

Prolactin and Growth Hormone Responses to Hypoglycemia in Patients with Rheumatoid Arthritis and Ankylosing Spondylitis

JOZEF ROVENSKY, RICHARD IMRICH, FRANTISEK MALIS, MARTIN ZLNAY, LADISLAV MACHO, JURAJ KOSKA, and MILAN VIGAS

ABSTRACT. Objective. Prolactin (PRL) and growth hormone (GH) are pituitary hormones with immunomodulating properties. Their upregulated secretion may play a role in the pathogenesis of chronic inflammatory diseases. We evaluated PRL and GH responses to secretion stimulus in patients with rheumatoid arthritis (RA) and ankylosing spondylitis (AS).

Methods. Insulin hypoglycemia (0.1 IU/kg) was induced in 15 women with RA, 18 men with AS, and healthy controls matched for age, sex and body mass index. Plasma concentrations of glucose, PRL, GH, interleukin 6 (IL-6), and tumor necrosis factor alpha (TNF- α) were analyzed.

Results. RA patients had significantly lower area under the curve (AUC) of PRL ($p = 0.049$) compared to RA controls. During hypoglycemia double or higher increase of plasma PRL occurred in 5 RA (33%) patients and in 8 RA controls (57%). Using the General Linear Model procedure, no significant differences in PRL or GH responses were observed in patients with RA and AS. TNF- α was higher in patients with RA compared to RA controls ($p < 0.05$). There was no significant difference in TNF- α concentrations between AS patients and AS controls. IL-6 was higher in RA patients compared to controls ($p < 0.05$) and in AS patients compared to controls ($p < 0.01$). Significant positive correlation was found between TNF- α levels and AUC of PRL in AS patients ($r = 0.46$, $p = 0.047$), but not in the 2 control groups or in RA patients.

Conclusion. Our results indicate no upregulated PRL or GH responses to stimulation in premenopausal women with RA or men with AS. (J Rheumatol 2004;12:2418–21)

Key Indexing Terms:

PROLACTIN
RHEUMATOID ARTHRITIS

GROWTH HORMONE
ANKYLOSING SPONDYLITIS

Several lines of evidence suggest involvement of neuroendocrine perturbations in the pathogenesis of rheumatoid arthritis (RA) and, hypothetically, also in ankylosing spondylitis (AS)^{1–4}. Although the autocrine/paracrine action of prolactin (PRL) and growth hormone (GH) in the immune system has been recognized for several years^{5,6}, production of these hormones by the pituitary may also participate in modulation of immune response, and thus in the control over the inflammatory process in RA and AS.

Dysregulated secretion of PRL under basal conditions or during stress response has been suspected to contribute to deterioration of the clinical course of RA⁷. Reports of

hyperprolactinemia found in about 40% of RA patients⁸, a positive effect of PRL-lowering therapy⁹, and increased demand for glucocorticoids in hyperprolactinemic RA patients¹⁰ support this proposal. Investigations on dysregulated stimulated PRL secretion yield conflicting results, showing higher^{11,12}, normal¹³, and, paradoxically, lower¹⁴ responses to various stimuli. In contrast to RA, there is a paucity of data on the regulation of PRL and GH secretion in AS.

We evaluated PRL and GH responses in premenopausal women with RA and in men with AS, and analyzed the responses with regard to patients' inflammatory status.

MATERIALS AND METHODS

We studied 15 women with RA fulfilling the revised criteria of the American College of Rheumatology¹⁵, and 18 men with AS (European Spondylarthropathy Study Group criteria¹⁶). Fourteen female and 16 male healthy volunteers matched for age and body mass index served as RA and AS controls, respectively (Table 1). No patient or control had a history of diabetes or impaired glucose tolerance. The disease activity of patients was evaluated by clinical examination (number of affected and swollen joints, duration of morning stiffness) and laboratory measures (erythrocyte sedimentation rate, C-reactive protein). Patients' RA and AS disease activity was found to be low to moderate. No subject had been treated with anti-tumor necrosis factor- α (TNF- α) agents or glucocorticoids during the past 5 years. All subjects gave informed written consent and the study was

From the National Institute of Rheumatic Diseases, Piestany; and the Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia.

