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Circadian Periodicity of Serum Prolactin Concentration in Man

J. NOKIN, M. VEKEMANS, M. L'HERMITE, C. ROBYN

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Summary

Immunoreactive human serum prolactin of pituitary origin has been measured by a radioimmunoassay developed for ovine prolactin. Blood samples were collected at four-hour intervals during a 24-hour period from 12 non-pregnant women, three pregnant women, and seven adult men. A circadian periodicity was found in serum prolactin concentration, with peak values during the night, between 1 a.m. and 5 a.m. for the non-pregnant women, and at 5 a.m. for the adult men. Mean serum levels of prolactin were 1.5 times higher in non-pregnant women than in men. In women investigated during the last month of their pregnancy the mean serum prolactin levels were 2.3 times higher than in the non-pregnant women, but there was no circadian periodicity.

Introduction

A circadian periodicity in serum prolactin concentration has been found in rats (Clark and Baker, 1964; Dunn *et al.*, 1972). The purpose of the present study was to investigate whether a similar rhythm exists in man.

Subjects and Methods

Blood samples were collected at four-hour intervals during 24 hours from 22 normal adult volunteers—12 non-pregnant women aged 19-44 years, 7 men aged 23-64 years, and 3 pregnant women aged 27-33 years who were investigated during the last month of their pregnancy. Among the non-pregnant women two were in the follicular phase and 10 were in the luteal phase of their menstrual cycle.

Human prolactin was measured in serum using a radioimmunoassay method initially developed for ovine prolactin (Davis *et al.*, 1971) and extended to human prolactin (L'Hermite *et al.*, 1972a; Midgley *et al.*, 1972). Highly purified ovine prolactin (LER-860-2) was labelled with ¹²⁵I (Radiochemical Centre, Amersham, England) at a dose of 1 mCi/2.5 µg, using

the chloramine-T method (Greenwood *et al.*, 1963). The labelled prolactin was separated from free iodide by chromatography on Sephadex G-100 (Pharmacia, Uppsala, Sweden) equilibrated with 0.05 M phosphate buffer at pH 7.0. Thereafter the fractions of the ¹²⁵I-prolactin peak were diluted with phosphate buffer in saline containing 1% egg white (Sigma Chemical Co., St. Louis, Mo.) in order to obtain a working solution containing 50,000 c.p.m./100 µl. The assays were conducted according to the double antibody method using 200 µl of sample or standard with 200 µl of anti-ovine prolactin serum 770 at a dilution of 1:72,000 in normal rabbit serum diluted at 1:600 in phosphate-buffered saline (pH 7.0) containing 0.05 M ethylenediaminetetra-acetic acid (EDTA), 300 µl of phosphate buffer in saline containing 1% egg white, 100 µl of radioactive tracer solution, and 200 µl of sheep anti-rabbit immunoglobulins serum at a dilution of 1:100 in phosphate-buffered saline containing 0.05 M EDTA.

Non-equilibrium conditions were employed as described previously (L'Hermite and Midgley, 1971). No cross-reaction was detected in this system with human growth hormone and with human chorionic somatomammotrophin (L'Hermite *et al.*, 1972b; Midgley *et al.*, 1972). All assays were conducted with reference to pooled sera collected from pregnant women. We conferred an immunological activity of 1.0 mU on the amount of prolactin contained in 1.0 µl of this pool used as laboratory standard. All samples from the same subject were run in triplicate in the same assay. The assay results were calculated according to the recommendations of Rodbard *et al.* (1968).

Results

The mean serum prolactin levels observed over the 24-hour period are shown in the Chart for men and non-pregnant women, and in the Table for pregnant women. The data were submitted to a variance analysis (Snedecor, 1956) of the between-subjects variation and the within-subject variation due to the influence of time. There was a highly significant variation between the subjects in serum levels of prolactin—non-pregnant women $F = 8.27$, $P < 0.001$; adult men $F = 3.81$, $P < 0.001$; pregnant women $F = 8.09$, $P < 0.01$. In addition in the non-pregnant women the levels of prolactin were higher ($F = 68.99$; $P < 0.001$) at 1 a.m. (mean 500 mU/ml) and 5 a.m. (504 mU/ml) than at 9 a.m. (332 and 312 mU/ml), 1 p.m. (273 mU/ml), 5 p.m. (299 mU/ml), and 9 p.m. (331 mU/ml). There was no significant difference in serum levels of prolactin found at 9 a.m. between the first and the second day ($F = 0.24$; $P > 0.05$). The prolactin levels started to increase at 9 p.m., but this rise was not significant ($F = 1.76$; $P > 0.05$) compared with the values seen at 1 p.m. and 5 p.m.

In the men there was also a peak in serum prolactin levels

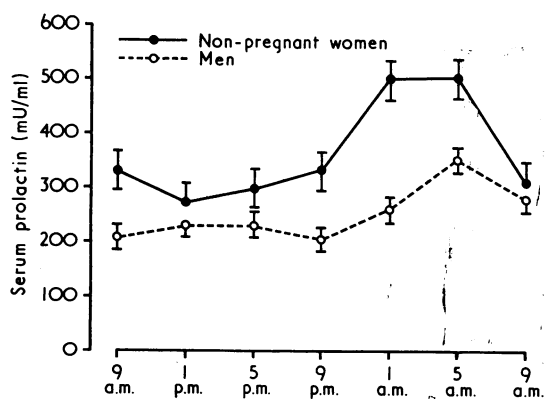
Human Reproduction Research Unit, Hôpital Saint Pierre, University of Brussels, Belgium

J. NOKIN, M.D., Postdoctoral Fellow

M. VEKEMANS, M.D., Resident

M. L'HERMITE, M.D., Resident

C. ROBYN, M.D., Research Worker, Fonds National de la Recherche Scientifique



Mean immunoreactive serum prolactin levels in blood samples collected at 4-hour intervals during a 24-hour period from 12 non-pregnant women and 7 men. The common standard errors of the means calculated by variance analysis are shown.

