Deceased prolactin response to hypoglycaemia in patients with rheumatoid arthritis: correlation with disease activity


Objective: To compare basal and stimulated prolactin levels between patients with rheumatoid arthritis and healthy controls, and to assess the effects of antirheumatic treatment on prolactin concentrations.

Methods: Serum prolactin was assessed under basal conditions and during an insulin tolerance test (ITT) in 20 patients with recently diagnosed active rheumatoid arthritis and 20 age and sex matched controls. The patients were reassessed after two weeks’ treatment with naproxen and after six months’ additional treatment with either sulfasalazine or methotrexate. Disease activity was assessed by the disease activity score (DAS).

Results: Basal levels of prolactin were not significantly different between patients with rheumatoid arthritis and controls. Prolactin responses to hypoglycaemia were less in untreated rheumatoid patients than in controls. DAS scores correlated negatively with the area under the curve (AUC) for prolactin concentrations during the ITT. Treatment with naproxen for two weeks did not influence either basal or stimulated prolactin levels. After six months of antirheumatic treatment, prolactin responses to hypoglycaemia increased significantly to levels observed in controls. At the same time point, DAS had improved considerably. The improvement correlated with the increase in AUC of prolactin during the ITT (r = 0.48, p = 0.05).

Conclusions: Patients with active rheumatoid arthritis have a decreased prolactin response to hypoglycaemia induced stress. The response recovers following treatment with antirheumatic drugs.

Prolactin, a hormone produced by the anterior pituitary gland, is known for its ability to stimulate lactation, but it has also been shown to play a role in regulating immune function and it has potent pro-inflammatory effects.1-3 Secretion of prolactin is restrained by the hypothalamus, where the most important prolactin inhibiting factor, dopamine, is produced. Other factors are also involved in prolactin secretion, including proinflammatory cytokines. Depending on the animal species studied and the severity of inflammation, proinflammatory cytokines can either stimulate or depress pituitary prolactin release.3-4 Prolactin levels are higher in women than in men, though there is considerable overlap in the ranges. Prolactin levels rise in response to all kinds of stress.

It has been suggested that excessive prolactin secretion may contribute to the pathogenesis of rheumatoid arthritis.5 A characteristic feature of rheumatoid arthritis is remission of the disease during gestation with exacerbation in the postpartum period. During pregnancy prolactin concentrations are low, but start to rise during the second trimester, preparing the breasts for lactation. They reach their peak at the end of pregnancy, and this has been related to the postpartum exacerbation of rheumatoid arthritis. Furthermore, there is an increased likelihood of rheumatoid arthritis developing in the postpartum period, particularly in breast feeding mothers.5-7

Previous studies of prolactin concentrations in patients with rheumatoid arthritis have had inconsistent results, the values being either increased,5-8-10 decreased,11-14 or unchanged.15-20 There could be several explanations for the contradictory reports.

First, it is possible that non-steroidal anti-inflammatory drugs (NSAIDs) may influence prolactin levels. In healthy subjects, treatment with NSAIDs can result in increased,21-22 decreased,23-24 or unchanged prolactin concentrations.25-26 However, the effects of NSAIDs on prolactin have not been studied in patients with rheumatoid arthritis, and in most previous studies the subjects had been taking NSAIDs.

Second, treatment with glucocorticoids may influence prolactin concentrations. This is suggested by a study by Mateo et al, who found raised serum prolactin in 91 men with rheumatoid arthritis. Many of their patients (n = 74) used glucocorticoids, and they found that a higher cumulative glucocorticoid dose was associated with higher prolactin levels.25 In another study in 50 healthy volunteers, Dinan et al showed that prolactin responses to buspirone were correlated with baseline cortisol levels.27 To exclude a possible influence of glucocorticoids on prolactin levels in our study, all patients who used glucocorticoids were excluded.

Third, disease modifying antirheumatic drugs (DMARDs) may influence prolactin levels. To our knowledge, the effects of DMARDs on prolactin levels have not been studied.

Our aim in this study was to compare serum prolactin between patients with rheumatoid arthritis and healthy controls, and investigate whether the concentrations change after antirheumatic drug treatment. The patients were studied before and after the use of the NSAID naproxen, and after the additional use of a DMARD. We studied basal serum prolactin levels and also carried out an insulin tolerance test, a standardised form of stress, to assess the prolactin response to hypoglycaemia induced stress.

