EXTENDED REPORT

Low levels of dehydroepiandrosterone sulphate in plasma, and reduced sympathoadrenal response to hypoglycaemia in premenopausal women with rheumatoid arthritis

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Objectives: To evaluate the function of the hypothalamic-pituitary-adrenal axis and sympathoadrenal system in premenopausal women with rheumatoid arthritis (RA).

Methods: Insulin-induced hypoglycaemia (0.1 IU/kg) was produced in 15 glucocorticoid-naive patients with long term RA with low disease activity and in 14 healthy women matched for age and body mass index. Concentrations of glucose, adrenocorticotropic hormone (ACTH), cortisol, Δ4-androstenedione (ASD), dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulphate (DHEAS), 17α-hydroxyprogesterone (17OHP), epinephrine (EPI), norepinephrine (NE), interleukin 6 (IL6), and tumour necrosis factor α (TNFα) were analysed in plasma.

Results: Patients had comparable responses of glucose, cortisol, ACTH, ASD, and 17OHP to hypoglycaemia, without any signs of hypothalamic insufficiency. Patients had lower basal DHEAS than controls (3.03 (0.37) μmol/l *v* 5.1 (0.9) μmol/l, respectively; $p<0.05$); borderline lower basal DHEA levels ($p=0.067$); while the response of DHEA to hypoglycaemia was comparable to that of controls. Patients with RA had lower EPI ($p=0.005$) and NE ($p<0.001$) responses to hypoglycaemia. TNFα and IL6 were higher ($p<0.05$) in patients with RA (TNFα 8 (2.8) pg/ml in RA *v* 1.1 (0.5) pg/ml in controls and IL6 15.1 (6.7) pg/ml *v* 1.4 (0.7) pg/ml).

Conclusions: Lower basal DHEAS levels, without concomitant differences or changes in DHEA, ASD, 17OHP, and cortisol responses to hypoglycaemia in patients with RA, indicate an isolated decrease in adrenal androgen production. Significantly lower responses of EPI and NE to hypoglycaemia may suggest sympathoadrenal hyporeactivity in patients with RA.

The neuroendocrine system is an important effector system helping to maintain homoeostasis. Precise coordination with other central (for example, neuronal, behavioural) and peripheral (for example, metabolic, immune) responses is necessary to effectively overcome constant perturbations of internal and external environments.[1] Immune challenge and subsequent immune response may lead to specific activation of regulatory mechanisms, including neuroendocrine control. Inflammatory cytokines, particularly interleukin (IL) 1, IL6, tumour necrosis factor α (TNFα), released during the immune response can activate the hypothalamic pituitary and sympathoadrenal systems, with subsequent release of many immunomodulating hormones.[2]

Adrenal glucocorticoids, secreted in response to pituitary adrenocorticotropic hormone (ACTH) stimulation, are considered to be one of the key factors involved in the regulation of immune responses. Thus it has been suspected that dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis plays a part in the pathogenesis of chronic inflammatory diseases, such as rheumatoid arthritis (RA).[3] Studies in patients with RA have suggested that activity of the HPA axis is inappropriately normal in the presence of continuing inflammation,[4–8] and cannot control the inflammatory responses. Data in women with premenopausal onset of RA support the hypothesis on HPA axis dysfunction resulting from relative adrenal insufficiency.[5–8] Inappropriately normal cortisol and low levels of adrenal androgen dehydroepiandrosterone sulphate (DHEAS) seen in this group of patients were regarded as signs of adrenal hypocompetence, which may even precede the onset of the disease.[8 9] Furthermore, a tendency towards normalisation of adrenal androgen production seen in patients with RA during anti-TNFα treatment suggests that TNFα may have a role in HPA perturbations in chronic inflammation.[10]

The first aim of our study was to evaluate function of the HPA axis in premenopausal women with RA and its association with their inflammatory status. We performed a stimulation test of the HPA axis, insulin-induced hypoglycaemia, and subsequently measured responses of ACTH and adrenal hormones at the several intermediate steps of steroidogenesis.

