Effects of Luteinizing Hormone-Releasing Hormone on Pulsatile Prolactin Secretion in Adult Hyperprolactinemic Female Rats

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Abstract. **Regulation of prolactin secretion involves complex interactions of multiple inhibitory and stimulatory factors. Among them, luteinizing hormone–releasing hormone (LHRH) has been shown, when analyzed in single samples, to exert both stimulatory and inhibitory influences on prolactin secretion. In the present study, we have further examined the effects of LHRH on prolactin secretion by studying the pulsatile pattern of the hormone after the administration of the neuropeptide. For this porpose, adult sham-operated and pituitary-grafted female rats, exhibiting diestrus smears were bled for 3 hr during the morning (1030 to 1330 hr). Two pulses of LHRH (10 ng/kg body wt) were administered 60 and 120 min after starting the bleeding period. Pituitary grafting increased the mean serum prolactin levels, absolute amplitude of the hormone peaks, and its mean half-life, compared with control animals. In sham-operated rats, LHRH administration diminished mean serum prolactin levels, the absolute pulse amplitude and frequency of prolactin peaks. In pituitary-grafted animals, LHRH administration only decreased the pulse frequency of prolactin peaks. These data suggest that LHRH significantly suppressed pulsatile prolactin secretion, and that this effect was blunted by exposure to previously elevated circulating prolactin levels.**
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Prolactin is released in an episodic fashion (1–3). This pattern represents the net results of an unknown, complex neuroendocrine signaling that comprises interactions of multiple inhibitory and stimulatory factors (4–6). Among them, luteinizing hormone–releasing hormone (LHRH) is involved in the regulation of pulsatile pattern of prolactin, but its exact role remains controversial, with evidence for both stimulatory (7, 8) and inhibitory (9–11) effects having been reported. Both prolactin and LH secretory patterns can be identically influenced by LHRH analogs (11), but these effects might be modified by differential responsiveness of the lactotrophs induced by its previous secretory activity and by the prevailing inhibitory inputs (4, 12). Also, prolactin release can be influenced by previously increased plasma prolactin levels (13) through mechanisms that are not fully understood. Therefore, the present study was designed to further examine the effects of exogenous administered LHRH on the pulsatile prolactin secretion and its possible modulation by previously increased plasma prolactin levels. For this purpose, adult female rats were rendered hyperprolactinemic by the transplantation of two pituitary glands under the kidney capsule, and aged-matched animals were sham-operated to be used as controls.

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Materials and Methods

Animals. Adult female Sprague-Dawley CD rats, weighing 240–280 g, were used in all experiments. They were housed in animal facilities with overhead fluorescent lighting provided between 0600 and 2000 hr. Room temperature was maintained at 22° ± 2°C. Laboratory chow and water was available *ad libitum*. Vaginal smears were taken daily, and only rats demonstrating at least two consecutive 4-day estrus cycles were used. Only rats exhibiting a diestrus smear, at the day of sacrifice, were used in the experimental design.

Induction of Hyperprolactinemia. Rats in the estrus phase of the cycle were anaesthetized with 2.5% tribromoethanol (1 ml/100 g body wt). Two pituitary glands from female rats (retired breeders, showing a diestrus smear) of the same strain were transplanted under the right kidney capsule, according to the method of Mena *et al.* (14), modified by Tresguerres and Esquifino (15). Another group of rats was sham-operated to be used as controls.

Experimental Design, Cannula Implantation, and Blood Sampling. Four groups of rats were studied: Groups 1 and 2, adult sham-operated or pituitary-grafted rats treated with saline during the bleeding period; Groups 3 and 4, adult sham-operated or pituitary-grafted rats, given two pulses LHRH (Sigma Chemical Co., St. Louis, MO), 10 ng/kg body wt, 60 and 120 min after starting the bleeding period. Eight animals per group were included. Eight days after pituitary grafting and 40 hr before the experiment, atrial cannulas were implanted through the external jugular vein, using tribromoethanol anaesthesia (16). This procedure allows the animals to run free in their cages during the period of bleeding. All rats were given 350 IU heparin 20 min before the start of bleeding period to avoid coagulation and were continuously infused with 0.9% (w/v) NaCl (0.5 ml/hr) for 4 hr beginning at 0900 hr. Sample collection was performed between 1030 and 1330 hr, through a peristaltic pump set at a flow rate of 50 μl/7 min (26 samples during the bleeding period) (16–18). Hematocrits remained stable with this bleeding protocol (38%–41%). The samples were added to assay tubes containing 200 μl of phosphate-buffered saline plus 0.1% gelatine, centrifuged, and the serum kept frozen for subsequent measurements of prolactin.

