Effects of naproxen and sulphasalazine or methotrexate on hypothalamic-pituitary-adrenal axis activity in patients with rheumatoid arthritis

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Abstract

Objective

To study the effects of antirheumatic drugs on hypothalamic-pituitary-adrenal (HPA) axis activity in patients with rheumatoid arthritis (RA).

Methods

Twenty patients with recent-onset active RA were studied before antirheumatic treatment, after 2 weeks of naproxen, and after 5½ months of additional treatment with sulphasalazine or methotrexate. The results before treatment were compared with those obtained in 20 age and sex-matched healthy controls (HC). Activity of the HPA-axis was assessed under basal conditions and during insulin tolerance tests (ITT). The ex-vivo production of interleukin (IL)-1β, tumour necrosis factor-α (TNF-α) and IL-6 in whole blood samples was measured with and without stimulation by LPS.

Results

At baseline, plasma ACTH and cortisol levels were not different between patients with RA and HC. The unstimulated production of IL-6 was significantly higher in RA patients than in HC. After 2 weeks of treatment with naproxen, urinary cortisol excretion decreased significantly (p=0.03), and the area under the curve for plasma cortisol during the ITT was significantly lower (p=0.015). The LPS stimulated production of IL-1β was significantly lower compared with baseline. After 6 months, basal plasma, salivary and urinary cortisol levels, and plasma cortisol and ACTH levels during the ITT, were all unchanged in comparison to the pre-treatment period. The unstimulated ex-vivo production of IL-1β was significantly lower than before treatment.

Conclusion

Our results suggest that the non-steroidal anti-inflammatory drug naproxen suppresses the HPA-axis in the first weeks of treatment. After 6 months, this suppressive effect is no longer present, suggesting the existence of adaptive mechanisms.

Key words

non-steroidal anti-inflammatory agents, hypothalamic-pituitary unit, pituitary-adrenal system, rheumatoid arthritis
Effects of NSAIDs on HPA-axis in RA / A.M.M. Eijsbouts et al.

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Introduction
One of the factors that may contribute to the chronicity of joint inflammation in patients with rheumatoid arthritis (RA) is a deficient activity of the hypothalamic-pituitary-adrenal (HPA) axis (1-4). Non-steroidal anti-inflammatory drugs (NSAIDs) have been shown to influence the interactions between the immune system and the HPA-axis in animal studies (5, 6). However, the effects of NSAIDs on the activity of the HPA-axis have not been studied extensively in patients with RA. In one small study, the use of NSAIDs was shown to be associated with decreased adrenocorticotropic hormone (ACTH) levels (7). There is evidence that pro-inflammatory cytokines stimulate the HPA-axis at either the hypothalamic, pituitary or adrenal level (1, 8, 9); e.g. IL-1 and IL-6 can stimulate the adrenal cortex to produce cortisol independently from ACTH (10). Prostaglandins may be involved in these stimulatory effects as in rats it was shown, that the responses of ACTH to IL-1β and IL-6 are blunted by pretreatment with indomethacin (11-13) ketoprofen (14) and a selective cyclooxygenase-2 inhibitor (14).

The aim of our study was to examine the effects of antirheumatic treatment on HPA-axis activity in patients with RA. HPA-axis activity was assessed both under basal conditions and after stimulation by insulin-induced hypoglycemia during the insulin tolerance test (ITT), a standardised form of stress. Because of the possible role of pro-inflammatory cytokines in stimulating the HPA-axis in RA, the ex-vivo production of interleukin (IL)-1β, tumour necrosis factor-α (TNF-α) and IL-6 was also assessed.