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J. Rovensky, MD, DSc, Professor, National Institute of Rheumatic Diseases; R. Imrich, MD, PhD, Institute of Experimental Endocrinology; F. Malis, MD, CSc; M. Zlnay, MD, National Institute of Rheumatic Diseases; L. Macho, MD, DSc; J. Koska, MD, PhD; M. Vigas, MD, DSc, Institute of Experimental Endocrinology.

Address reprint requests to Prof. J. Rovensky, National Institute of Rheumatic Diseases, Nabr. I. Krasku 4, 921 23 Piestany, Slovakia.
E-mail: rovensky@vurch.sk

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Table 1. Basic characteristics of patients and healthy controls. Data are mean \pm SEM.

	RA	RA Controls	AS	AS Controls
N	15	14	18	16
Age, yrs	41.2 \pm 1.5	44 \pm 2.8	37.9 \pm 1.8	34.4 \pm 2.4
Male/female	0/15	0/14	18/0	16/0
Body mass index, kg/m ²	21.6 \pm 1.1	23 \pm 1.1	23.7 \pm 0.7	22.8 \pm 0.6
Disease duration, yrs	8.2 \pm 2.4	—	7.2 \pm 1.9	—
Radiographic changes	2.3 \pm 0.8*	—	3.9 \pm 1.2**	—
ESR	20.3 \pm 12	—	25.3 \pm 4	—
C-reactive protein, ng/ml	15.4 \pm 13	—	—	—
Rheumatoid factor, IU	936 \pm 630	—	—	—
Patients taking NSAID	15	—	18	—
Patients taking methotrexate	10	—	3	—
Patients taking DMD	12	—	0	—

ESR: erythrocyte sedimentation rate, NSAID: nonsteroidal antiinflammatory drugs, DMD: disease modifying drugs. * Steinbrocker criteria; ** sacroiliitis stage.

approved by the Ethical Committee of the National Institute of Rheumatic Diseases.

The investigations started at 8:00 A.M. after an overnight fast. An indwelling catheter was inserted into the cubital vein for blood sampling; basal samples were drawn 30 min after inserting the catheter. Intravenous injection of insulin (0.1 IU/kg; Actrapid HM, Novo Nordisk A/S, Bagsvaerd, Denmark) was administered afterwards. At time intervals shown in Figure 1 blood samples were collected into polyethylene tubes containing EDTA. After centrifugation, plasma aliquots were stored at -20°C until analyzed. PRL and GH were assayed by immunoradiometric assay, IL-6 by radioimmuno assay, and TNF- α by ELISA kits (all by Immunotech SA, Paris, France). Intraassay variation coefficients were 2.6% for PRL, 1.1% for GH, 6.1% for IL-6, and 8.6% for TNF- α . Interassay variation coefficients were 6.8% for PRL, 13.6% for GH, 11.6% for IL-6, and 12.4% for TNF- α . Plasma glucose was analyzed by the glucose-oxidase method (Hitachi, Japan).

T-test for independent samples was used for between-group comparison of basal values. The General Linear Model procedure was used to determine the differences in responses during hypoglycemia between patients and controls (SPSS 11.01, SPSS Inc., Chicago, IL, USA). The frequency of PRL responders in patients with RA and AS compared to the respective control groups was assessed using the Fisher exact test (SigmaStat 2.0, Jandel Corp., San Rafael, CA, USA).

All data were expressed as the mean \pm SEM. The limit for statistical significance was set at $p < 0.05$.

RESULTS

Glucose concentration in patients immediately before insulin administration was not significantly different from that in controls. After insulin administration all subjects had sufficient decrease ($> 50\%$ of basal values at 30 min) of plasma glucose. Insulin administration resulted in comparable decrease of plasma glucose concentrations in RA patients and RA controls (Figure 1). The general linear model test revealed significant difference in the course of the changes of plasma glucose in patients with AS ($F = 3.3$, $p < 0.01$) compared to AS controls (Figure 2).