Prolactin Radioimmunoassay. Serum prolactin concentrations were determined by a specific double antibody radioimmunoassay system, using reagents kindly supplied by the NIADDK (Baltimore, MD). Prolactin values were expressed in terms of the rat NIADDK PRL RP-3 reference preparation. The sensitivity of the assay was 0.025 ng/ml. Samples were analyzed within the same assay to avoid interassay variations. To ascertain the variability of the assay, series of 10 replicates corresponding to five different concentrations of prolactin in the standard curve were used. The mean intraassay coefficient of variation (CV) was 8.0%. This method has been described elsewhere (3, 13).

Data Analysis. To identify and characterize pulses appearing in the hormonal profile of each rat, a computer program (Ultra-analysis) described by Van Cauter (19) and reviewed by Richard *et al.* (20) was used. In this program, a pulse was defined as a significant increase exceeding a multiple of the dose-adjusted coefficient of variance (CV), followed by a significant decrease. The intraassay CVs were calculated from values of five different concentrations of prolactin in its standard curve. Thus, the CV and the mean hormone level were determined for prolactin values that comprised the ascending and descending phases of each potential pulse. The pulse was defined when this CV was triple that of the intraassay CV determined at a comparable mean prolactin level. To test the specificity of pulse detection, a series of 26 samples from a pool of serum was analyzed using a threshold of three CVs for prolactin peaks. Extensive simulation studies using computer-generated series have indicated that for series that have large and frequent pulses, threshold of three CV will minimize both false positive and false negative errors (21).

Pulsatile prolactin secretion pattern was characterized by the mean hormone level, absolute and relative amplitude of the peaks, their frequency, and pulse duration. Also, the program calculates the mean half-life of the hormone. The 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 the absolute pulse amplitude and the preceding nadir value. Pulse frequency was the number of pulses per hour. Pulse duration was the time between the beginning of the ascending and the end of the descending phase of the peak.

The mean hormone level was calculated from the mean of all samples collected from each rat during the 3-hr period and the average for the experimental group from the individual means.

Statistical Analysis of the Data. Comparison of values for the pulsatile parameters was done by analysis of variance followed by a paired Student's t test. The results were considered significant at $P < 0.05$. All values represent the mean ± SEM.

Results

A representative pulsatile secretion pattern of prolactin in one rat of each experimental group is shown in Figure 1. In all groups studied prolactin secretion

was pulsatile. No differences were observed in prolactin pulsatility of sham-operated animals during the LHRH's pre-administration compared with sham-operated rats treated with saline during the whole bleeding period. A similar finding was observed for pituitary-grafted animals.

As expected, pituitary grafting was followed by an increase in the mean serum prolactin levels (Fig. 2) as measured by comparing the values obtained during the first hour of the bleeding period (previously to LHRH stimulatory test) with those found in sham-operated rats. LHRH administration decreased the mean values of prolactin in sham-operated rats ($P < 0.05$) but not in pituitary-grafted animals (Fig. 2). Second administration of LHRH did not potentiate the inhibitory effects of first administration of the neuropeptide in either sham-operated or pituitary-grafted rats.

In sham-operated rats, the pulsatile pattern of prolactin before the LHRH administration was documented by the existence of over 2.20 pulses (in 1 hr) and over 2.28 peaks (in 1 hr) in pituitary-grafted rats (Fig. 3). There was a reduction in the number of pulses in both sham-operated and pituitary-grafted rats after LHRH administration (Fig. 3) ($P < 0.05$). The second administration of LHRH did not potentiate the inhibitory effect of the first administration of the neuropeptide.