Subjects and methods

Subjects
Twenty patients with active, recent-onset (<1 year) RA and 20 age- and sex-matched healthy controls (HC) were included. All patients fulfilled the revised criteria for RA of the American College of Rheumatology (15) and were IgM rheumatoid factor positive. Active disease was defined as a disease activity score (DAS) ≥3.5 (16). None of the patients had ever been treated with oral, intramuscular or intra-articular glucocorticoids, or a disease modifying anti-rheumatic drug (DMARD). Treatment with NSAIDs was discontinued in all patients one week prior to the study. All patients with RA were studied at baseline, after 2 weeks and after 6 months. At baseline all patients were without treatment. Thereafter, all patients were started on naproxen 500 mg twice a day and studied again after 2 weeks. A DMARD was then added by the rheumatologist in charge of the patient, if indicated. The initial DMARD was sulphasalazine which could be switched to methotrexate if necessary. After 6 months of therapy patients were assessed again.

Subjects, all aged between 18 and 65 years, were excluded if they had any condition or medication (including oral contraceptives) known to influence HPA-axis activity. Other exclusion criteria were anaemia (Hb ≤6.5 mmol/l), renal or hepatic disorders, and contraindications for undergoing the stress of an ITT, such as cardiovascular disorders, hypertension and epilepsy. All subjects voluntarily signed an informed consent form. The study protocol was approved by the hospital’s ethics committee.

Methods

– Disease activity
Disease activity was assessed at baseline, after 2 weeks and after 6 months with a composite disease activity score, 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 (16).

– HPA-axis activity
The activity of the HPA-axis was assessed under basal conditions and during an ITT in all subjects at baseline, and after 2 weeks and 6 months in patients with RA. For the assessments under basal conditions, blood was drawn at 9:00 am while patients were fasting, and at 4:00 pm for determination of plasma total cortisol, free cortisol, cortisol binding globulin (CBG) and ACTH concent-
trations. Urine was collected for determination of 24-hour cortisol excretion. On the same day salivary samples were collected every 4 hours starting at 8:00 am, for determination of the 24-hour cortisol rhythm.

ITTs were performed on separate days with the subjects fasting and in a supine position. At 8:30 am a catheter was inserted in an antecubital vein and kept patent by saline solution. After a 30 minute rest, insulin (Actrapid® 100 IU/ml, Novo-Nordisk, Denmark) was administered 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 for determination of cortisol and ACTH concentrations. The test was considered adequate if a glucose level <2.0 mmol/l was reached.

Blood for measurement of ACTH and cortisol was collected in pre-chilled EDTA tubes and centrifuged for 10 minutes at 1500g (4°C). The plasma obtained was aliquoted and Trasylol® (250 KIU; Bayer, Germany) was added. ACTH was measured by an immunoradiometric assay based on two polyclonal antibodies (EuroDiagnostics, The Netherlands). Standard curves were prepared by spiking ACTH-free plasma with ACTH₁₈₋₃₉ (MRC 74/555, NIBSC, Potters Bar, Hertfordshire, UK). The sensitivity of the assay was 0.5 pmol/l and the within- and between-assay coefficients of variation (CV) were 4.4% and 7.2% respectively. Normal range at 8:00 am: 1.3-9.2 pmol/l.

Plasma cortisol levels were measured by a radioimmunoassay which has been described before (17). 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 0.21 μmol/l. Normal range at 8:00 am 0.19-0.55 μmol/l and at 4:00 pm. 0.06-0.38 μmol/l.

Plasma 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.

The plasma free cortisol fraction was measured by indirect equilibrium dialysis by a method described earlier for androstenedione (18). Briefly, H-cortisol is added to the plasma sample. An aliquot of 180 μl of this mixture is dialysed against PBS for 4 hours. After dialysis, 50 μl aliquots were taken from the dialysates and counted for radioactivity. The fraction or percent free cortisol was calculated while taking into account the dilution inherent in equilibrium dialysis. Multiplication of the free fraction by the total concentration yields the free cortisol concentration. The within- and between-assay CV were 8.5% and 5.5%, respectively, at an average free cortisol fraction of 5.0%.

Salivary cortisol as well as 24-hour urinary free cortisol were measured by radioimmunoassay after previous extraction and paper chromatography (19).

