SLEEP-DEPENDENT HYPERPROLACTINEMIA AND *CORPUS LUTEUM* PATHOGENESIS

HUGO E. SCAGLIA *, NÉSTOR DRÓN **, GRACIELA RAMOS *, MÓNICA BERMÚDEZ *, MARTA ARRASCAETA **, NANCY BRIASCO **

*Centro de Referencia de Radioinmunoensayo **,*
Centro de Especialistas en Análisis Biológicos, La Plata
*Department of Gynecology ***, National University of La Plata Medical School*

Prolactin (PRL) secretion has been reported to be significantly increased under certain physiological conditions, basically due to an increment in the amplitude of sleep-induced secretion pulses[8]. The same effect has been observed in tumor-induced or idiopathic hyperprolactinemia[3]. High serum PRL levels may not modify the reproductive function in women, but can produce a wide variety of dysfunctions, including the amenorrhea-galactorrhea syndrome[12].

These alterations can be prevented by the administration of dopamine (DA) agonists. Since DA-agonist therapy has also been reported to be successful in some normoprolactinemic infertile women having an altered menstrual cycle[13], the aim of the present study was to investigate a hypothetical pathological PRL secretion pattern during sleep in this type of patients.

MATERIALS AND METHODS

Six women between 23 and 35 years of age with a diagnosis of sterility due to a luteal phase deficiency (LPD) were studied. The luteal phase deficiency was determined by at least two of the following parameters: *a.* endometrial biopsy, *b.* basal temperature and *c.* plasma progesterone serial determinations on appropriate days during the second half of the menstrual cycle.

Prolactin was assayed every 30 min for 12h (from 19^{00} to 07^{00}) in plasma samples obtained during the early follicular phase using an intravenous cath-

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eter. In order to ensure a good adaptation of the patient, the catheter was inserted 3h prior to the first extraction. The catheter permeability between extractions was maintained by a physiological solution.

Five female volunteers with normal menstrual cycle and proved fertility were used as control group, employing the same extraction procedure and schedule as in the experimental group. In both groups sleep was physiological and none of the patients awoke during the sample extraction. All the women were adequately informed on the purpose of the study and all gave their consent.

PRL was measured by an already described[9] double-antibody radioimmunoassay (RIA). Iodination was performed using hypochloride as oxidant[10]. Human PRL (75-504 preparation), used as a standard, was donated by MRC (Great Britain). Purified hypophyseal human PRL used for iodination and the antibody (hPRL rabbit antiserum) were generously donated by NIAMDD-NIH (USA). The sheep anti-rabbit γ-globulin, used as a second antibody, was prepared in our laboratory. The assay sensitivity was 1.7 ng/ml and the intra- and inter-assay variation coefficients were 6.8 and 9.7%, respectively.

In order to avoid the inter-assay error, all samples from the same patient were evaluated in the same assay.

For statistical analysis, the following parameters were evaluated: *1.* mean value and standard error of the mean (SEM) of PRL levels in wake conditions; *2.* mean value and SEM of PRL plasma levels in sleep conditions; *3.* all these values were calculated by the Student's *t* test and $p < 0.05$ was considered as a statistically significant difference; *4.* the highest amplitude pulse was considered as the difference between the mean values in sleep and wake states.

RESULTS

Figure 1 shows the PRL values obtained during wake and sleep in the control group. In all the patients there was a significant increase in PRL values during sleep. The sleep period lasted for between 04^{30} and 06^{00}. In relation to

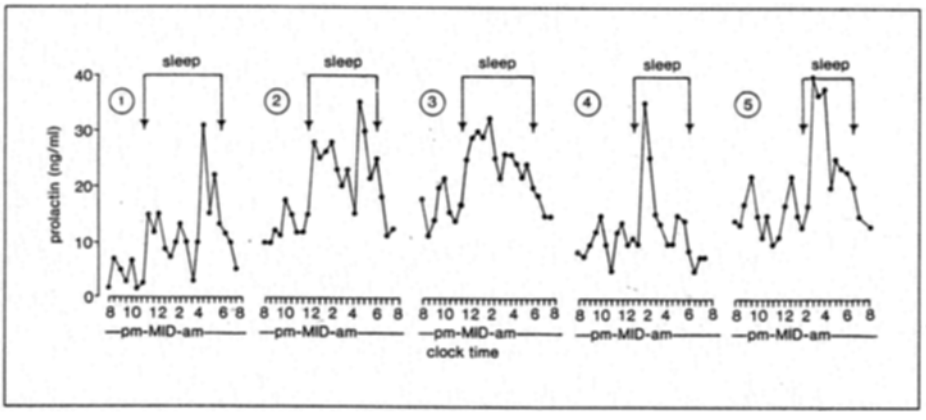

Fig. 1 - Twelve-h profile of serum PRL levels obtained in 5 normal women. Arrows indicate the total sleep periods.