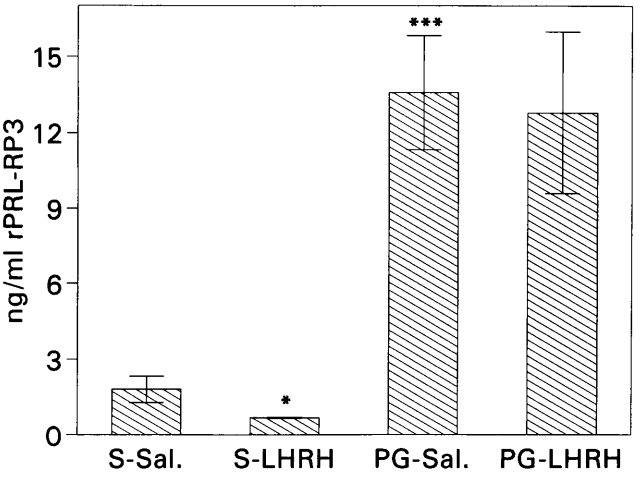

Figure 2. Mean serum prolactin levels in sham-operated and pituitary-grafted rats before and after LHRH administration. Values are expressed as mean ± SEM. *$P < 0.05$, ***$P < 0.001$ vs sham-operated rats.

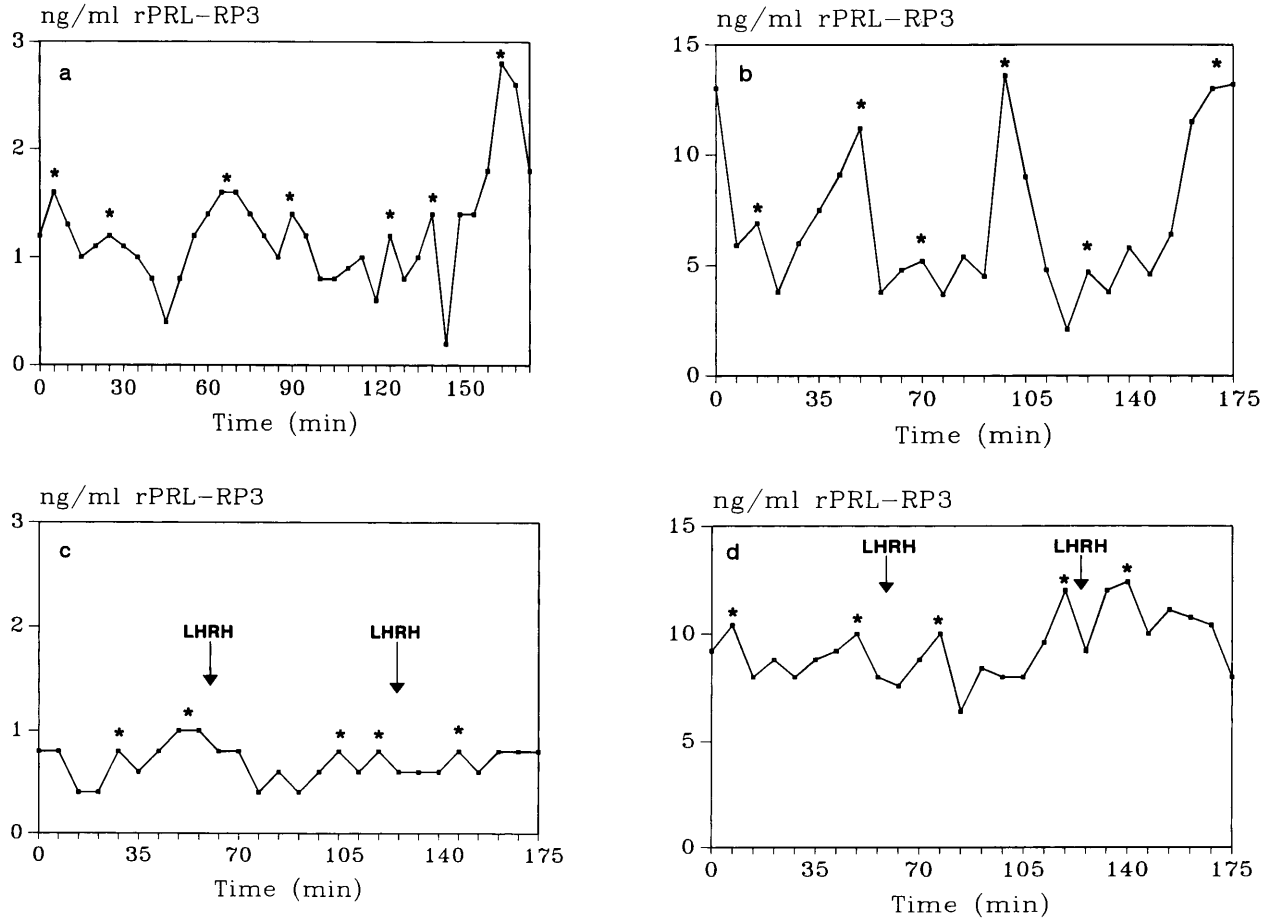

Figure 1. Individual pulsatile prolactin patterns in sham-operated and pituitary-grafted female rats with saline (Panel a and b, respectively) or after two pulses of 10 ng/kg body wt of LHRH administration (Panel c and d, respectively). Values were given in terms of r-PRL-RP-3. *The appearance of prolactin pulses during the period studied.

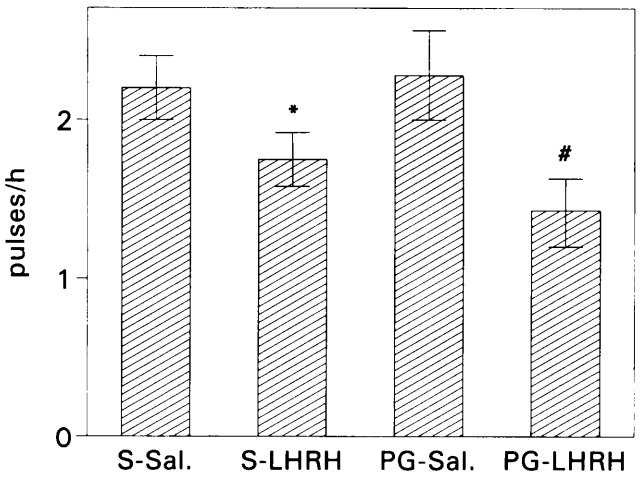

Figure 3. Frequency of the prolactin pulses in sham-operated and pituitary-grafted animals before and after LHRH administration. Values are expressed as mean ± SEM. $^{*}P < 0.05$ vs sham-operated rats. $^{\#}P < 0.05$ vs pituitary-grafted rats.

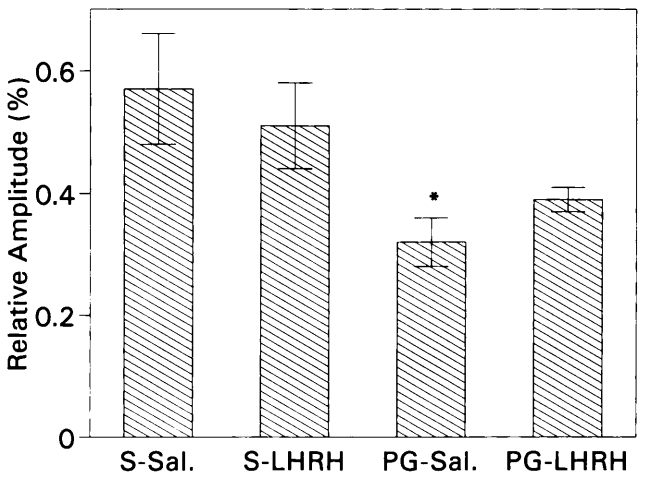

Figure 5. Relative pulse amplitude of prolactin in sham-operated and pituitary-grafted rats before and after LHRH administration. Values are expressed as mean ± SEM. $^{*}P < 0.05$ vs sham-operated rats.

The absolute amplitude of the prolactin peaks increased after pituitary grafting (Fig. 4) ($P < 0.001$), compared with sham-operated controls prior to LHRH administration. There was a decrease in the absolute pulse amplitude of prolactin peaks after the administration of the neuropeptide in sham-operated (Fig. 4) ($P < 0.05$) but not pituitary-grafted rats (Fig. 4). The relative amplitude of the prolactin pulses decreased in pituitary-grafted animals (Fig. 5) ($P < 0.05$), compared with sham-operated rats prior to neuropeptide stimulatory test. After LHRH administration, this parameter did not change in either sham-operated or pituitary-grafted rats (Fig. 5).

Prolactin pulse duration was over 26 min in sham-operated and 30 min in pituitary-grafted rats (Fig. 6). LHRH administration did not change the pulse duration in any group studied (Fig. 6). Pituitary grafting increased mean half-life of circulating prolactin, compared with that of sham-operated animals (Fig. 7) ($P < 0.05$). LHRH administration did not change this parameter in either sham-operated or pituitary-grafted animals (Fig. 7).