– Cytokines
The ex-vivo production of IL-1β, TNF-α and IL-6 was measured in whole blood samples collected at 9:00 am (fasting) and at 4:00 pm. Blood was collected in two 4 ml EDTA tubes containing 250 kIU Trasylol®. Fifty microliters LPS (final concentration 10 μg/ml; E. coli serotype 055:b5; Sigma St. Louis, USA) was added under sterile conditions to one tube; the other tube was incubated without LPS. The tubes were incubated for 24 hours at 37°C and centrifuged thereafter. Aliquots were stored at -20°C until assay. IL-1β, IL-6 and TNF-α were measured in duplicate by radioimmunoassay as described previously (20).

Statistical analysis
Responses of ACTH and cortisol to hypoglycemia were integrated over time as area under the response curve (AUC) from 0 to 180 minutes. The calculated AUCs were divided by 180 minutes to obtain a mean integrated level of ACTH and cortisol during the ITT. The maximal rise in ACTH and cortisol after insulin administration was calculated as the difference in concentration between the levels of these hormones at 0 minutes and at the time point with the highest concentration during the ITT. An AUC was also calculated for the 24-hour cortisol measurements in salivary samples.

Comparisons between HC and patients with RA were made with the unpaired t-test or with the Mann-Whitney U-test if data were not distributed normally. In patients with RA, changes from baseline after 2 weeks and after 6 months were analysed with the Wilcoxon signed-rank test. All p-values are based on 2-tailed tests and considered significant at the 0.05 level. Due to the explorative nature of this study the Bonferroni correction was not used.

Results
Both the RA patient group and HC group contained 3 men and 17 women. The patients and HC had a mean (SD) age of 49.0 (12.0) and 47.5 (9.8) years, respectively. All patients with RA were studied again after 2 weeks treatment with naproxen. Subsequently, 1 patient was treated with systemic corticosteroids and 2 patients received no DMARD therapy. These 3 patients did not participate in the assessments after 6 months.

Disease activity
After 2 weeks of naproxen the DAS decreased significantly with a mean value of 0.7 (p=0.0003, Table I). The individual components of the DAS also showed significant decreases, except for the ESR. After 5½ months of additional treatment with either sulphasalazine or methotrexate, the DAS showed further improvement (1.6 units, p=0.0002 versus baseline). The ESR however still showed no significant change compared to baseline.

HPA-axis activity: basal values
At baseline, patients with RA tended to have higher mean basal ACTH levels (p=0.09 at 9:00 am and p=0.10 at 4:00 pm) than HC (Table II). Other basal hormone levels were not different between patients and HC. After 2 weeks of naproxen, a significant decrease in urinary cortisol excretion (p=0.03) was observed. Other hormone levels were unchanged. After 5½ months of additional DMARD treatment, urinary cortisol excretion had returned to its pre-treatment level. ACTH levels at 9:00 am showed a significant decrease (p=0.005) compared with baseline but at 4:00 pm ACTH levels were unchanged.
Effects of NSAIDs on HPA-axis in RA / A.M.M. Eijsbouts et al.

Table I. Disease activity: baseline values and changes from baseline after 2 weeks and 6 months of antirheumatic drug therapy.

<table>
<thead>
<tr>
<th></th>
<th>Baseline (n=20)</th>
<th>Change from baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean (SD)</td>
<td>After 2 weeks (n=20)</td>
</tr>
<tr>
<td>DAS (units)</td>
<td>4.3 (0.7)</td>
<td>-0.7 (0.7)</td>
</tr>
<tr>
<td>ESR (mm/hr)</td>
<td>21.6 (24.7)</td>
<td>3.4 (8.1)</td>
</tr>
<tr>
<td>RAI (units)</td>
<td>16.7 (7.8)</td>
<td>-4.3 (4.9)</td>
</tr>
<tr>
<td>Number of swollen joints</td>
<td>11.4 (6.3)</td>
<td>-2.4 (5.1)</td>
</tr>
<tr>
<td>VAS general well-being (mm)</td>
<td>52.3 (18.9)</td>
<td>-12.8 (19.1)</td>
</tr>
</tbody>
</table>
| * for comparison with baseline. DAS: Disease activity score; ESR: Erythrocyte sedimentation rate; RAI: Ritchie articular index; VAS: Visual analogue scale.

