ratio before the administration of CRF was also significantly decreased in the patients with RA, in agreement with previously published data (Straub *et al.* 2002). Together, these data suggest an abnormal regulation of the HPA axis in a subpopulation of patients with RA. The lack of any significant increase in ACTH in the patients who mounted a cortisol response may be attributable to a number of factors; possible candidates might be POMC fragments, an alteration in adrenal sensitivity, or an increased sympathetic nervous system activity at the adrenal medulla that modulates cortisol release. Before the administration of CRF, the ACTH:cortisol ratio was threefold greater in the patients compared with that in the controls (2.55 compared with 0.83 respectively), suggesting hyposensitivity to ACTH at the level of the adrenal cortex in patients with RA. Further studies are needed to address the responsiveness of the ACTH-independent systems to CRF.

Significantly increased baseline concentrations of ACTH and cortisol after dexamethasone and before the administration of CRF were evident in the patients with RA compared with the controls; we did not determine plasma concentrations of these hormones before the administration of dexamethasone. This increase in baseline concentration may suggest that dexamethasone was less effective at suppressing plasma cortisol in the patients with RA than in the controls. The role of the HPA axis in RA has recently been reviewed, leading to the conclusion that most studies to date have not revealed major differences in HPA axis activity in patients with RA compared with that in control individuals (Harbuz & Jessop 1999). However, subtle differences may exist that influence the time course or severity of inflammation. Comparison of controls and patients with RA after dexamethasone with and without CRF challenge may help elucidate these differences.

The present data confirm the findings of previous studies in which normal individuals have consistently been shown to be unable to respond to CRF after dexamethasone pretreatment. In contrast, patients with depression are able to respond to CRF in this context, suggestive of abnormal regulation of the HPA axis in these individuals (Von Bardeleben & Holsboer 1991, Steckler *et al.* 1999). The mechanisms underlying this escape in patients with depression are unknown, but it has been suggested

Figure 1 Plasma cortisol (ng/ml) in control individuals (thick line; mean $\pm$ S.E.M. for $n=6$ individuals) and individual patients with RA ($n=7$) in the dexamethasone/CRF test. Time represents clock time, beginning at 1300 h. CRF was infused at 1500 h.

that there is a shift in the dose–response curve towards decreased glucocorticoid receptor sensitivity in depressed patients, reflecting feedback resistance at the level of the PVN and pituitary (Modell *et al.* 1997). The involvement of corticosteroid receptors is supported by the evidence of decreased hippocampal mineralocorticoid and glucocorticoid receptor mRNA levels in suicide victims with a

Table 2 Health assessment questionnaire (HAQ) score, number of tender and swollen joints and C-reactive protein concentrations (CRP) in the patients with RA. The patients are grouped according to whether they were able to mount a cortisol response to CRF challenge (Responders) or not (Non-responders).

	HAQ score	Tender (No.)	Swollen (No.)	CRP (mg/L units)
Patient No.				
Non-responders				
1	2.25	17	17	32
3	2.125	16	11	60
4	2.25	8	6	17
7	1.75	26	15	20
Responders				
2	1.75	12	6	10
5	2.25	8	4	32
6	1.75	10	5	22

history of depression (Lopez *et al.* 1998). Alcohol abusers who are undergoing withdrawal also exhibit increased cortisol concentrations in response to the dexamethasone/CRF test. After successful withdrawal of alcohol, the cortisol response to CRF was normal (Hundt *et al.* 2001). At the level of the PVN, the activity of the HPA axis may be mediated by AVP that is synthesised in a subpopulation of CRF cells within the PVN. An increase in the number of CRF cells co-localising AVP has been reported in postmortem studies of patients with depression (Purba *et al.* 1996). An increase in AVP activity has also been reported in rats with adjuvant-induced arthritis (Harbuz *et al.* 1992, Chowdrey *et al.* 1995). These data suggest that abnormal HPA axis regulation may be a feature of other chronic illnesses besides psychiatric disease, but that this may not reflect an inherent defect in HPA axis regulation, as HPA axis activity appears to be normal in patients who recover from illness.

Recently, the dexamethasone/CRF test has been used to investigate abnormal HPA axis regulation in patients with the autoimmune disease, multiple sclerosis (MS). These patients exhibited an exaggerated increase in plasma cortisol on challenge with CRF that was dependent on the clinical course of the MS. Those with relapsing–remitting disease had a modest increase in cortisol, those with secondary progressive MS were intermediate, and the

greatest response was seen in patients with chronic primary progressive MS (Kumpfel *et al.* 1999, Then Bergh *et al.* 1999). In the present study, all the RA patients were admitted to hospital as a consequence of a flare of their disease. We were unable to determine any differences in terms of age, HAQ scores or measures of severity of inflammation (tender or swollen joint counts or C-reactive protein concentrations) between the responders and non-responders. Although we did not rate the degree of depressed mood, none of our patients was receiving treatment for depression. No correlation with depressed mood was found in the MS study (Then Bergh *et al.* 1999), suggesting that depression is unlikely to be a factor explaining the observations in the responsive group of patients with RA.

In a study of patients with remission from depression, an increased cortisol response was associated with a greater risk of relapse (Zobel *et al.* 2001). In the present investigation, we were unable to study our patients over a long follow-up. It would be intriguing in future studies to determine if there were any long-term alterations in disease activity or severity, comparing the responders and the non-responders to the dexamethasone/CRF test.

This paper is the first to report that a subpopulation of patients with RA are able to escape the effects of dexamethasone suppression and mount a cortisol response to CRF challenge. We were unable to determine any immediate differences in disease severity between the responders and the non-responders, but these differences may emerge when larger numbers are identified and the course of their disease is studied in more detail. Further investigation will be required to assess the underlying mechanisms and the implications of these differences.

Acknowledgement

We would like to thank Dr M Sharif for measurement of C-reactive protein concentrations.

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Received 24 December 2002

Accepted 27 March 2003