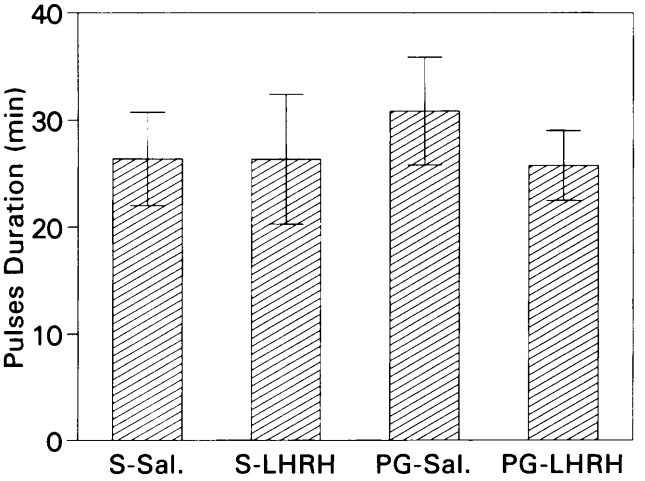

Figure 6. Pulse duration of prolactin in sham-operated and pituitary-grafted rats before and after LHRH administration. Values are expressed as mean ± SEM.

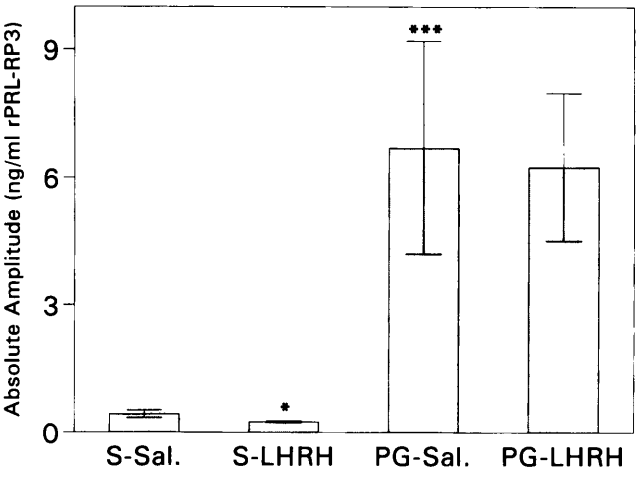

Figure 4. Absolute pulse amplitude of prolactin in sham-operated and pituitary-grafted rats before and after LHRH administration. Values are expressed as mean ± SEM. $^{*}P < 0.05$, $^{***}P < 0.001$ vs sham-operated rats.

Discussion

The results of this study provided a detailed characterization of prolactin pulsatile pattern in sham-operated and pituitary-grafted rats under basal conditions and after the administration of two pulses of LHRH during the bleeding period.

The data obtained indicate that LHRH inhibits the pulsatile secretory pattern of prolactin in sham-operated rats, as measured by a decrease of the absolute amplitude and frequency of prolactin peaks and the mean values of the hormone. The presence of previously elevated plasma prolactin levels blunted the inhibitory action of LHRH on the pulsatile pattern of the hormone that characterizes to control animals.

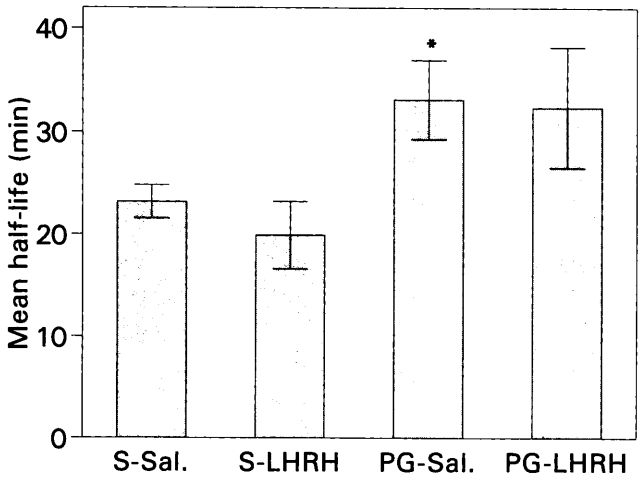

Figure 7. Mean half-life of prolactin in sham-operated and pituitary-grafted rats before and after LHRH administration. Values are expressed as mean ± SEM. *$P < 0.05$ vs sham-operated rats.