Abbreviations: ACR, American College of Rheumatology; AUC, area under the curve; DAS, disease activity score; DMARD, disease modifying antirheumatic drug; ITT, insulin tolerance test; NSAID, non-steroidal anti-inflammatory drug
METHODS

Subjects
We included 20 patients with rheumatoid arthritis of recent onset (less than one year) who had active disease, and 20 age and sex matched healthy controls. All patients were aged between 18 and 65 years. The patients fulfilled the revised ACR criteria for rheumatoid arthritis. All patients with rheumatoid arthritis and the 20 age and sex matched healthy controls. All subjects were aged between 18 and 65 years. The patients fulfilled the revised ACR criteria for rheumatoid arthritis. They all had newly diagnosed rheumatoid arthritis and were consecutive attendees at a rheumatology outpatients clinic who were willing to participate in the study. We recruited only IgM rheumatoid factor positive patients to avoid including any with non-rheumatoid forms of arthritis. Active disease was defined by a disease activity score (DAS) of 3.5 or more. Patients had not been treated with a DMARD or with oral, intramuscular, or intra-articular glucocorticoids. Treatment with NSAIDs was discontinued in all patients one week before the study.

The patients with rheumatoid arthritis were studied at baseline, after two weeks, and after six months. At baseline they were untreated, as described above. Thereafter, all patients started treatment with naproxen 500 mg twice a day and were studied again after two weeks. A DMARD was then added to the therapeutic regimen, if indicated, by the rheumatologist in charge of the patient. The initial DMARD was sulfasalazine, which could be switched to methotrexate if necessary. The final assessments were done after six months of treatment.

Subjects were excluded if they had any condition or drug treatment that is known to influence blood prolactin concentrations. Subjects using oral contraceptives were also excluded. Other exclusion criteria were anaemia (Hb < 6.5 g/dl), renal or hepatic disorders, and contraindications to undergoing the stress of an insulin tolerance test, such as cardiovascular disorders, hypertension, or epilepsy. All participants voluntarily signed an informed consent form. The study protocol was approved by the hospital’s ethics committee.

Procedures
Disease activity
In patients with rheumatoid arthritis, disease activity was assessed using a composite disease activity score which included the erythrocyte sedimentation rate (ESR), the Ritchie articular index, the number of swollen joints, and a visual analogue scale for general wellbeing.

Prolactin concentrations
Serum prolactin concentrations were measured under basal conditions and, on a separate day, during an insulin tolerance test (ITT). Blood was collected at 09:00 and 16:00 hours for determination of basal prolactin levels. On the day of the ITT, subjects were fasting and placed in a supine position. At 08:30 a catheter was inserted in an antecubital vein and kept patent by saline solution. After a 30 minute rest, insulin (Actrapid®, Novo-Nordisk, Bagsvaerd, Denmark) was given as a bolus injection in a dose of 0.1 units/kg body weight. Blood samples for measurement of glucose and prolactin levels were collected at 0, 20, 30, 45, 60, 90, 120, and 180 minutes after injection. During the test, heart rate and blood pressure were measured using an automatic blood pressure recorder. The test was considered adequate if a glucose level of <2.0 mmol/l was achieved. If not, the test was repeated with a 50% higher dose of insulin. This occurred in one patient, but a glucose level of <2.0 mmol/l was achieved when the test was repeated.

All blood samples for measurement of prolactin levels were collected in dry tubes. Blood was centrifuged at 2000 g for 10 minutes and serum was stored at −20°C until analysis. Serum prolactin concentrations were measured using the Spectria competitive solid phase radioimmunoassay from Farmos Diagnostica (Turku, Finland), as described earlier. The sensitivity of the assay was 30 mIU/l. The within and between assay coefficients of variation were 5.8% and 10% at 430 mIU/l, and 4.9% and 8.5% at 1200 mIU/l, respectively (normal range 100 to 700 mIU/l).

Statistical analysis
Responses of prolactin to hypoglycaemia were integrated over time as the area under the response curve (AUC) from 0 to 180 minutes. The calculated AUCs are divided by 180 minutes to obtain an integrated level of prolactin during the ITT.

The primary outcome measure was the integrated serum prolactin level during the ITT. Secondary outcomes included unstimulated (basal) serum prolactin and values of prolactin at individual time points during the ITT. Comparisons between healthy controls and patients with rheumatoid arthritis were made with the unpaired t test or the Mann–Whitney U test if the data were not normally distributed. In patients with rheumatoid arthritis, the assessments after two weeks and after six months of treatment were compared with baseline using paired t tests or the Wilcoxon signed rank test if data were not normally distributed. All p values are based on two tailed tests and were considered significant at the 0.05 level.

Spearman correlation coefficients were calculated between baseline DAS and the AUC for prolactin, and between the change in DAS after six months and the change in AUC for prolactin after six months.