Table II. Basal plasma levels of adrenocorticotropic hormone (ACTH), total and free cortisol and cortisol binding globulin (CBG), and total 24 hrs urinary excretion of cortisol: baseline and changes from baseline after 2 weeks and 6 months of antirheumatic treatment.

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls (n=20)</th>
<th>Rheumatoid arthritis After 2 weeks (n=20)</th>
<th>After 6 months (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean (SD)</td>
<td>mean (SD) median p*</td>
<td>mean (SD) median p**</td>
</tr>
<tr>
<td>9:00 AM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACTH (pmol/l)</td>
<td>3.7 (2.2)</td>
<td>5.5 (3.5) 0.09</td>
<td>-1.2 (2.9) 0.4</td>
</tr>
<tr>
<td>total cortisol (µmol/l)</td>
<td>0.44 (0.11)</td>
<td>0.45 (0.12) &gt;0.2</td>
<td>-0.04 (0.12) -0.04</td>
</tr>
<tr>
<td>free cortisol (nmol/l)</td>
<td>20.1 (4.7)</td>
<td>20.5 (4.6) &gt;0.2</td>
<td>-1.7 (9.3) -2.6</td>
</tr>
<tr>
<td>CBG (µmol/l)</td>
<td>0.83 (0.10)</td>
<td>0.83 (0.11) &gt;0.2</td>
<td>-0.02 (0.10) 0</td>
</tr>
<tr>
<td>4:00 PM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACTH (pmol/l)</td>
<td>3.3 (1.6)</td>
<td>4.3 (2.4) 0.10</td>
<td>-0.2 (1.9) -0.5</td>
</tr>
<tr>
<td>total cortisol (µmol/l)</td>
<td>0.27 (0.10)</td>
<td>0.26 (0.09) &gt;0.2</td>
<td>-0.01 (0.11) -0.01</td>
</tr>
<tr>
<td>Free cortisol (nmol/l)</td>
<td>12.3 (4.3)</td>
<td>12.3 (4.4) &gt;0.2</td>
<td>-0.4 (5.8) -1.2</td>
</tr>
<tr>
<td>CBG (µmol/l)</td>
<td>0.83 (0.15)</td>
<td>0.84 (0.17) &gt;0.2</td>
<td>-0.02 (0.15) 0.01</td>
</tr>
<tr>
<td>Urinary cortisol nmol/24 hrs</td>
<td>69.9 (26.5)</td>
<td>67.5 (36.0) &gt;0.2</td>
<td>-20.4 (37.1) -13.4</td>
</tr>
</tbody>
</table>

* for the comparison between healthy controls and rheumatoid arthritis patients; ** for the comparison with baseline in rheumatoid arthritis patients; *available for only 10 patients at 9:00 AM and 9 patients at 4:00 PM. nd: not done.

compared with baseline (p>0.2). Salivary cortisol levels at baseline were not significantly different between HC and RA patients (Fig. 1). When salivary cortisol levels after 2 weeks and after 6 months were compared with baseline values, no significant changes were found in RA patients, neither at individual time points nor in AUC (p>0.2, data not shown).

Table III. Disease activity: baseline values and changes from baseline after 2 weeks and 6 months of antirheumatic drug therapy.