Innervation of almost all tissues affected in the immune response by sympathetic nerves as well as the presence of functional adrenergic receptors on circulating immune cells, splenocytes, and other cells of the immune system provide strong evidence for sympathetic regulation of the immune processes.[11] However, the role of sympathetic dysregulation in the pathogenesis of RA is even less clear than that of the HPA axis. At the systemic level, epinephrine (EPI) may affect circulating immune cells.[12] Locally, norepinephrine (NE) and some other neurotransmitters appear to suppress Th1-type and enhance Th2-type immune responses.[13] It is generally accepted that the predominance of Th1 responses plays a part in the pathogenesis of RA. It was proposed that sympathetic hypoactivity might participate in a shift towards Th1 related responses.[11] Activation of the sympathoadrenal system

Abbreviations: ACTH, adrenocorticotropic hormone; ASD, Δ4-androstenedione; AUC, area under the curve; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulphate; EPI, epinephrine; HPA, hypothalamic-pituitary-adrenal; 3β-HSD, 3β-hydroxysteroid dehydrogenase; IL, interleukin; 17OHP, 17α-hydroxyprogesterone; NE, norepinephrine NSAIDs, non-steroidal anti-inflammatory drugs; RA, rheumatoid arthritis; SNS, sympathetic nervous system; TNFα, tumour necrosis factor α

during insulin induced hypoglycaemia provides an opportunity to assess sympathetic reactivity in patients with RA by measuring plasma catecholamines. Therefore the second aim of our study was to prove whether reduced responsiveness of the sympathoadrenal system contributes to RA pathology.

SUBJECTS AND METHODS

Fifteen female patients fulfilling the revised criteria of the American College of Rheumatology for RA (1988) were studied. They were recruited from the National Institute of Rheumatic Diseases in Piestany and the 1st Clinic of Internal Medicine, Medical Faculty of Comenius University in Bratislava, Slovakia. Fourteen healthy female subjects matched for age and body mass index, recruited from laboratory staff of the Institute of Experimental Endocrinology, Slovak Academy of Sciences Bratislava, Slovakia, served as controls. None of the patients and controls had a history of diabetes or impaired glucose tolerance. The disease activity of patients with RA was evaluated by clinical examination (number of affected and swollen joints, duration of morning stiffness), and laboratory measures (erythrocyte sedimentation rate, C reactive protein) were determined. The activity of RA was low to moderate. Table 1 shows the characteristics of all subjects. None of the patients had been treated with glucocorticoids or other drugs known to interfere with the neuroendocrine function during the past 5 years, except for non-steroidal anti-inflammatory drugs (NSAIDs) and disease modifying antirheumatic drugs. To minimise the effect on hormonal levels of treatment with an NSAID, the last dose of the drug was given 24 hours before the investigation.

All subjects gave informed written consent and the study was approved by the ethics committee of the National Institute of Rheumatic Diseases.

Patients and controls were all investigated under the same conditions. The investigations started at 8 00 am after an overnight fast. An indwelling catheter was inserted into the cubital vein for blood sampling. To eliminate the stress effect of a venepucture the blood sample for basal values was taken 30 minutes after inserting the catheter. An intravenous bolus of insulin (0.1 IU/kg, Actrapid HM; Novo Nordisk A/S Bagsvaerd, Denmark) was given afterwards. At intervals shown in fig 1, blood samples were collected into polyethylene tubes containing EDTA as anticoagulant and immediately cooled. Heparin as anticoagulant was used for measurement of catecholamines. After centrifugation, plasma aliquots were stored at −20°C until analysed.

Table 1 Basic characteristics of patients with RA and of healthy controls.

	RA	Controls
Number of subjects	15	14
Age (years)	41.2 (1.5)	44 (2.8)
Body mass index (kg/m^2)	21.6 (1.1)	23 (1.1)
Disease duration (years)	8.2 (2.4)	–
Number of affected joints	5.1 (2–10)*	–
Morning stiffness (min)	36 (0–120)*	–
Radiographic changes (Steinbrocker's criteria)	2.3 (1–3)*	–
Erythrocyte sedimentation rate (mm/1st h)	20.3 (12)	–
C reactive protein (mg/l)	15.4 (13)	–
Rheumatoid factor (IU)	936 (16–5120)*	–
Number of patients using NSAIDs	15	–
Number of patients using methotrexate	10	–
Number of patients using DMARDs	12	–

Data are mean (SEM) or mean (range)*.
NSAIDs, non-steroidal anti-inflammatory drugs; DMARDs, disease modifying drugs.

