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Effects of Hyperprolactinemia on Prolactin and LH Pulsatile Pattern in Female Rats

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Prolactin (PRL) and luteinizing hormone (LH) secretions are very closely-related. To further understand these mechanisms, the pulsatile secretion pattern of both hormones in experimentally-induced hyperprolactinemia has been studied in adult female rats. Hyperprolactinemia was induced by the transplanting of two pituitary glands. Nine days after the transplant operation, rats were bled (75 or 100 µl/7 min for 3 h). Serum samples were analyzed for prolactin and LH values by RIA. Hyperprolactinemia modifies pulsatile PRL secretion by increasing the absolute amplitude and duration of the peaks together with a decrease in their frequency. Also, the mean values of the hormone during the whole studied period were increased. Hyperprolactinemia was followed by an increase in the mean values of LH and in the absolute amplitude of the peaks. All these results suggest that hyperprolactinemia induced by pituitary grafting in adult female rats, is followed by a significant change in prolactin and LH pulsatility, which may explain, to some extent, the effects of hyperprolactinemia on reproduction.

Key words: Pulsatile secretion, Prolactin, Luteinizing hormone, Hyperprolactinemia.

Hyperprolactinemia (HyperPRL) is one of the most common clinical disorders of hypothalamic-pituitary axis origin (9, 35). HyperPRL is frequently associated with an impaired reproductive function (3, 7, 21, 32). Pituitary grafting modifies luteinizing hormone (LH) (4, 8, 14, 25, 36) and the endogenous prolactin (PRL) secretion (16, 24, 27). HyperPRL may influence pituitary responsiveness to hypothalamic regulatory factors which reg-

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ulate PRL (2, 11) and LH (15, 28) secretion patterns, suggesting that both mechanisms are closely related. In fact, both hormones are secreted in an episodic fashion (10, 18), so that these patterns could be modified by hyperprolactinemia. This work was undertaken to evaluate if hyperprolactinemia is able to modify PRL and LH pulsatility secretion patterns. For that purpose adult female rats were rendered hyperprolactinemic by pituitary grafting, under the kidney capsule and 9 days after the transplant operation PRL and LH pulsatility was studied.

Materials and Methods

Animals. — Adult female Sprague-Dawley CD rats, weighing 220-280 g, were used in all experiments. Animals were maintained on a 14 h light, 10 h dark schedule (lights on from 06.00 to 20.00 h), controlled temperature (22 ± 2 °C) and feed rat chow and water was provided *ad libitum*. Vaginal smears were taken daily, and only rats demonstrating at least two consecutive 4 day estrous cycles were used. On the days of the operations and the experiment vaginal smears were also recorded to properly assess the actual stage of the estrous cycle during the experimental manipulations.

Induction of hyperprolactinemia and cannula implantation. — Rats in estrus phase of the cycle were anesthetized with 2.5 % tribromoethanol (1 ml/100 g BW). Two pituitary glands of a matter donor were transplanted under the right kidney capsule, according to the method of MENA *et al.* (22). Rats were sham transplanted in the control or normoprolactinemic group. Eight days later rats were cannulated under tribromoethanol anesthesia forty hours before the experiment, between 18.00-19.30 h, and two polyethylene cannulae (PE-50) were inserted into or near the right atrium via the external jugular vein in each rat. The tip of the first cannula was inserted 10 mm beyond the tip of the second and was used for infusion of saline with a Sage syringe pump. The second cannula was used for continuous blood sampling. This arrangement allows animals to be continuously bled and infused at the same time without saline withdrawal.

Experimental Protocols. — On the day of the experiment, conscious and free moving rats were continuously infused either with 0.9 % saline (0.5 ml/h) for 4 h, beginning at 09.30 h. One hour after the intravenous infusion started, rats were bled continuously through a peristaltic pump set at a flow rate of 75 or 100 µl/7 min. All rats were given 350 U heparin 20 min prior to the onset of bleeding. Blood samples were collected in Hamilton microliter syringes every 7 min for 3 h from 10.30 to 13.30 h. The samples were added to assay tubes kept on ice and containing phosphate-buffered saline plus 0.1 % gelatin. Hematocrits remain stable with this bleeding protocol. At the end of this 3 hour period, the samples were centrifuged, and the serum was frozen for subsequent measurement of PRL and LH.

Two groups of rats were studied: group 1: sham-operated rats, infused with saline; group 2: pituitary-transplanted rats infused with saline.

Radioimmunoassay. — Serum samples were analyzed for PRL by the double antibody radioimmunoassay using reagents supplied by the NIADDK. PRL values are expressed in terms of the rat NIADDK PRL RP-3 Standard. The minimum sensitivity of the assay was (5 pg/tube). Samples were evaluated within the same assay to avoid interassay variation. To ascertain the variability of the assay, a plasma series of 10 replicates corresponding to approximately 5 different concentrations of PRL in the standard curve, were used to obtain the intraassay coeffi-

cient of variation (CV) which was 8.1 %. The NIADDK rat LH kit (RP-3 standard) was used to determine serum LH concentrations. The intraassay coefficient of variation was 6.8 %, and the sensitivity of the LH assay was 3.3 pg/tube.

