Possible Changes in the Regulatory Mechanisms of Pulsatile Luteinizing Hormone Secretion in Adult Pituitary-Grafted Female Rats (43891)

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Abstract. **It is well known that LH is released in an episodic fashion. However, the effects of hyperprolactinemia on luteinizing hormone (LH) pulsatility are not fully understood. The present study was undertaken to describe hyperprolactinemia effects on the regulatory mechanism of LH pulsatility. For this purpose hyperprolactinemia was induced in female rats by the transplantation of two pituitary glands beneath the kidney capsule. Age-matched sham-operated animals were used as controls. We have evaluated the pulsatile pattern of LH in both groups of animals. As expected, pituitary grafting increased mean serum prolactin levels compared with the values found in control animals. Luteinizing hormone-releasing hormone (LHRH) administration did not change serum prolactin levels in control or in pituitary-grafted rats. Thyrotropin-releasing hormone (TRH) administration increased prolactin secretion in both groups. There is an increase in mean values of LH and in the absolute amplitude of LH peaks in pituitary-grafted compared with control rats. No other parameters of LH pulsatility were changed by pituitary grafting. After LHRH administration, LH release was increased and a priming effect after the second administration of LHRH is observed in control rats. In pituitary-grafted animals the responses of LH to LHRH administration was diminished compared with the response observed in control rats. The administration of two pulses of TRH to control rats only decreases the duration of LH pulses. However, in pituitary-grafted animals, TRH administration was followed by a decrease in the mean values of LH and in the absolute amplitude of the LH peaks. Vasoactive intestinal peptide (VIP) administration increased mean values of LH and the absolute and relative amplitudes of LH pulses in sham-operated animals, whereas only the relative amplitude of LH peaks was modified by VIP in pituitary-grafted rats. All these data suggest that hyperprolactinemia induced by pituitary-grafting interfere with the neuromodulator effects on the pulsatile regulatory center of the hypothalamus.** [P.S.E.B.M. 1995, Vol 209]

It is widely accepted that pituitary hormones are secreted in an episodic fashion (1, 2) as a consequence of the pulsatile secretion of the hypothalamic regulatory factors involved in their regulation. The pulsatile secretion of luteinizing hormone (LH) has been widely studied (3–5) and is the consequence of the pulsatile secretion of LH-releasing hormone (LHRH) from the hypothalamus (6). Other regulatory factors, such as vasoactive intestinal peptide (VIP) and thyrotropin-releasing hormone (TRH) have also been found to be involved in the modulation of LH pulsatile secretion (7–10). Also dopamine (DA)-mediated effects have been reported (11).

On the other hand, it is well known that prolactin and LH secretion mechanisms are very closely related (12–15). Hyperprolactinemia is associated with diminished (12), increased (15), or not modified (16) circulating LH levels. Although effects of hyperprolactin-

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emia on LH pulsatility is less known, hyperprolactinemia could modulate LH pulsatility by indirect effects through hypothalamic DA (17) and/or LHRH (6) release or by paracrine effects between lactotrophs and gonadotrophs in the pituitary (18).

An experimental model of hyperprolactinemia widely used to study the interrelationship mechanisms between prolactin and gonadotropin secretions is obtained by the grafting of one or more extra pituitaries in a normal (nonhypophysectomized) host (12). This grafted pituitary is the main source of circulating prolactin (14), while LH secreted to the circulation is mainly coming from the *in situ* pituitary (12).

This work was designed to study hyperprolactinemia effects on the regulatory mechanism of LH pulsatile secretion. For this purpose, the pusatile pattern of LH was studied in sham-operated and pituitary-grafted female rats. The responses of LH to LHRH, TRH and/or VIP were evaluated.

Materials and Methods

Animals. Adult female rats of the Sprague-Dawley CD strain, weighing 240–280 g, were used in all experiments. They were maintained in a room with controlled photoperiod (14:10-hr light:dark cycle; lights on from 06:00 hr to 20:00 hr), and temperature (22° ± 2°C) and with rat chow and water available *ad libitum*. Vaginal smears were taken daily, and only rats demonstrating at least two consecutive 4-day estrous cycles were used in this study.