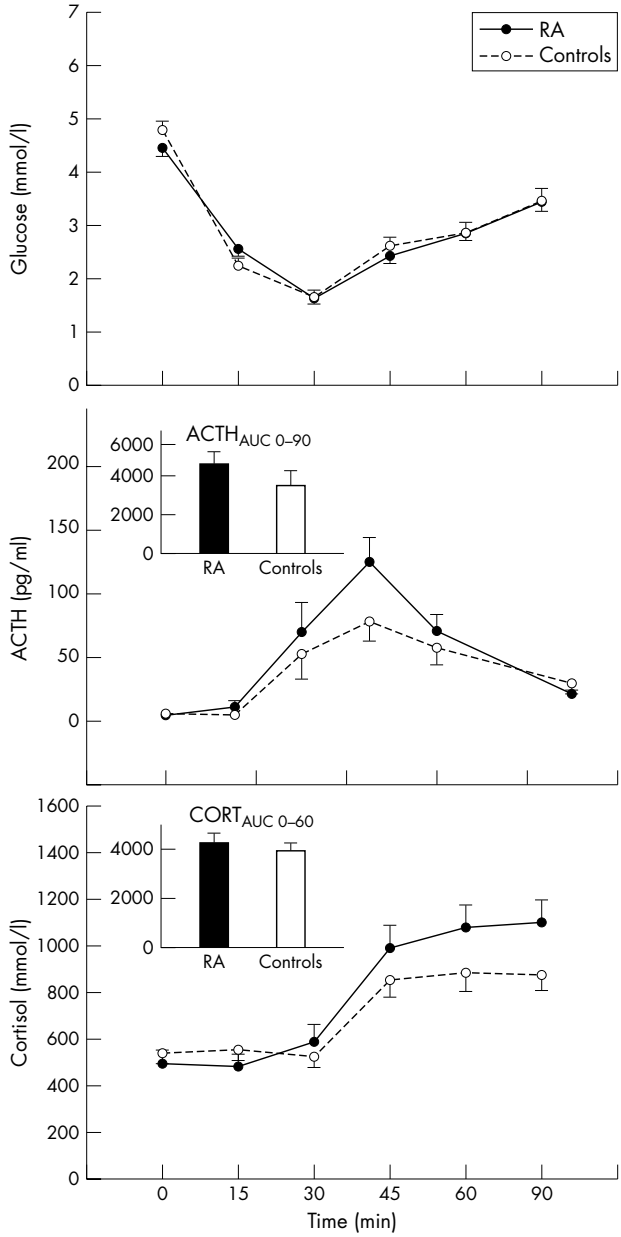

Figure 1 Concentrations of glucose, adrenocorticotropic hormone (ACTH), and cortisol in plasma of 15 patients with RA and 14 healthy controls during insulin-induced hypoglycaemia; the ACTH data were measured in 12 patients with RA and 11 healthy controls. Data are means, error bars = SEM. Inserts in graph panels indicate values of areas under the response curve (AUCs) of ACTH from 0 to 90 minutes and AUCs of cortisol from 0 to 60 minutes in patients with RA and healthy controls.

Concentrations of cortisol and ACTH were assayed by immunoradiometric assay; 3α-diol-glucuronide, DHEAS, 17α-hydroxyprogesterone (17OHP), Δ4-androstenedione (ASD), testosterone, estradiol, and IL6 by radioimmunoassay; dehydroepiandrosterone (DHEA) by radioimmunoassay after extraction in diethyl ether; and TNFα by enzyme linked immunosorbent assay (ELISA). All kits were manufactured by Immunotech SA, Marseille, France. Concentrations of EPI and NE were measured by a radioenzymatic method.[14] Plasma glucose concentrations were analysed by the glucose oxidase method (Hitachi, Japan).

Statistical evaluation was performed using SIGMASTAT 2.0 (Jandel Scientific, USA) software. Comparisons of basal

hormone and cytokine concentrations were made by unpaired *t* test or Mann-Whitney rank sum test, depending on data normality. Two way analysis of variance for repeated measurements with consecutive post hoc tests was used to determine the differences in endocrine responses to hypoglycaemia between patients and controls. One way analysis of variance for repeated measurements was used to determine differences from baseline within each group. All data are expressed as mean (SEM). Significance was set at $p<0.05$.