RESULTS
The 20 patients with active rheumatoid arthritis and the 20 healthy controls were matched for age and sex, and both groups included three men and 17 women. The healthy controls and the patients with rheumatoid arthritis had a mean (SD) age of 47.5 (9.8) years and 49.0 (12.0) years, respectively. All 20 patients with rheumatoid arthritis were studied again after two weeks of treatment with naproxen. Subsequently, one patient was treated with systemic corticosteroids and two received no DMARD therapy. These three

Table 1 Disease activity: baseline values and changes from baseline after two weeks and six months of antirheumatic drug treatment

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline (n = 20)</th>
<th>Change from baseline</th>
<th>After 2 weeks (n = 20)</th>
<th>p Value*</th>
<th>After 6 months (n = 17)</th>
<th>p Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAS (units)</td>
<td>4.3 (0.7)</td>
<td>–0.7 (0.7)</td>
<td>0.0003</td>
<td>–1.6 (1.2)</td>
<td>0.0002</td>
<td></td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>28.4 (24.7)</td>
<td>8.4 (8.1)</td>
<td>0.013</td>
<td>2.6 (32.2)</td>
<td>0.030</td>
<td></td>
</tr>
<tr>
<td>RA (units)</td>
<td>16.7 (7.8)</td>
<td>–4.3 (4.9)</td>
<td>0.001</td>
<td>–10.1 (6.5)</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Number of swollen joints</td>
<td>11.4 (6.3)</td>
<td>–2.4 (5.1)</td>
<td>0.02</td>
<td>–4.6 (8.1)</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>VAS general wellbeing (mm)</td>
<td>52.3 (1.9)</td>
<td>–12.8 (19.1)</td>
<td>0.007</td>
<td>–25.1 (23.9)</td>
<td>0.002</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean (SD). *For comparison with baseline. DAS, disease activity score; ESR, erythrocyte sedimentation rate; RA, Ritchie articular index; VAS, visual analogue scale (0–100 mm).
patients did not participate in the assessments after six months.

Of the 20 women in the control group, 11 were premenopausal, and of the 20 women in the rheumatoid group, 10 were premenopausal.

**Disease activity**

Table 1 shows the baseline disease activity and the changes from baseline after two weeks and six months of treatment in the patients with recent onset rheumatoid arthritis. After two weeks’ use of naproxen, the DAS decreased significantly, with a mean value of 0.7 (p = 0.0003). All the individual components of the DAS also showed significant decreases with the exception of the ESR, which remained unchanged. After six months of additional treatment with either sulfasalazine or methotrexate, the DAS showed further improvement (mean decrease 1.6 units, p = 0.0002 vs baseline). The ESR, however, again remained unchanged when compared with baseline values.

**Prolactin levels**

Table 2 summarises the prolactin concentrations at baseline and the changes after two weeks and six months of antirheumatic treatment. At baseline the unstimulated prolactin levels were not different between the rheumatoid patients and the healthy controls.

There were no significant differences between the groups in the hypoglycaemia nadir reached during the ITT. The serum prolactin concentrations during the ITT were, however, lower in the rheumatoid patients than in the controls between 30 and 90 minutes after the bolus injection of insulin (fig 1). This also resulted in a lower AUC for prolactin during the ITT in the rheumatoid patients. After two weeks of treatment with naproxen, no significant changes occurred in either basal or stimulated serum prolactin concentrations. After six months of antirheumatic drug treatment serum prolactin concentrations during the ITT were higher than at baseline between 60 and 120 minutes after the bolus injection of insulin. The AUC for prolactin was also higher at six months than at baseline.

At baseline, the DAS correlated negatively with the AUC for prolactin (r = −0.58; p = 0.008) in patient with rheumatoid arthritis. After six months of treatment, the improvement in DAS correlated with the change in AUC for prolactin (r = 0.48; p = 0.05).

**DISCUSSION**

In our study, untreated newly diagnosed patients with active rheumatoid arthritis had basal serum prolactin levels that were not different from healthy controls, but their prolactin responses to stress induced by hypoglycaemia were decreased compared with the controls. Ideally, we should have measured the premenopausal women at the same phase of the menstrual cycle at each time point of the study, but for practical reasons this was not possible. However, because of the large number of patients and the similar number of premenopausal and postmenopausal subjects in each group, we expected that this would not influence our results. In our view, the fact that we did not find any difference in basal prolactin levels between the groups confirms that assumption.