Pituitary-grafted animals exhibited an increase in the absolute amplitude of the prolactin pulses and in the mean half-life of the hormone, thus leading to an increase in the mean serum prolactin levels and a decrease in the relative pulse amplitude of prolactin, compared with sham-operated animals. Thus, the results of the present study agree with previous findings by our group (13, 22) and other workers (23). These changes in prolactin pulsatility in grafted animals might be due to the well-known ability of hyperprolactinemia to increase tuberoinfundibular dopamine turnover (24) and thus suppressing the release of prolactin from the *in situ* gland (24). For this reason, the ectopic pituitary gland greatly contributes to the pulsatility of prolactin secretion in pituitary-grafted animals (25, 26), suggesting the persistence of an intrinsic pulsatility of the ectopic lactotrophs similar to that demonstrated in *in situ* pituitaries (27). Also, the increase in the number of lactotrophs in the ectopic gland observed by Iturriza *et al.* (28) and by previous studies in our laboratory (Esquifino, unpublished observations) might contribute to the higher mean values of the hormone, observed during the studied period.

In agreement with previous work from our laboratory (15), LHRH administration reduced the mean serum prolactin levels in sham-operated rats (10–15, 29–30), and this effect might be explained by the changes in the absolute amplitude and frequency of the prolactin peaks. When all the parameters of prolactin pulsatility are analyzed, an inhibitory role of LHRH on prolactin secretion emerges. However, controversial effects of LHRH on prolactin secretion have been observed (7, 8, 31–32). Analysis of all available data suggests that LHRH can stimulate or inhibit prolactin release depending on the prevailing inputs arriving to the pituitary, before the administration of the neuropeptide. Also, it has to be considered that most studies on the modulatory role of LHRH on prolactin secretion were performed under pathophysiological conditions, which could have conditioned lactotroph responsiveness to external stimulii (32–34), or by the administration of LHRH analogs that may not exactly mimic the effects of the neuropeptide (33). However, direct effects of the neuropeptide on the *in situ* lactotrophs modifying their intrinsic pulsatility (27) or through interactions with TIDA (5) and/or other neuromodulatory neurones (12) at the hypothalamus have to be considered.

The ability of LHRH to suppress pulsatile prolactin release was blunted in pituitary-grafted rats, as suggested by the lack of change in the absolute pulse amplitude of prolactin peaks or in the mean values of the hormone after the administration of the neuropeptide (30, 34). Changes in paracrine interactions at the hypophyseal level, induced by hyperprolactinemia, can account for the differential effects of LHRH on prolactin secretion (7, 29).

The differential effect of LHRH on prolactin pulsatility in sham-operated and pituitary-grafted rats shown in this study might be related to the data obtained by Rubio *et al.* (34) demonstrating that LHRH agonist administration decreased serum prolactin levels in patients with microprolactinomas, but not in those with macroprolactinomas. The lack of fall in serum prolactin in the macroprolactinoma patients may simply reflect greater tumor cell autonomy or the presence of a larger number of lactotroph cells, which might be less affected by altered gonadotroph cell function after LHRH agonist administration. In this regard, the transplantation of two pituitary glands instead of one as in previous studies (22) may more closely resemble the situation in patients with macroprolactinomas.

This possibility agrees with recent data from our laboratory showing a higher activity of gonadotrophs at the ectopic pituitary compared with the *in situ* gland, using immunohystochemical (Esquifino *et al.*, unpublished observations) or *in vitro* luteinizing hormone release studies (30, 35). The administration of exogenous LHRH might change the activity of these cells, which through paracrine effects could contribute to minimizing the changes in prolactin pulsatile secretion, and thus confirm previous studies (7).

In conclusion, all the above-mentioned data might suggest that exogenous LHRH administration is most effective in influencing prolactin release from pituitaries with an increased absolute or relative number of lactotrophs, which would agree with previous work from our laboratory (30), and that local regulatory mechanisms developed at the ectopic gland differentially affect prolactin release in pituitary-grafted and control rats, thus confirming previous works from our laboratory which used single samples to measure circulating levels of the hormone (25, 26).

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