<table>
<thead>
<tr>
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</tr>
<tr>
<td>RAI (units)</td>
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<td>-4.3 (4.9)</td>
</tr>
<tr>
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<td>-2.4 (5.1)</td>
</tr>
<tr>
<td>VAS general well-being (mm)</td>
<td>52.3 (18.9)</td>
<td>-12.8 (19.1)</td>
</tr>
</tbody>
</table>

* for comparison with baseline. DAS: Disease activity score; ESR: Erythrocyte sedimentation rate; RAI: Ritchie articular index; VAS: Visual analogue scale.

HPA-axis activity: insulin tolerance tests

In response to insulin injection adequate hypoglycemia (glucose level <2.0 mmol/l) occurred in all patients with a nadir at 30 minutes. Figure 2 shows the ACTH responses to hypoglycaemia in the different groups and periods. There were no significant differences between the HC and the patients with RA. In patients with RA no significant changes occurred after 2 weeks and after 6 months of treatment when compared with baseline. The mean integrated ACTH levels during the ITT were not different between HC and patients with RA and they did not change significantly from baseline after 2 weeks and after 6 months in patients with RA (p>0.2, data not shown).

At baseline, the mean integrated level of cortisol during ITT tended to be

Fig. 1. Circadian levels of cortisol in salivary samples during 24 hours. HC: healthy controls (n=20); RA: rheumatoid arthritis (n=20). No significant differences were found when RA patients were compared with HC or when values after 2 weeks (n=20) or 6 months (n=17) of antirheumatic treatment were compared with baseline values in RA patients.
Effects of NSAIDs on HPA-axis in RA

A.M.M. Eijsbouts et al.

lower in patients with RA than in HC ($p=0.07$, Fig. 3). In patients with RA, the mean integrated cortisol level was significantly lower after 2 weeks naproxen compared with baseline ($p=0.015$). After 6 months, it had returned to pre-treatment values.

At baseline no significant differences in plasma cortisol during ITT at individual time points were found between patients and HC (Fig. 4), and the mean (SD) maximal rise in plasma cortisol also did not differ between HC and patients with RA: 0.31 (0.14) μmol/l and 0.31 (0.15) μmol/l respectively ($p>0.2$).

In patients with RA treated for 2 weeks with naproxen plasma cortisol levels were significantly lower than baseline levels at 45 ($p=0.02$), 60 ($p=0.03$), 90 ($p=0.03$) and 120 ($p=0.045$) minutes after insulin injection. After 6 months of treatment cortisol levels at individual time points and the maximal rise in cortisol during the ITT were not significantly different compared with baseline ($p>0.2$).

Cytokines

Table III shows the whole blood production of IL-1β, TNF-α and IL-6 after 24 hrs of incubation with or without LPS. Baseline values for IL-1β production were not significantly different between RA patients and HC. After 2 weeks use of naproxen, the LPS-stimulated production of IL-1β decreased significantly compared with baseline, both at 9:00 am ($p=0.03$) and at 4:00 pm ($p=0.01$). The spontaneous production of IL-1β did not change significantly compared with baseline. After 6 months of treatment however, the spontaneous production of IL-1β had decreased significantly ($p=0.01$ at 9:00 am and $p=0.02$ at 4:00 pm) while the LPS-stimulated production of IL-1β was no longer significantly different from baseline.

At baseline, both spontaneous and LPS-stimulated production of TNF-α, did not significantly differ between RA patients and HC. No significant changes occurred in spontaneous TNF-α production after 2 weeks and after 6 months. Compared to baseline the LPS-stimulated production of TNF-α had decreased after 2 weeks (at 4:00 pm only, $p=0.02$), but not after 6 months.
Effects of NSAIDs on HPA-axis in RA / A.M.M. Eijssenbouts et al.

Table III. Spontaneous and LPS* stimulated ex-vivo whole blood production of IL-1β (ng/ml), TNF-α (ng/ml) and IL-6 (ng/ml): baseline and changes from baseline after 2 weeks and 6 months of antirheumatic treatment.