Resting PRL concentrations were within normal range in all subjects, with no significant difference between patients and controls. Insulin administration resulted in a significant ($p < 0.01$) rise in PRL concentrations after 45 min in AS

patients and in both control groups, but not in RA patients ($p = 0.069$). The general linear model test did not reveal a significant ($F = 1.05$, $p = 0.39$) interaction between the 2 factors (i.e., time \times disease) of the PRL response to hypoglycemia as a dependent variable in the RA patient and control groups. The area under the response curve (AUC) for PRL was lower in RA patients compared to RA controls ($p = 0.049$). The PRL response in AS patients was comparable to that in AS controls. During hypoglycemia, a double or higher increase of plasma PRL (the PRL responders) occurred in 5 RA patients, 8 RA controls, 9 AS patients, and 15 AS controls. The frequency of PRL responders was not significantly different in the RA and AS patients compared to the respective control groups.

Basal GH concentrations as well as hypoglycemia induced increases in plasma GH concentration were comparable between the patients and the controls. Insulin administration resulted in a significant ($p < 0.001$) rise in plasma GH concentration in all groups of patients and controls (Figures 1 and 2).

The concentration of TNF- α was higher in RA patients compared to RA controls (8.0 ± 2.8 pg/ml in RA vs 1.1 ± 0.5 pg/ml in controls; $p < 0.05$). There was no significant difference in the TNF- α levels between AS patients and AS controls. Positive correlation between TNF- α levels and the AUC for PRL was found in AS patients ($r = 0.46$, $p = 0.047$; Figure 3) but not in the control groups ($r = -0.065$, $p = 0.81$ in AS controls, $r = -0.13$, $p = 0.73$ in RA controls) or in patients with RA ($r = 0.11$, $p = 0.75$).

The concentration of IL-6 was higher in RA patients compared to RA controls (15.1 ± 6.7 pg/ml vs 1.4 ± 0.7 pg/ml; $p < 0.05$), and higher in AS patients compared to AS controls (20.9 ± 3.82 pg/ml vs 5.9 ± 1.72 pg/ml; $p < 0.01$).

DISCUSSION

Considering the enhancing effects of PRL and GH on inflammatory responses, their upregulated secretion from