RESULTS

Glucose concentration immediately before and after insulin administration in patients with RA was not significantly different from that in controls. Insulin administration resulted in a decrease (>50% of basal values; $p<0.001$) of plasma glucose concentrations in all subjects (fig 1).

Plasma ACTH levels were measured in 12 patients with RA and 11 controls. Basal ACTH concentrations and hypoglycaemia-induced increases in plasma ACTH concentration were comparable in the patients and controls. Insulin administration resulted in a significant ($p<0.001$) rise in plasma ACTH concentration in patients with RA and in controls (fig 1).

The mean basal cortisol levels were comparable in patients with RA and in healthy controls. During insulin-induced hypoglycaemia plasma cortisol concentration significantly increased in RA and in the control group ($p<0.001$). The increment of cortisol from 0 to 60 minutes ($\Delta CORT_{0\text{-}60}$) was higher in patients with RA than in controls (586 (71) nmol/l *v* 344 (69) nmol/l, respectively; $p = 0.022$). The area under the response curve (AUC) of cortisol ($CORT_{AUC\ 0\text{-}60}$), $CORT_{AUC\ 0\text{-}90}$ (data not shown), and the response curve of cortisol to hypoglycaemia did not differ in the RA group from those in controls (fig 1).

Basal 17OHP concentrations and hypoglycaemia-induced increases in plasma 17OHP concentration were comparable in the patients and controls. Insulin administration resulted in a significant ($p<0.001$) rise in plasma 17OHP concentration in patients with RA and in controls (fig 2).

Basal ASD concentrations as well as hypoglycaemia-induced increases in plasma ASD concentration were comparable in patients and controls. Insulin administration resulted in a significant ($p<0.001$) rise in plasma ASD concentration in patients with RA and in controls (fig 2).

Basal DHEA concentration tended to be lower in patients with RA than in healthy controls (3.86 (0.34) ng/ml *v* 5.27 (0.81) ng/ml, respectively; $p = 0.067$). Insulin administration resulted in a significant ($p<0.001$) rise in the mean plasma DHEA concentration in RA and in controls. Neither $DHEA_{AUC\ 0\text{-}90}$ nor the responses of DHEA were significantly different between RA and controls (fig 2).

Basal DHEAS levels were lower in patients with RA than in controls (3.03 (0.37) µmol/l *v* 5.1 (0.9) µmol/l, respectively; $p<0.05$).

No significant differences in 3α-diol-glucuronide, estradiol, and testosterone levels were found between patients with RA and controls.

Plasma EPI and NE levels were measured in 12 patients with RA and 11 controls. The mean basal EPI concentration tended to be lower in patients with RA than in healthy controls (11.8 (2.6) pg/ml *v* 25 (7) pg/ml, respectively; $p = 0.099$). Insulin administration resulted in a significant ($p<0.001$) rise in the mean plasma EPI concentration in RA and in controls. An analysis of variance test showed a significant ($F = 4.02$, $p = 0.005$) interaction between two factors—namely, time and the disease, indicating a significant reduction of the EPI response in patients with RA compared with controls. $EPI_{AUC\ 0\text{-}60}$ was also diminished ($p<0.05$) in patients with RA compared with controls (fig 3).

