Circadian Periodicity of Serum Prolactin Concentration in Man

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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 concentrations, 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 mg/g/25 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, Upsalla, 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./μl. The assays were conducted according to the double antibody method using 200 μl of sample or standard with 200 μl of antiovine 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 antirabbit 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 a pooled serum 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 to the non-pregnant women the levels of prolactin were higher (F = 68·99; P < 0·001) at 1 a.m. (mean 500 μU/ml) and 5 a.m. (504 μU/ml) than at 9 a.m. (352 and 312 μU/ml), 1 p.m. (273 μU/ml), 5 p.m. (299 μU/ml), and 9 p.m. (331 μU/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 women there was also a peak in serum prolactin levels
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 thyrotrhphin 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 thyrotrhphin—indeed, as for prolactin, peak values of serum thyrotrhphin occurred during the night around 5 a.m. (Vanhaelst et al., 1972). Further studies are required to elucidate whether the release of both thyrotrhphin 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 pathophysiological and experimental conditions cannot be based exclusively 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 lactotropic 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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References

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 galactorrhea, and during treatment with psychotropic drugs such as chlorpromazine and sulpiride (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, 1972b). Peak values of serum prolactin were found during the night between 1 a.m. and 5 a.m. 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).