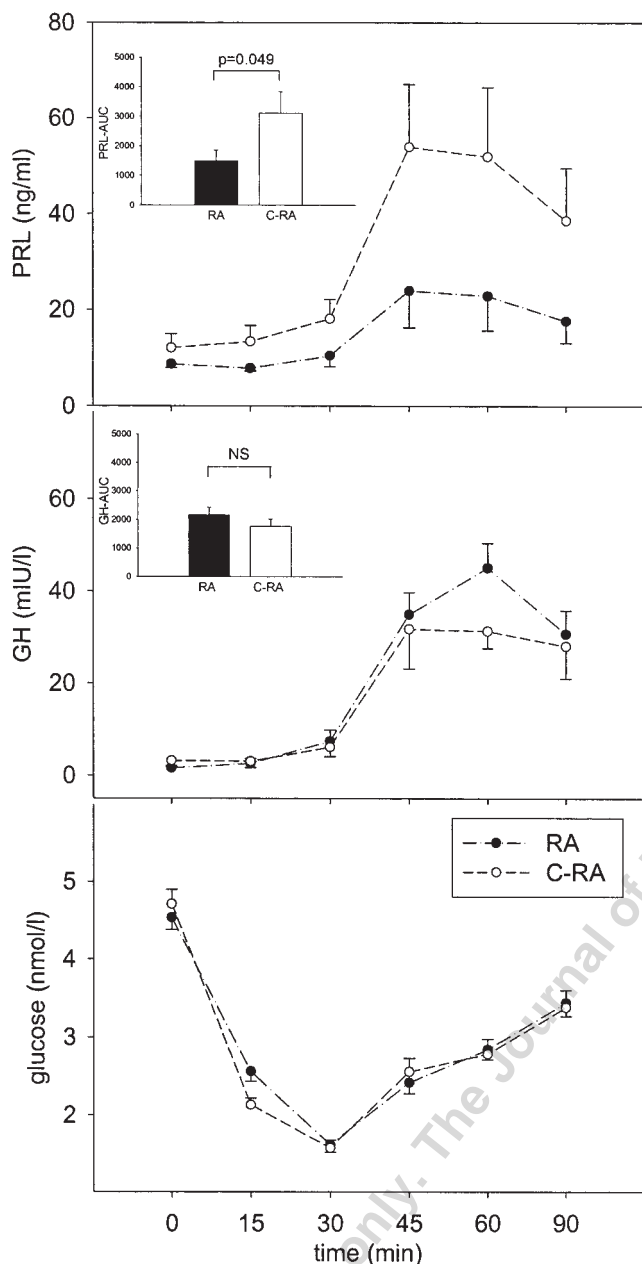


Figure 1. Concentrations of prolactin (PRL; top panel), growth hormone (GH; middle), and glucose (bottom) in plasma samples from 15 women with RA and 14 healthy female controls (C-RA) during insulin induced hypoglycemia. Data are means; error bars show SEM. Inset graphs show values of AUC for PRL and GH from 0 to 90 min in patients and controls.

the pituitary may be implicated in the development of chronic inflammatory diseases^{5,6}. Evidence supporting the hypothesis of upregulated secretion is, however, inconclusive in the case of PRL¹¹⁻¹⁴ and GH^{14,17}. Our results in premenopausal women with RA, together with our previous findings on notably lower PRL responses to hypoglycemia in different cohorts of older patients with RA¹⁴, do not indicate upregulated secretion of PRL in RA.