Previous studies of prolactin levels in patients with rheumatoid arthritis have given contradictory results. As discussed above, several explanations may be offered to account for the different results in published reports. The possibility that NSAIDs influence prolactin secretion now seems unlikely to us, as our longitudinal results indicate that two weeks of treatment with the NSAID naproxen had no effect on either basal or stimulated prolactin concentrations. However, we are aware that this does not exclude an effect of other NSAIDs. A possible influence of glucocorticoids on prolactin levels was ruled out in this study, as patients using glucocorticoids were excluded. The effects of DMARDs on prolactin levels have not been studied before. In our study prolactin responses to hypoglycaemia induced stress increase significantly after six months of treatment with either sulfasalazine or methotrexate, to levels comparable with the healthy controls. We hypothesise that this increase in prolactin response after six months was related to the
considerable decrease in disease activity observed at that time point. The significant negative correlation between disease activity and prolactin levels that we found at baseline and the positive correlation between the improvement in the DAS and the increase in prolactin levels after six months of treatment supports this view. Gudbjörnsson et al.14 in a study in 18 patients with active untreated rheumatoid arthritis, found a decreased prolactin response to the MRH (multiple releasing hormone) test, but a normalisation of the prolactin response after treatment with corticosteroids, which supports an impaired prolactin response due to inflammatory stimuli, consistent with our hypothesis. In a recent study by Pool et al.15 basal prolactin levels in patients with rheumatoid arthritis and systemic lupus erythematosus did not differ from healthy volunteers, but in response to exercise induced stress both groups showed only small and insignificant increases in prolactin concentrations, in contrast to healthy volunteers who showed a large increase, comparable with our findings in response to ITT induced stress.

The exact mechanism by which the prolactin response to stress is decreased in active rheumatoid arthritis remains unclear. One could speculate that raised concentrations of proinflammatory cytokines such as interleukin (IL) 6 and IL1β, which are found during active disease in rheumatoid arthritis, may have an inhibitory effect on prolactin secretion. This is supported by a study in rats which showed that IL1β was able to inhibit prolactin release.16 However, inhibitory as well as stimulatory effects of cytokines on prolactin release have been described, and future research will be needed to clarify this issue.

Another possible explanation for the discrepancies in published reports is the use of different prolactin stimulation tests. Chikanza et al studied prolactin responses after major surgery, a non-standardised stimulus for prolactin release. The increased prolactin responses they found could not be reproduced in a similar study.17 Other investigators have employed thyrotropin releasing hormone (TRH) tests.18 19 20 Tempel et al19 and Gutiérrez et al20 found normal prolactin responses to TRH, while Jorgensen et al found increased responses.21 ITTs have not been used before to assess prolactin responses to stress in patients with active rheumatoid arthritis. We conclude that prolactin responses to stress is decreased in patients with active rheumatoid arthritis. The disturbance may be caused by hypothalamic rather than pituitary defects in these subjects. This hypothesis should be studied in more detail in future investigations.

To our knowledge, this is the first longitudinal study of prolactin concentrations in patients with newly diagnosed rheumatoid arthritis. We conclude that prolactin responses to hypoglycaemia are decreased in patients with active rheumatoid arthritis. The disturbance may be a result of active disease, because prolactin responses increase towards normal values when disease activity was reduced after six months of antirheumatic treatment.

Our data oppose the hypothesis that increased prolactin levels play a role in the pathogenesis of rheumatoid arthritis. Instead, we found decreased prolactin responses to stress in active rheumatoid arthritis, which may be considered a part of the normal anti-inflammatory defence mechanism.

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REFERENCES


Committee on Publication Ethics Seminar 2005
Friday 11 March 2005, 9.30 am – 5 pm, BMA House, London

This year’s seminar will focus on COPE’s new Code of Conduct for Editors and interactive workshops on common ethical and editorial dilemmas. The seminar is for editors, authors, and all those interested in increasing the standard of publication ethics.

The Code aims to set a new basic standard for the ethical conduct of editors and sets out guidelines for quality and correcting the record, standing by decisions made, ethics committee approval, consent for publication confidentiality of submitted material, guidance to authors, pursuing misconduct, relationship to publishers, owners, and advertisers, and conflict of interest. The code also creates a mechanism to refer a complaint to COPE if an editor has breached the code.

The seminar will include:
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- Dr Iona Heath, Chair BMJ Ethics Committee—research, audit, and ethics committee approval
- COPE’s new website—full text and keyword searching for COPE’s advice on specific issues, for example research misconduct, conflict of interest, and deception
- Interactive workshops—common ethical and editorial dilemmas for editors
- Opportunities to network with other editors and share your experiences and challenges

The seminar is free for COPE members and £30.00 for non-members. Numbers are limited and early booking is advisable. For registrations or more information please contact Sam Knottenbelt at cope@bmjgroup.com or call 020 7383 6602. For more information on COPE see www.publicationethics.org.uk/