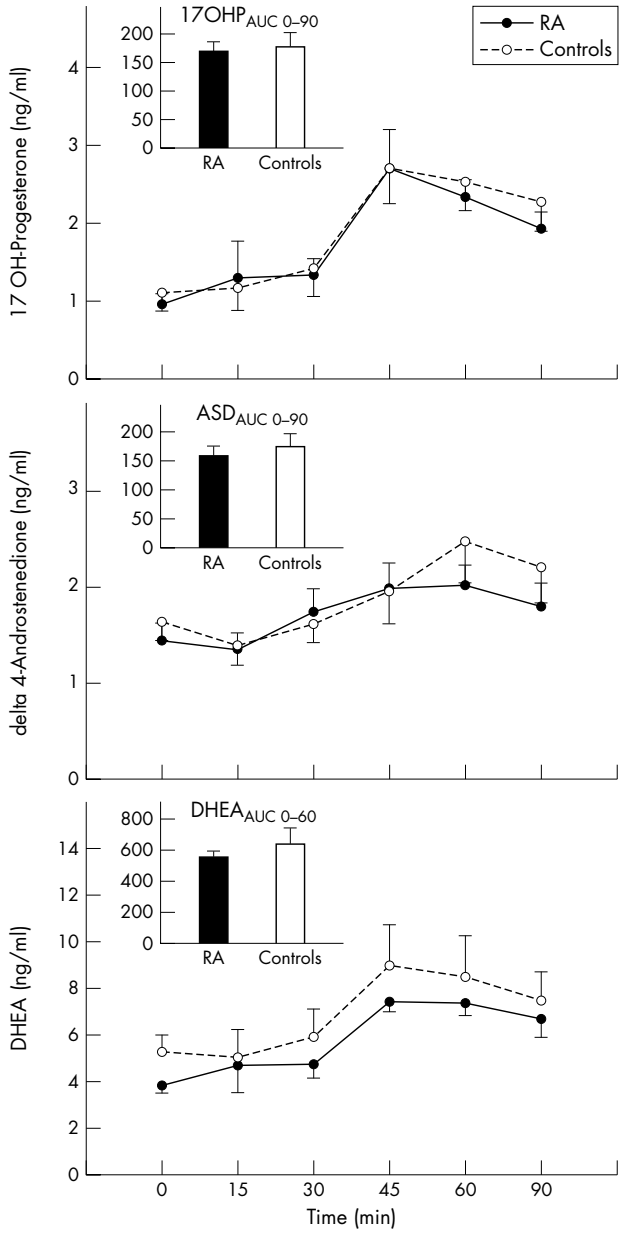

Figure 2 Concentrations of 17α-hydroxyprogesterone, Δ4-androstenedione, and dehydroepiandrosterone in plasma of 15 patients with RA and 14 healthy controls during insulin-induced hypoglycaemia. Data are means, error bars = SEM. Inserts in graph panels indicate values of areas under response curve (AUCs) of respective hormones from 0 to 90 or 0–60 minutes in patients with RA and healthy controls.

The mean basal NE concentrations were comparable in patients with RA and in healthy controls. Insulin administration resulted in a significant ($p = 0.014$) rise in mean plasma NE concentration in controls but not in patients with RA. An analysis of variance test showed a significant ($F = 5.11$, $p<0.001$) interaction between two factors—namely, time and disease, indicating a significantly different pattern of response of NE in patients with RA compared with controls. $NE_{AUC\ 0\text{-}60}$ was lower ($p<0.05$) in patients with RA than in controls (fig 3).

Plasma levels of IL6 were higher in patients with RA (15.1 (6.7) pg/ml *v* 1.4 (0.7) pg/ml, respectively; $p<0.05$). Plasma levels of TNFα were also higher in patients with RA (8 (2.8) pg/ml *v* 1.1 (0.5) pg/ml, respectively; $p<0.05$).

As expected, several significant correlations were found among basal levels of adrenal and gonadal steroids as well as