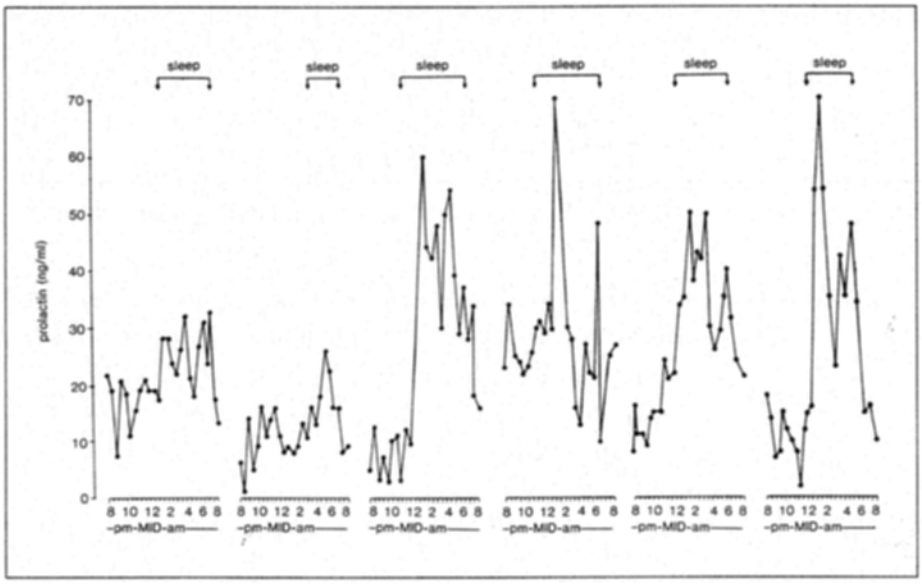

Fig. 2 - Twelve-h profile of serum PRL levels obtained in 6 women with luteal phase deficiency. Arrows indicate the total sleep periods.

the last value obtained during sleep, plasma PRL levels dropped 77, 56, 32, 67 and 46% within the 2h immediately following awaking.

In fig. 2 the profile of PRL levels of patients with luteal phase deficiency shows an increment during sleep in all the cases studied. The sleep period lasted for 4-5h. After awaking, plasma PRL levels dropped 60, 50, 57, 44, 48 and 79%, respectively, for each patient studied, in comparison with the last value obtained during the sleep period.

Figure 3 shows mean PRL values in wake and sleep states in both control and experimental groups. Under wake conditions there were no significant differences in serum PRL values of patients in the experimental group when compared with those of the control group ($p > 0.05$), with the exception of patient 4, who showed a significant difference ($p < 0.01$), although also this value was within the normal range. During sleep a significant difference was observed in patients 3, 5 and 6 of the LPD group; patients 1 and 2 showed no statistical differences ($p > 0.05$), while patient 4 showed a higher mean value, but no statistically significant differences ($p < 0.05$) in comparison with values of the control group.

Considering the highest PRL pulse in the wake state, patients 1, 2, 3 and 6 of the LPD group showed values within the same range as the mean value of the highest pulse of the control group. Patients 4 (34 ng/ml) and 5 (26 ng/ml) showed 1.9 and 1.4 times more elevated PRL pulses, respectively, when compared with the control group (fig. 4). During sleep the highest PRL pulses in patients 1 and 2 were similar to those of the control group. The values of patients 3, 4, 5 and 6 were statistically significant; these values were clearly pathological.