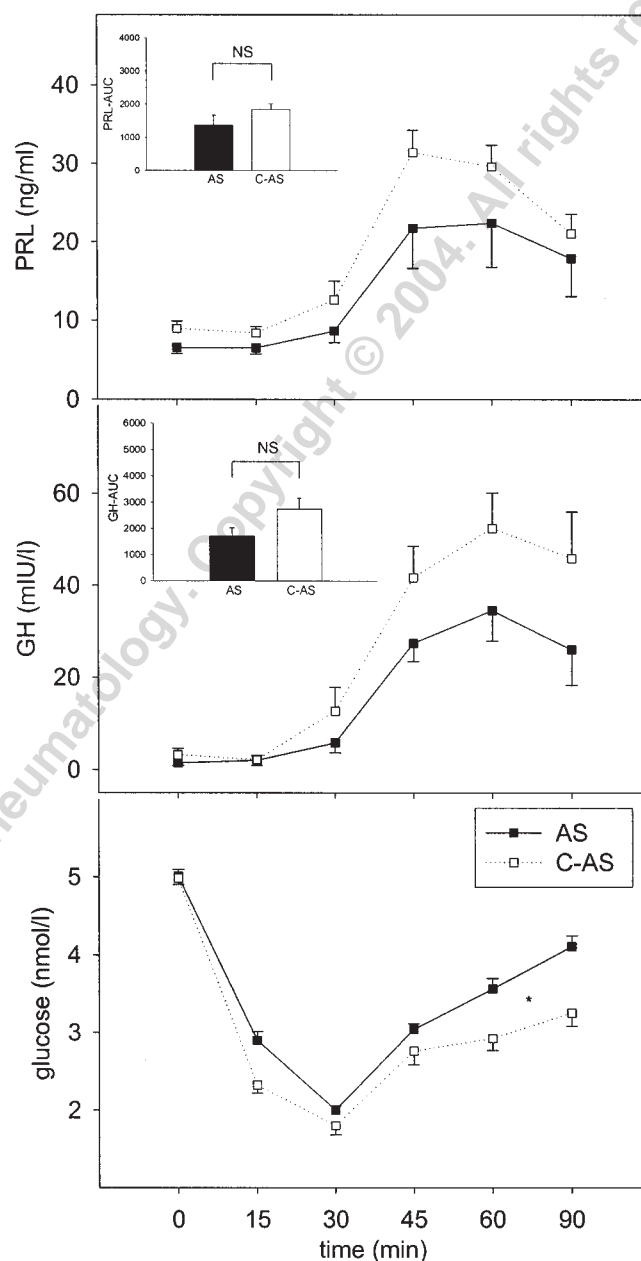


Figure 2. Concentrations of prolactin (PRL; top panel), growth hormone (GH; middle), and glucose (bottom) in plasma samples from 18 men with AS and 16 healthy male controls (C-AS) during insulin induced hypoglycemia. Data are means; error bars show SEM. Inset graphs show values of AUC for PRL and GH from 0 to 90 min in patients and controls. *Significant time \times disease interaction (general linear model, $F = 3.3$, $p < 0.01$).

A low AUC for PRL in RA patients compared to healthy controls might suggest decreased secretion capacity of pituitary PRL in RA patients. However, this result must be analyzed in context of the finding of a lower number of PRL responders in the RA patient group compared to the RA control group. Rather than changes in PRL secretion itself, this may be indicative of a higher threshold for PRL release to

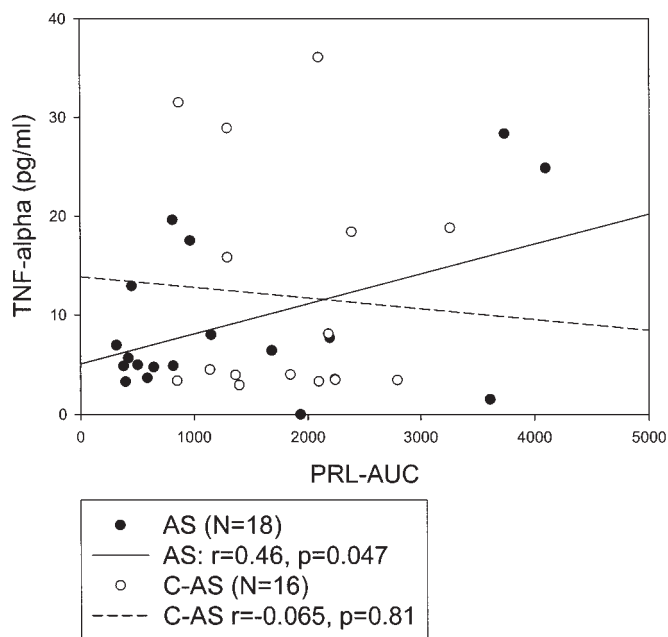


Figure 3. Scattergram with regression lines of correlations between AUC for prolactin (PRL) and TNF- α concentrations in 18 men with AS and 16 controls (C-AS).

hypoglycemia in RA patients, possibly due to altered regulation of PRL release. Utilizing a stimulus acting directly on the pituitary level, Gutierrez, *et al* observed normal responses of PRL to thyrotropin-releasing hormone stimulation in patients with early RA¹³. The results of that study¹³ support our assumption of a normal capacity of the pituitary for PRL secretion.

Difficulties in standardization of surgical stress as a stimulus for PRL secretion, relatively small numbers of patients, and stimulus-specificity might explain discrepancies reported in other studies^{11,12}. The 2 latter factors must be also taken into account when analyzing GH responses in patients with RA; they were comparable in our present study, but higher in a different group of 38 patients with RA using the same stimulus in our previous study¹⁴. In contrast, the response of GH to growth hormone-releasing hormone stimulation was found to be decreased in patients with newly diagnosed untreated RA¹⁷.

Our study demonstrates for the first time the stimulated responses of PRL and GH in patients AS, which were apparently normal. Since the neuroendocrine and immune systems are profoundly linked by multiple interactions, it seems rational to analyze the neuroendocrine responses within the context of immune activity, especially in chronic inflammatory diseases. Of note, the PRL response in patients with AS was positively correlated with TNF- α concentrations. Although this finding may be circumstantial, it may reflect the complexity of neuroendocrine-immune relations that deserves further investigation.