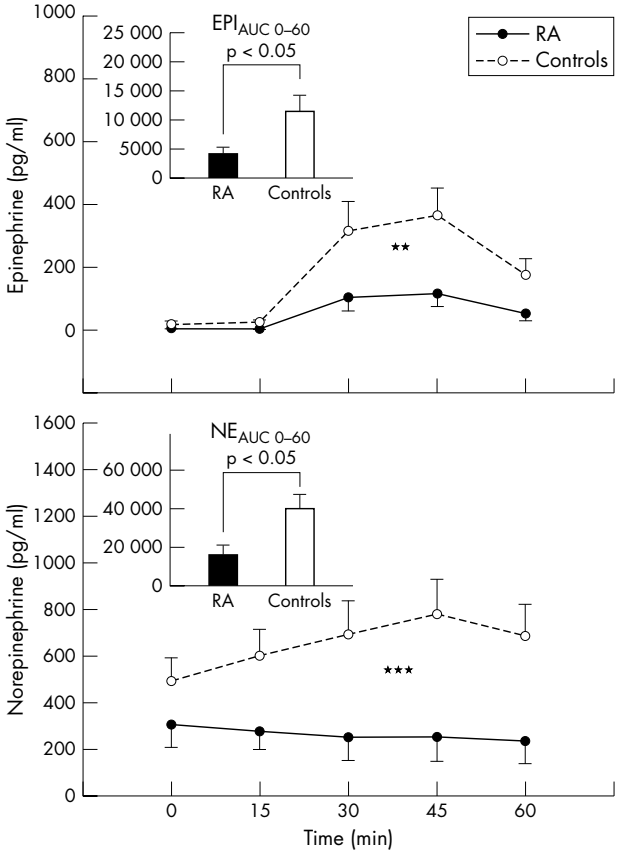

Figure 3 Concentrations of epinephrine and norepinephrine in plasma of 12 patients with RA and 11 healthy controls during insulin-induced hypoglycaemia. Data are means, error bars = SEM. Inserts in graph panels indicate significantly lower values of areas under response curve (AUCs) of both epinephrine and norepinephrine from 0 to 60 minutes in patients with RA compared healthy controls at $p<0.05$. Asterisks indicate the level of statistical significance between patients with RA and healthy controls assessed by two way analysis of variance for repeated measurements (**$p=0.005$, ***$p<0.001$).

AUCs of respective hormones in both RA and control groups (data not shown). When age and body mass index as possible confounders were controlled, no significant correlation was found between either clinical or inflammatory variables and the studied endocrine parameters.

DISCUSSION

This study aimed at acquiring insight into functional aspects of the adrenal androgens in premenopausal women with RA. In line with previous reports,[5–7] no significant difference in the basal cortisol concentrations was seen despite raised levels of inflammatory cytokines and significantly lower DHEAS and borderline DHEA levels. The response of DHEA in the group of patients with RA during hypoglycaemia was, however, comparable with that in the control group. In a similar attempt to evaluate secretion capacity of DHEA in premenopausal women with RA, Cutolo and coworkers found a lower response of this androgen to ACTH administration.[5] Findings in their study[5] are consistent with our results, confirming decreased production of adrenal androgens in premenopausal women with RA. No differences in basal cortisol, DHEA, or DHEAS levels, but no positive relationships of ACTH with these adrenal hormones were found in patients with new onset of synovitis, including patients with RA, compared with healthy subjects.[15] Authors of the latter study concluded that the HPA axis of patients

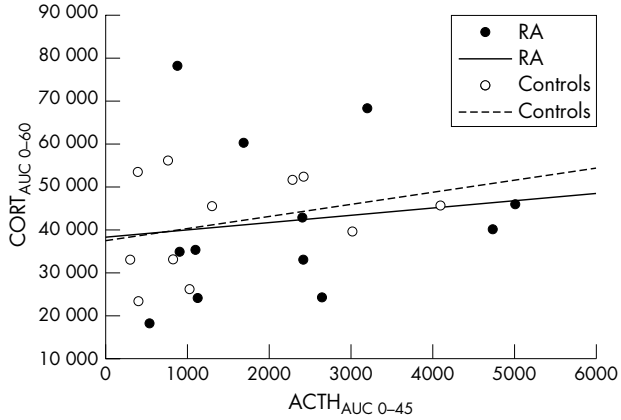

Figure 4 Scattergram and linear regression lines of areas under the response curve (AUC) of ACTH from 0 to 45 minutes (ACTH $_{AUC\ 0\text{-}45}$) *v* AUC of cortisol from 0 to 60 minutes (CORT $_{AUC\ 0\text{-}60}$) in 12 patients with RA ($r^2=0.019$) and 11 healthy controls ($r^2=0.094$) indicating comparable HPA function during insulin-induced hypoglycaemia.

with RA cannot respond properly to inflammatory cytokines as early as within the first year of the disease and that chronic activation of the axis in the course of the disease may be one of the factors leading to progressive decrease in production of adrenal androgens.[15] Indeed, the low level of adrenal androgens found in our group of patients with long term RA supports this view.

A higher $\Delta CORT_{0\text{-}60}$ found in our RA group is not consistent with the results of two previous studies demonstrating lower "interval-specific" cortisol response in patients with RA using the same stimulus.[8 16] A tendency of cortisol towards higher peak concentrations and the lack of difference in DHEA response in our group of patients with RA might be due to a moderately enhanced ACTH response to hypoglycaemia in comparison with controls. However, no significant differences in the ACTH response alone (fig 1) or ACTH *v* cortisol response (fig 4) were found between RA and controls. The relatively small number of patients studied and differences in activity or duration of the disease may explain the discrepancy.