Immunoreactive Human Serum Prolactin (in mU/ml) assayed in Blood Samples collected at various Times during 24-hour Period from Three Women during the Last Month of Pregnancy

Case No.	9 a.m.	1 p.m.	5 p.m.	9 p.m.	1 a.m.	5 a.m.	9 a.m.
1	911	824	947	725	787	708	774
2	817	863	743	739	765	687	825
3	953	773	1,261	1,189	953	937	1,031
Mean*	894	820	984	884	835	777	877

* Common standard deviation calculated by variance analysis = 119 mU/ml.

during the night but it was of less amplitude. The mean levels at 1 a.m. (270 mU/ml) and 5 a.m. (354 mU/ml) were higher ($F = 17.03$; $P < 0.001$) than those at 9 a.m. (209 and 280 mU/ml), 1 p.m. (222 mU/ml), 5 p.m. (222 mU/ml), and 9 p.m. (205 mU/ml). The mean prolactin level at 9 a.m. was significantly higher the second day than the first day ($F = 4.74$; $P < 0.05$); also the value at 5 a.m. was higher than that at 1 a.m. ($F = 8.37$; $P < 0.01$). Mean serum prolactin levels during the period of observation were higher ($F = 19.09$; $P < 0.001$) in non-pregnant women (364 mU/ml) than in men (250 mU/ml). Mean levels were also slightly higher during the luteal phase (372 mU/ml) than during the follicular phase (323 mU/ml), but this difference was not statistically significant ($F = 2.06$; $P > 0.05$).

Finally, as indicated in the Table, no circadian periodicity could be found in the three pregnant women investigated during the last month of their pregnancy.

The values at 1 a.m. (835 mU/ml) and 5 a.m. (777 mU/ml) were slightly lower than those at 9 a.m. (894 and 877 mU/ml), 5 p.m. (984 mU/ml), and 9 p.m. (884 mU/ml). This difference, however, was not statistically significant ($F = 3.01$; $P > 0.05$). The mean serum prolactin levels during the period of observation were higher ($F = 96.8$; $P < 0.001$) in pregnant women (867 mU/ml) than in non-pregnant women (364 mU/ml).

Discussion

Immunoreactive serum prolactin, as measured by the radioimmunoassay method used here, has been found to be raised in lactating women where, in addition, peak values have occurred in response to breast-feeding (L'Hermite *et al.*, 1972c), in cases of various types of galactorrhoea, and during treatment with psychotropic drugs such as chlorpromazine and sulphiride (L'Hermite *et al.*, 1972a). From the data obtained recently with the same assay method serum levels of prolactin increase also during pregnancy (L'Hermite *et al.*, 1972c), after intravenous injection of thyrotrophin releasing hormone (L'Hermite *et al.*, 1972b), and in response to insulin-induced hypoglycaemia (L'Hermite *et al.*, 1972a, 1972d). Peak values of serum prolactin were found during the night between 1 a.m. and 5 a.m.

both in men and in non-pregnant women. A release of prolactin from the pituitary, occurring with a circadian periodicity, was most likely responsible for this rhythmic fluctuation of serum levels. The mechanism of control could be either a transitory suppression of the prolactin inhibiting factor (Sulman, 1970a) or an increased secretion of a prolactin releasing hormone (Sulman, 1970b).

Synthetic thyrotrophin releasing hormone—that is, (pyro)-GLU-HIS-PRO (NH₂)—induces not only a release of thyrotrophin but also a release of prolactin (Bowers *et al.*, 1971; Jacobs *et al.*, 1971; L'Hermite *et al.*, 1972b). Moreover, circadian periodicity exists also in serum levels of thyrotrophin—indeed, as for prolactin, peak values of serum thyrotrophin occurred during the night around 5 a.m. (Vanhaelst *et al.*, 1972). Further studies are required to elucidate whether the release of both thyrotrophin and prolactin, occurring with a circadian periodicity, would be associated in time and therefore possibly controlled by the same hypothalamic releasing hormone. Owing to the existence of a circadian periodicity in prolactin secretion variations of the serum levels of this hormone occurring in physiopathological and experimental conditions cannot be based entirely on single samples collected during the day. A more precise evaluation of prolactin secretion should be based on frequent blood sampling during the day and night or possibly on evaluation of the total output in 24-hour urine collections. The fact that lactotrophic activity has been found by biological methods in urinary extracts (von Berswordt *et al.*, 1967) favours this last solution.

The disappearance of the circadian periodicity in serum prolactin concentration during pregnancy has to be confirmed by increasing the number of cases studied. It would also be of interest to investigate at what age of pregnancy this periodicity is lost and when it appears again after delivery.

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Requests for reprints should be addressed to C. Robyn, Department of Gynaecology and Obstetrics, Hôpital Saint Pierre, 322, rue Haute, 1000 Brussels, Belgium.

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