DISCUSSION

The results demonstrate that in 4 out of 6 patients with LPD showing normal PRL levels in the wake state there is a sleep-dependent hyperprolactinemia which is essentially due to a significantly wider range in the secretion pulses. BOARD et al.[2] found that 7 patients with short luteal phase and normal PRL levels had an increased nocturnal PRL secretion. Polytomography of the sellar region revealed the existence of microadenomas in 4 cases. The short luteal phase has been associated with hyperprolactinemia[4,6,7] and treatment with bromocryptine was followed by pregnancy in a significant number of cases. ANDERSEN et al.[1] observed pregnancy in 5 out of 10 women with LPD and normal diurnal PRL levels. Our results and those of BOARD et al.[2] strongly suggest that the short luteal phase in these women may be associated with nocturnal hyperprolactinemia. LPD is, by definition, the *corpus luteum* which is defective in progesterone production; this defect is caused not by a single etiological factor, but by multiple etiology[11]. The development of the *corpus luteum* implies the continued development of the follicle; consequently, any factor affecting follicular growth and development also affects the *corpus luteum* function.

From the clinical point of view, LPD has been connected with: *1.* a primary ovarian defect; *2.* a defect in the hypothalamus-pituitary axis that involves an alteration in the tonic secretion of FSH-LH or LH mid-cycle peak. It has been recently suggested that hyperprolactinemia is associated with LPD either by a direct action on the ovary or, indirectly, through the alteration of gonadotropin secretion; *3.* a metabolic defect produced by inadequate oxygenation of the blood; *4.* a specific defect of luteal steroidogenesis produced by certain substances that might be involved in the luteolysis.

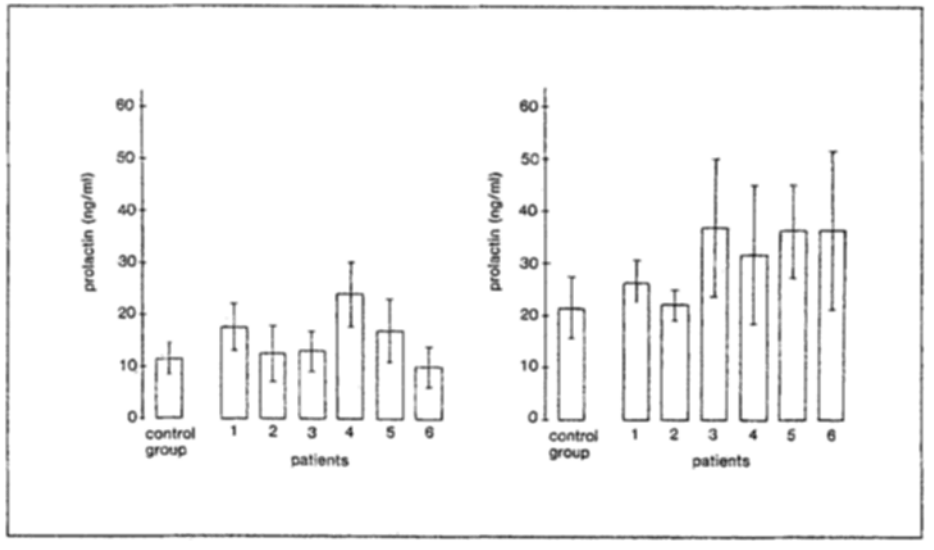

Fig. 3 - Serum PRL levels (means ± SD) in wake (left) and sleep (right) conditions in the control group and in patients with luteal phase deficiency.