DHEA represents an intermediate product of the "Δ5" pathway in steroidogenesis, whereas ASD and 17OHP are "Δ4" intermediates. Conversion of Δ5 to Δ4 steroids is assisted by the 3β-hydroxysteroid dehydrogenase (3β-HSD) enzyme system. Higher activity of 3β-HSD may be a possible explanation for the proposed shifts from adrenal androgens to glucocorticoids in RA. Basal concentrations of ASD and 17OHP were similar in both groups of our study, as were the dynamics of these intermediates of the Δ4 pathway, failing to suggest preferential activation of 3β-HSD in our RA subjects. Rather than supporting the idea of a production shift from androgens to cortisol, our results are indicative of an isolated impairment of DHEAS production. Decreased sulphotransferase activity might also partially explain low DHEAS and only borderline DHEA in the RA group. However, we did not find any significant difference in the DHEAS:DHEA ratio in patients with RA in comparison with controls. Further studies will be necessary to examine the precise dynamics at each individual step of steroidogenesis, including the Δ5 pathway, in the subgroup of patients with RA with DHEAS levels at a low physiological range.

Plasma EPI predominantly produced by the adrenal medulla is a very sensitive and reliable indicator of sympathoadrenal activity in many models of stress, including hypoglycaemia, as well as in real life situations.[17] Our findings of a significantly lower EPI response to insulin-induced

hypoglycaemia in patients with RA support the hypothesis of sympathoadrenal hyporeactivity present in RA.[11] The absence of NE response observed in the group of patients with RA may also indicate an attenuated sympathoneural reactivity. However, the NE response to hypoglycaemia did not usually reach the proportions found in other situations, such as exposure to cold stimulus or physical exercise.[17 18]

Interpretation of systemic sympathetic nervous system (SNS) activity based on NE levels in antecubital venous blood is limited owing to a significant contribution of activity of the sympathetic nerves of the forearm and the hand. Moreover, NE concentration in venous blood under resting conditions depends besides sympathetic nervous activity (spillover) also on cell membrane transport and rapid removal of NE from plasma (reuptake). The adrenal medulla may contribute to the plasma NE pool during some stress stimulations, including hypoglycaemia.[18] Thus, lower responses of not only EPI but also of NE observed in our group of patients with RA may indicate their impaired secretion from the adrenal medulla. In view of the observed changes in adrenal androgen production in RA, it is of interest to mention that normal high intra-adrenal steroid levels are required for an adequate production of catecholamines in the adrenal medulla.[18]

Only a limited number of studies have dealt with the question of SNS reactivity in inflammatory arthritis. Most of them are related to cardiovascular[19 20 24] or other effects[21] of the SNS without actual measurement of plasma catecholamines. In accordance with our results, significantly lower EPI levels were found in 21 patients with RA in comparison with 137 patients with osteoarthritis during a 7 day follow up after hip or knee arthroplasty.[22] On the other hand, another study showed higher EPI levels before surgery compared with day-before values in patients with RA but not in patients with osteoarthritis.[23] Interestingly, in a real life environment cardiac sympathetic activity was found to be increased in patients with RA, whereas cardiac parasympathetic activity was normal.[24] Reduced cardiovascular responses associated with a lower NE response to tilt up were reported in children with juvenile rheumatoid arthritis.[25]

Processes leading to ''remodelling'' of SNS reactivity in chronic diseases are complex and poorly explored. Future well controlled studies on the effect on immune responses of manipulation with SNS may help to resolve the dilemma of a beneficial adaptation process *v* inadequate control of the immune system and thus may serve as a potential target for therapeutic intervention.

In conclusion, our study confirmed previous data showing lower basal DHEAS in patients with premenopausal onset of RA. No significant differences were seen in the responses of its direct precursor DHEA, steroid intermediates ASD and 17OHP, and of cortisol in RA in comparison with healthy controls. Significantly lower responses of EPI and NE to hypoglycaemia may suggest sympathoadrenal hyporeactivity in patients with RA. Further studies will be necessary to discriminate between stimulus-specific observation and generalised down regulation of stress related responses of the SNS.

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