Induction of Hyperprolactinemia and Cannula Implantation. Rats showing estrous smears were anesthetized with 2.5% tribromoethanol (1 ml/100 g body wt). Two pituitary glands from adult female rats (retired breeders) from the same strain were implanted under the right kidney capsule according to the method previously described (12). Rats of the same age were sham-operated to serve as controls.

Eight days after pituitary grafting and 40 hours before the day of the experiment, animals were anesthetized with tribromoethanol (2.5% tribromoethanol, 1 ml/100 g body wt) and atrial cannulas were implanted through the external jugular vein according to procedures used in previous studies (15, 36). This procedure allows the animals to move freely in their cages during the period of bleeding.

Blood Sampling. On the day of the experiment, conscious and freely moving sham-operated or pituitary-grafted rats were continuously infused with 0.9% saline (0.5 ml/hr) for 4 hr, beginning at 09:30 hr. One hour after intravenous infusion of saline and 15 min after the administration of 350 IU of heparin, rats were bled continuously through a peristaltic pump set at a flow rate of 50 μl every 7 min for 3 hr from 10:30 to 13:30 hr. The time intervals between samples were 7 min long. The samples (50 μl) were collected into assay tubes, kept on ice and containing phosphate buffer (0.01 *M*) with 0.1% gelatin, centrifuged (1500*g*) for 15 min at 4°C, and the serum was kept frozen at −20°C until analyzed.

Also, plasma from the trunk blood was collected to measure circulating values of prolactin. Hematocrits remained stable with this bleeding protocol (42%–38%).

Experimental Design. On the day of experiment, sham-operated and pituitary-grafted rats were bled for 3 hr during (i) saline administration; (ii) two iv pulses of LHRH (Peninsula Laboratories, Inc., Belmont, CA) of 10 ng/100 g body wt administered 60 and 120 min after starting the bleeding period; (iii) two iv pulses of TRH (Sigma Chemical Co., St. Louis, MO) of 1 μg infused 60 and 120 min after starting the bleeding period; and (iv) one pulse of VIP (Sigma) of 20 μg/rat administered 60 min after starting the bleeding period. Saline, LHRH, TRH, and VIP were administered through the jugular catheter.

Hormone Measurements. LH levels, in each series of samples from each rat and serum prolactin, were measured by specific double antibody radioimmunoassay techniques. The reagents were kindly supplied by the NIDDK (Baltimore, MD). Prolactin (PRL) values (ng/ml) are expressed in terms of the rat NIDDK PRL RP-3 reference preparation. The sensitivity of the assay was 5 pg/tube. The intra-assay coefficient of variation was 8.6%. LH levels (ng/ml) are expressed in terms of the rat NIDDK LH RP-3 reference preparation. The sensitivity of the LH assay was 0.5 pg/tube, with an intra-assay coefficient of variation of 7%. All samples were measured within the same assay to avoid interassay variations.

Data Analysis. To identify and characterize pulses appearing in the hormonal profile of each rat, a computer program (Ultra-analysis) described by Van Cauter (37) was utilized. In this program, a pulse is defined as a significant increase exceeding a multiple of the dose-adjusted coefficient of variance (CV), followed by a significant decrease. The intra-assay CVs were calculated from values of five different concentrations of LH in the standard curve. Thus, the CV and the mean hormone level were determined for the LH values which comprised the ascending and descending phases of each potential pulse. The pulse was defined when this CV was three times that of the intra-assay CV determined at a comparable mean LH level. To test the specificity of pulse detection, a series of 26 samples from a pool of serum was analyzed using a threshold of 3 CV for this hormone.

Pulsatile LH secretion pattern was characterized by the mean hormone level, absolute and relative amplitude of the peaks, their frequency and pulse duration. 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 per 3 hr. 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.

The mean hormone level was calculated by 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 Duncan's multiple range test or Student's t test. The results considered significant at $P < 0.05$. All values represent the mean ± SEM.

Results

A representative profile of the pulsatile pattern of LH in one rat of each experimental group is given in Figure 1 and 2.