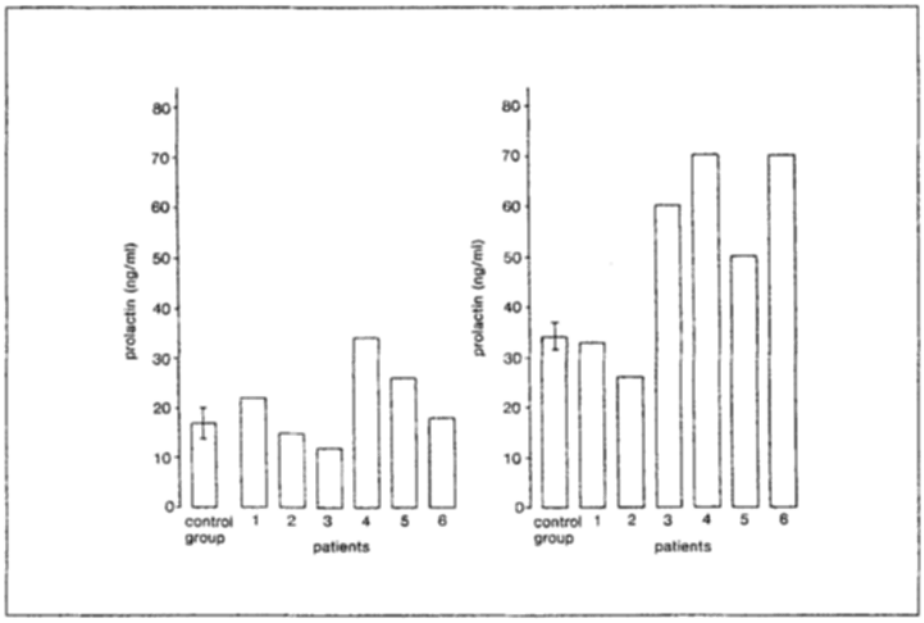

Fig. 4 - Serum highest PRL pulses obtained in wake (left) and sleep (right) conditions in the control group (mean ± SD) and in patients with luteal phase deficiency.

Our results agree with the concept of multiple etiology in that a nocturnal sleep-dependent hyperprolactinemia could be an etiological cause of this pathology; in 2 out of 4 patients studied, pregnancy followed only the treatment with a dopaminergic agonist.

Under normal conditions the PRL circadian secretion pattern is bimodal, with an increase at the end of the afternoon and a greater increment during sleep, reaching its maximum level in the middle of the sleep period and remaining high up to wake period, when it rapidly decreases[5].

The results profile for the normal control group as well as for the pathological group is similar to that described by other authors. For this reason we consider that although the sleep state was subjectively reported (it was not possible to monitor it by encephalography), this similarity between our and previously reported results fails to invalidate our findings.

Our results stress the need of a more exhaustive study on the circulating PRL levels, since in all cases the means as well as the pulses during the wake state study were normal. Samples taken in the wake state in our experimental group agree with the normal increment in the afternoon hours. In all the cases values did not statistically differ from the normal. Our results demonstrate that the pathological PRL secretion in our patients takes place during sleep and is characterized by pulses of greater amplitude.

Sleep has REM and non-REM periods in which different neurotransmitters are produced. VAN CAUTER et al.[14] demonstrated by spike analysis that the relationship between episodic fluctuations of PRL plasma levels and state of sleep is purely coincidental. These results lend validity to our experimental

design, since our interest lies in determining PRL concentrations during sleep rather than in evaluating it in the different sleep periods. It is likely that sleep-dependent hyperprolactinemia may be conditioned by an alteration in the secretion of specific neurotransmitters affecting the PRL production.

From the practical point of view this type of investigation is difficult to carry out. However, if our preliminary results are confirmed and sleep-dependent hyperprolactinemia is accepted as a new pathological entity involved in the etiology of the short luteal phase, experiments should be designed in order to obtain pertinent informations, such as on PRL concentrations in diurnal and nocturnal urine samples.

SUMMARY

The results obtained in wake and sleep conditions by PRL determinations performed in 6 normoprolactinemic infertile women with luteal phase deficiency (LPD) are reported. Infertility was apparently due to LPD. PRL levels were determined by RIA in blood samples collected at 20-min intervals from 18^{00} to 08^{00}. LPD has been previously demonstrated by endometrial biopsy, basal temperature and circulating progesterone determinations. PRL levels were also determined in 5 normal women used as control subjects under the same experimental conditions. The results obtained, expressed as means ± SD of LPD *vs.* control group, were 15.9 ± 4.6 *vs.* 11.6 ± 3.3 ng/ml ($p > 0.1$) in wake conditions and 31.9 ± 5.9 *vs.* 21.4 ± 5.7 ng/ml ($p < 0.01$) in sleep conditions. PRL values during the highest pulse (HP) in sleep and wake conditions were 20.9 ± 5.2 *vs.* 17.0 ± 3.3 ng/ml ($p < 0.1$) and 51.1 ± 17.1 *vs.* 34.3 ± 2.4 ng/ml ($p < 0.01$), respectively. In 2 out of the 6 patients mean PRL values were 22.0 and 26.5 ng/ml during sleep, and 26.0 and 33.0 ng/ml during HP. These values were not statistically significant when compared with those obtained in the control group. The results obtained show that 4 out of the 6 patients with LPD and normal PRL levels in wake conditions had sleep-dependent hyperprolactinemia due to the pulses with a more significant amplitude. These findings suggest that in some cases sleep-induced hyperprolactinemia might be involved in LPD pathogenesis.

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Requests for reprints should be addressed to:

HUGO E. SCAGLIA
Centro de Referencia de Radioinmunoensayo
Calle 47 n. 1015
1900 La Plata - Argentina