Data analysis. — To identify and characterize pulses in the hormonal profile of each rat, the Ultra-analysis computer program according to VAN CAUTER (34) was used. In this program, a pulse is defined as a significant increase exceeding a multiple of the dose-adjusted CV, followed by a significant decrease. The intraassay CVs were calculated from values of five different concentrations of PRL and LH levels in standard curves. Thus, the CV and the mean hormone level were determined for all hormone values which comprised the ascending and descending phases of each potential pulse the pulse was defined when this CV was twice that of the intraassay CV determined at a comparable mean PRL level, and 3 times that of the intraassay CV determined at a comparable mean LH level. To test the specificity of pulse detection, a series of 26 samples from a pool of plasma was analyzed using a threshold of 2 CV (or 3 CV for LH), 1 false positive being detected.

Pulsatile PRL and LH release was characterized by mean hormone level, absolute and relative pulse amplitudes, frequency, and pulse duration. The hormone level was calculated by the mean of all samples collected from each rat during 3 h period, and the average for the experimental group from the individual means. The absolute pulse amplitude was defined as the difference between the hormone level at the maximum of the peak and the hormone level at the preceding nadir. The relative pulse amplitude was calculated as the quotient between absolute pulse amplitude and preceding nadir value. Pulse frequency was the number of pulses/3 h. Pulse duration was the time between the beginning of the ascending phase of the peak and the end of the descending phase of the peak.

Comparison of values for the pulsatile parameters was done by using the Student's test analysis. The results were considered significant at $p < 0.05$. All values represent the mean ± SEM.

Results

Short-term hyperprolactinemia was followed by an increase in the peripheral values of PRL (as a mean of all samples obtained during the studied period) as compared to the control group (table I). The pulsatility pattern was characterized by an

Table I. *Quantitative parameters (mg/ml) of pulsatile PRL and LH secretion (serum PRL levels, absolute pulse amplitude and relative pulse amplitude) in pituitary-grafted and sham-operated rats.*
Values are expressed as mean ± SEM. Number of animals per group, 8. The relative pulse amplitude was calculated as the quotient between absolute pulse amplitude and preceding nadir value.

Group	PRL or LH	Abs. Amplitude	Rel. Amplitude
PRL			
Sham	1.23 ± 0.16	0.73 ± 0.08	1.16 ± 0.24
Graft	9.99 ± 1.74***	8.50 ± 1.53***	1.14 ± 0.11
LH			
Sham	0.07 ± 0.001	0.16 ± 0.03	5.99 ± 0.89
Graft	0.39 ± 0.05***	0.68 ± 0.17**	5.27 ± 1.27

** $p < 0.01$; *** $p < 0.001$ vs. sham-operated rats.

increased absolute amplitude (table I and fig. 1,a,b) and duration as compared to the control group (table II). A decrease in pulsatile frequency, after pituitary grafting, was also observed (table II and fig. 1,b). No differences were detected in pulsatile relative amplitude (table I). Hyperprolactinemia was associated with increased mean values of LH during the studied period (table I). The absolute amplitude of the peaks was increased in pituitary grafted animals (table I and fig. 1,d).

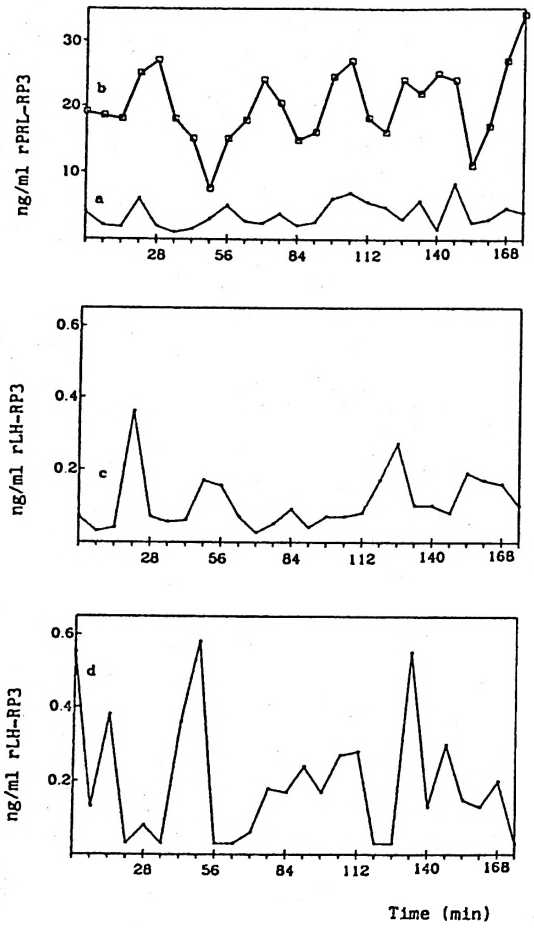

Fig. 1. *Representative profiles of pulsatile PRL and LH secretion of one animal from each experimental group in sham operated (a,c) and pituitary-grafted (b,d) rats.*

Table II. *Qualitative parameters of pulsatile PRL and LH secretion in pituitary-grafted and sham-operated rats.*

Values are expressed as mean ± SEM. Number of animals per group, 8.