REFERENCES

1. Masi AT, Bijlsma JW, Chikanza IC, Pitzalis C, Cutolo M. Neuroendocrine, immunologic, and microvascular systems interactions in rheumatoid arthritis: physiopathogenetic and therapeutic perspectives. *Semin Arthritis Rheum* 1999;29:65-81.
2. Straub RH, Cutolo M. Involvement of the hypothalamic-pituitary-adrenal/gonadal axis and the peripheral nervous system in rheumatoid arthritis: viewpoint based on a systemic pathogenetic role. *Arthritis Rheum* 2001;44:493-507.
3. Straub RH, Struharova S, Scholmerich J, Harle P. No alterations of serum levels of adrenal and gonadal hormones in patients with ankylosing spondylitis. *Clin Exp Rheumatol* 2002;20:52-9.
4. Giltay EJ, van Schaardenburg D, Gooren LJ, Popp-Snijders C, Dijkmans BA. Androgens and ankylosing spondylitis: a role in the pathogenesis? *Ann NY Acad Sci* 1999;876:340-64.
5. Ben-Jonathan M, Mershon JL, Allen DL, Steinmetz RW. Extrapituitary prolactin: distribution, regulation, functions, and clinical aspects. *Endocrine Rev* 1996;17:639-68.
6. Matera L, Mori M, Geuna M, Buttiglieri S, Palestro G. Prolactin in autoimmunity and antitumor defence. *J Neuroimmunol* 2000;109:47-55.
7. Walker SE, Jacobson JD. Roles of prolactin and gonadotropin-releasing hormone in rheumatic diseases. *Rheum Dis Clin North Am* 2000;26:713-36.
8. Mateo L, Nolla JM, Bonnin MR, Navarro MA, Roig-Escofet D. Higher serum prolactin levels in men with rheumatoid arthritis. *J Rheumatol* 1998;25:2077-82.
9. Figueroa F, Carrion F, Martinez ME, Rivero S, Mamani I, Gonzales G. Effects of bromocriptine in patients with active rheumatoid arthritis. *Rev Med Chil* 1998;126:33-41.
10. Rovinsky J, Bakosova J, Payer J, Lukac J, Raffayova H, Vigas M. Increased demand for steroid therapy in hyperprolactinemic patients with rheumatoid arthritis. *Int J Tissue React* 2001;23:145-9.
11. Chikanza IC, Petrou P, Chrousos G, Kingsley G, Panayi GS. Excessive and dysregulated secretion of prolactin in rheumatoid arthritis: immunopathogenetic and therapeutic implications. *Br J Rheumatol* 1993;32:445-8.
12. Jorgensen C, Bressot N, Bologna C, Sany J. Dysregulation of the hypothalamo-pituitary axis in rheumatoid arthritis. *J Rheumatol* 1995;22:1829-33.
13. Gutierrez MA, Garcia ME, Rodriguez JA, Mardonez G, Jacobelli S, Rivero S. Hypothalamic-pituitary-adrenal axis function in patients with active rheumatoid arthritis: a controlled study using insulin hypoglycemia stress test and prolactin stimulation. *J Rheumatol* 1999;26:277-81.
14. Rovinsky J, Bakosova J, Koska J, Ksinantova L, Jezova D, Vigas M. Somatotrophic, lactotropic and adrenocortical responses to insulin-induced hypoglycemia in patients with rheumatoid arthritis. *Ann NY Acad Sci* 2002;966:263-70.
15. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31:315-24.
16. Dougados M, van der Linden S, Juhlin R, et al. The European Spondylarthropathy Study Group preliminary criteria for the classification of spondylarthropathy. *Arthritis Rheum* 1991;34:1218-27.
17. Templ E, Koeller M, Riedl M, Wagner O, Graninger W, Luger A. Anterior pituitary function in patients with newly diagnosed rheumatoid arthritis. *Br J Rheumatol* 1996;35:350-6.