<table>
<thead>
<tr>
<th></th>
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<th>Rheumatoid arthritis (n=20)</th>
<th>After 2 weeks (n=20)</th>
<th>After 6 months (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>SD</td>
<td>mean</td>
<td>SD</td>
</tr>
<tr>
<td>9:00 am</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1β, LPS-</td>
<td>0.059</td>
<td>0.017</td>
<td>0.058</td>
<td>0.058</td>
</tr>
<tr>
<td>IL-1β, LPS+</td>
<td>5.740</td>
<td>2.764</td>
<td>6.697</td>
<td>3.626</td>
</tr>
<tr>
<td>TNFα, LPS-</td>
<td>0.343</td>
<td>0.350</td>
<td>0.254</td>
<td>0.068</td>
</tr>
<tr>
<td>TNFα, LPS+</td>
<td>4.934</td>
<td>1.119</td>
<td>4.507</td>
<td>1.972</td>
</tr>
<tr>
<td>IL-6, LPS-</td>
<td>8</td>
<td>2</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>IL-6, LPS+</td>
<td>13538</td>
<td>7253</td>
<td>12002</td>
<td>7281</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>4:00 pm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1β, LPS-</td>
<td>0.065</td>
<td>0.015</td>
<td>0.061</td>
<td>0.069</td>
</tr>
<tr>
<td>IL-1β, LPS+</td>
<td>6.666</td>
<td>3.345</td>
<td>6.862</td>
<td>4.015</td>
</tr>
<tr>
<td>TNFα, LPS-</td>
<td>0.252</td>
<td>0.045</td>
<td>0.211</td>
<td>0.077</td>
</tr>
<tr>
<td>TNFα, LPS+</td>
<td>4.714</td>
<td>1.414</td>
<td>5.209</td>
<td>2.250</td>
</tr>
<tr>
<td>IL-6, LPS-</td>
<td>8</td>
<td>3</td>
<td>17</td>
<td>16</td>
</tr>
<tr>
<td>IL-6, LPS+</td>
<td>8540</td>
<td>4931</td>
<td>9691</td>
<td>5242</td>
</tr>
</tbody>
</table>

*LPS: measurement after 24 hrs incubation with (+) or without (-) lipopolysaccharide; **for the comparison between healthy controls and rheumatoid arthritis patients; ***for the comparison with baseline in rheumatoid arthritis patients.

At baseline, the spontaneous production of IL-6 was significantly higher in RA patients than in HC: mean values of 28 versus 8 ng/ml at 9:00 am and 17 versus 8 ng/ml at 4:00 pm (p=0.004 and p=0.04, respectively). LPS-stimulated production of IL-6 did not significantly differ between RA patients and HC. No significant changes occurred in stimulated or unstimulated IL-6 levels after 2 weeks and 6 months of treatment.

Discussion

The main finding of our study is the decrease responsiveness of the HPA-axis in patients with RA after 2 weeks of treatment with naproxen, which results in a decreased 24-hour urinary cortisol excretion and decreased plasma cortisol levels during ITTs compared with pre-treatment values. After 6 months, in which naproxen was continued and sulphasalazine or methotrexate were added to the treatment, these cortisol levels returned to pre-treatment values.

The significant decrease in plasma cortisol levels during the ITT between the assessments at baseline and after 2 weeks of treatment with naproxen, was not found in plasma samples that were collected for basal values on a day separate from the day of the ITT. Possibly, this is due to the less standardised conditions under which these samples were collected. Therefore, the decrease in basal plasma ACTH level at 9:00 am after 6 months of treatment compared with baseline, which was not confirmed under the more standardised conditions of the ITT, is most likely a spurious result.

Our findings cannot be explained by competition between naproxen and cortisol in binding to CBG or by a change in CBG levels, because the ratio between free and total cortisol as well as CBG levels remained unchanged after treatment with naproxen. Adaptation of patients to the test situation and improvement of disease activity are also unlikely to explain our results, especially because cortisol levels returned to pre-treatment levels after 6 months.