Group	Frequency (n. Pulses/3 h)	Duration (min)
PRL		
Sham	8.8 ± 0.60	19.0 ± 1.38
Graft	4.1 ± 0.42***	32.6 ± 3.92**
LH		
Sham	3.1 ± 0.24	34.7 ± 2.58
Graft	3.0 ± 0.33	33.0 ± 4.22

** $p < 0.01$; *** $p < 0.001$ vs. sham-operated rats.

No other studied parameters of LH pulsatility were modified by pituitary grafting.

Discussion

The present study shows a detailed analysis of ectopic pituitary grafting effect on pulsatile prolactin and LH secretions in adult female rats. Hyperprolactinemia differentially affects the pulsatile secretion of both hormones, by modifying different parameters which characterize their episodic release.

Regarding the characterization of the PRL pulsatile pattern, the data confirm previous ones from our group (17) and from other laboratories (18). The increase in pulse absolute amplitude and duration supports the present high values of PRL in the periphery and explains the peak occurrence low frequency when compared to controls.

The detailed analysis of the PRL pulsatile pattern in control female rats shows episodic PRL pulses, which are modified in grafted animals. This effect does not seem to be related to the well known actions of PRL on the hypothalamus which increases dopamine turnover (6, 12, 30). Under this experimental condition, the hypothalamic pituitary axis should be inhibited from the existence of an increased

dopamine turnover. This increase in dopamine release to the portal vessels inhibits *in situ* lactotrophs (19) as it has been widely shown. Based on these data, it might be deduced that the high mean serum PRL levels reached come from the ectopic pituitary, due to the fact that the latter is not under hypothalamic control (23).

However, regulatory mechanisms on the ectopic tissue cannot be excluded, as the treatment with dopaminergic agonists reduced further plasma prolactin levels (5) while a high dopamine content was measured in the pituitary graft (8, 13). Therefore a dopaminergic-mediated regulatory control might be present in the graft. Although the role of dopamine, regulating prolactin secretion from the ectopic tissue, is not well understood yet, the increase in the number of lactotrophs (13) in the graft might in some extent explain the high peripheral mean values of prolactin. The episodic pattern of prolactin release, in pituitary-grafted rats, could be due to an intrinsic pulsatile characteristic of the lactotrophs which was described *in situ* glands and which can persist in the ectopic gland (31).

The presence of norepinephrine in the graft could be a key role in the local regulatory mechanisms of prolactin secretion by the ectopic tissue, by increasing the release of the hormone (8) and perhaps modifying the absolute amplitude of the prolactin peaks.

Hyperprolactinemia has been associated in humans with increased, decreased and normal LH levels (4, 26, 29) whereas in rats most of the works show lower LH values (20, 25, 33). The increase in the mean values of LH serum during the studied period disagrees with earlier studies from our laboratory (33). The discrepancies could be due to the fact that most of the previous studies were done with a single measurement of the hormone and with a different strain of rats (33). The increase in the absolute amplitude of the LH peaks, shown in pituitary-grafted animals, accounts for the high mean values of the hormone. These effects could be explained through prolactin action on the hypothalamus which increases norepinephrine turnover (1).

In conclusion, all these data suggest that hyperprolactinemia induced by pituitary grafting in adult female rats, is followed by a significant change in prolactin and LH pulsatility, which may explain, to some extent, the effects of hyperprolactinemia on reproduction.

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Resumen

Se estudia en la rata hembra adulta la secreción pulsátil de prolactina (PRL) y hormona luteinizante (LH), cuyos mecanismos de secreción están estrechamente relacionados, en un modelo experimental de hiperprolactinemia, inducida por el trasplante de dos adenohipófisis. Nueve día después de la operación, se extraen dos muestras de sangre (75 ó 100 µl/7 min) durante 3 h. En las muestras de suero obtenidas se determina por RIA la concentración de PRL y LH. La hiperprolactinemia modifica la secreción pulsátil de PRL incrementando la amplitud absoluta de los pulsos, la duración de los mismos y los niveles medios de PRL sérica, a la vez que disminuye su frecuencia. En los animales hiperprolactinémicos se observa un incremento de la amplitud absoluta de los pulsos de LH y de los niveles séricos medios de LH. Todos estos datos sugieren que el trasplante de dos hipófisis produce un cambio significativo en los patrones pulsátiles de LH y de la propia PRL que explicaría, al menos en parte, los efectos de la hiperprolactinemia sobre la función reproductora.

Palabras clave: Secreción pulsátil, Prolactina, Hormona luteinizante, Hiperprolactinemia.

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