The effects of NSAIDs on ACTH and cortisol have not been studied extensively in patients with RA. As far as we know, this is the first study that shows an effect of NSAID treatment on hypoglycaemia stimulated cortisol levels in patients with RA. Hall et al. did a cross-sectional study in which they compared 8 untreated RA patients with 7 NSAID treated RA patients and 13 HC (7). Unlike in our longitudinal study, they found that plasma cortisol levels were not different between untreated and NSAID treated RA patients while the latter had lower plasma ACTH levels.

The most likely explanation for our observations is that naproxen has transient effects on HPA-axis activity in patients with active RA e.g. through inhibition of prostaglandin synthesis or induction of changes in pro-inflammatory cytokine production. Naproxen and other NSAIDs inhibit the enzyme cyclooxygenase (COX) and thereby decrease prostaglandin synthesis. Prostaglandins are known to be involved in the regulation of ACTH and cortisol secretion (1, 5). Studies of the effects of cyclooxygenase inhibitors on ACTH and cortisol levels in healthy humans have yielded conflicting results. NSAIDs had no effects on basal ACTH and cortisol levels (21-23). An iv infusion of sodium salicylate increased ACTH and cortisol responses to hypoglycaemia (24).

In contrast, a few days use of acetylsalicylic acid decreased ACTH and cortisol responses to hypoglycaemia (25). In normal cortisol levels, because the ratio between free and total cortisol as well as CBG levels remained unchanged after treatment with naproxen. Adaptation of patients to the test situation and improvement of disease activity are also unlikely to explain our results, especially because cortisol levels returned to pre-treatment levels after 6 months.

The effects of NSAIDs on ACTH and cortisol have not been studied extensively in patients with RA. As far as we know, this is the first study that shows an effect of NSAID treatment on hypoglycaemia stimulated cortisol levels in patients with RA. Hall et al. did a cross-sectional study in which they compared 8 untreated RA patients with 7 NSAID treated RA patients and 13 HC (7). Unlike in our longitudinal study, they found that plasma cortisol levels were not different between untreated and NSAID treated RA patients while the latter had lower plasma ACTH levels.

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of IL-1β after 2 weeks treatment with naproxen. Because IL-1β stimulates the adrenal gland to produce cortisol (10), this may have contributed to the transient decrease in cortisol levels. However, the spontaneous ex-vivo production of IL-1β had significantly decreased after 6 months compared with baseline. If anything, this should have reduced the activation of the HPA-axis and have resulted in a further decrease in cortisol levels. Furthermore, we observed an increased production of IL-6 in RA patients compared with HC. An increased IL-6 production would be expected to activate the HPA-axis. However, levels of cortisol during the ITT tended to be lower in our RA patients than in HC before treatment. This finding is consistent with the hypothesis that cortisol levels are inappropriately low in patients with RA (1-3). Apart from the potential influence on prostaglandin and cytokine production, NSAIDs might also establish an indirect effect on the HPA-axis via reduction of pain, inflammation and subsequent stress in patients with RA. Indeed, in healthy volunteers preliminary observations have shown that naproxen did not influence the activity of the HPA-axis (23).

A limitation of our study is that the only NSAID tested was naproxen; other NSAIDs and especially selective COX-2 inhibitors may have different effects. Besides, we chose to use the ITT to evaluate the integral HPA-axis, whereas other tests like the corticotropin releasing hormone and ACTH test are necessary to evaluate specific parts of the axis.

To our knowledge, this is the first longitudinal study of the effects of antiinflammatory treatment on HPA-axis activity in patients with RA. We conclude that naproxen treatment results in a transient suppression of HPA-axis activity. The degree of suppression is mild and probably without direct clinical consequences. It is nevertheless noteworthy because it adds up to the relative suppression of the HPA-axis seen in untreated patients with RA. Furthermore, our findings point to the fact that existing data should be re-interpreted due to possible influence of NSAIDs.

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