Pituitary grafting was followed by the expected

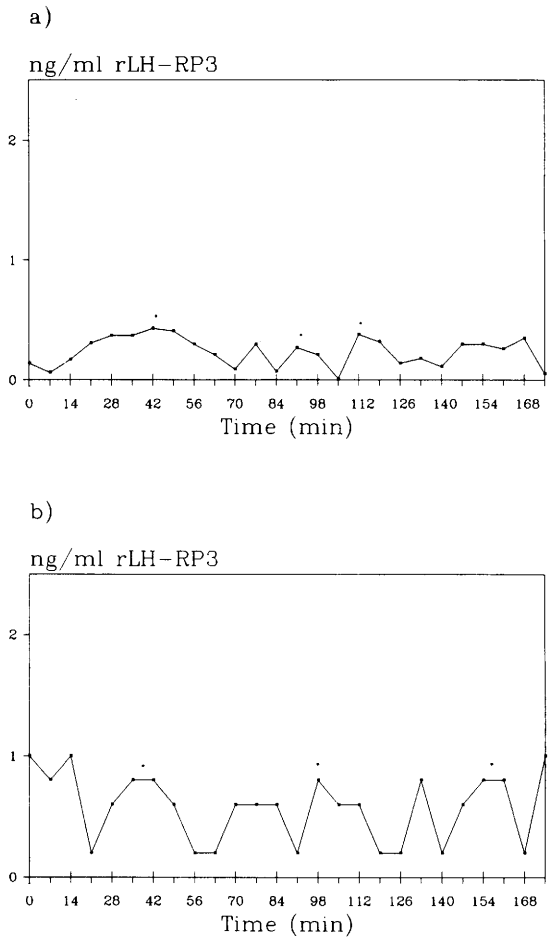

Figure 1. Individual pulsatile LH patterns. (a) Sham-operated rat infused with saline; (b) Pituitary-grafted rat infused with saline. Asterisks indicate the LH peaks during the period studied.

increase in the values of serum prolactin levels compared with sham-operated controls ($P < 0.001$, Table I). LHRH administration did not modify serum prolactin levels in either sham-operated or pituitary-grafted rats. However, TRH administration was followed by the expected increase in mean serum prolactin levels in both groups ($P < 0.01$ and $P < 0.05$, respectively, Table I). VIP administration markedly increased mean serum prolactin levels in pituitary-grafted animals but not in sham-operated rats ($P < 0.001$ versus pituitary-grafted rats with saline administration and sham-operated rats with VIP administration, Table I).

There was an increase in the mean values of serum LH (as mean value of all samples measured during the bleeding period) in pituitary-grafted infused with saline compared with sham-operated rats ($P < 0.001$, Table I), together with an increase in the absolute amplitude of the LH peaks ($P < 0.01$, Table I). No other parameters of LH pulsatility studied (relative pulse amplitude and frequency and duration of the LH peaks) were modified by pituitary grafting (Table I and II).

The administration of 2 pulses of LHRH, to sham-operated rats, 60 and 120 min, after starting the bleeding period, induced an increase in the mean values of LH ($P < 0.001$ versus control, Table I and Fig. 2a) and in the absolute and relative amplitude of LH peaks ($P < 0.001$ and $P < 0.01$ versus saline-treated rats, respectively, Table I and Fig. 2a). After the second administration of LHRH, a primming effect was observed with a higher increase in LH release than after the first administration of LHRH (Fig. 2a).

In pituitary-grafted rats, LHRH administration also induced an increase in the mean values of LH and in the relative amplitude of LH pulses ($P < 0.05$, Table I). No effects were detected in any other parameter studied of LH pulsatility (Fig. 2b).

The administration of two pulses of TRH to sham-operated rats 60 and 120 min after starting the bleeding period only decreased the duration of LH peaks ($P < 0.05$, Table II and Fig. 2c); whereas, in pituitary-grafted rats TRH decreased the absolute amplitude of LH peaks ($P < 0.05$, Table I) and the mean values of the hormone ($P < 0.05$, Table I and Fig. 2d).

The administration of one pulse of VIP to sham-operated rats 60 min after starting the bleeding period increased both the absolute and relative amplitude of LH pulses ($P < 0.001$, Table I) and mean serum values ($P < 0.001$, Table I and Fig. 2e). However, in pituitary-grafted rats VIP administration only increased the relative amplitude of LH peaks ($P < 0.05$, Table I and Fig. 2f).

Discussion

As expected, pituitary grafting was followed by an increase in the mean values of circulating prolactin

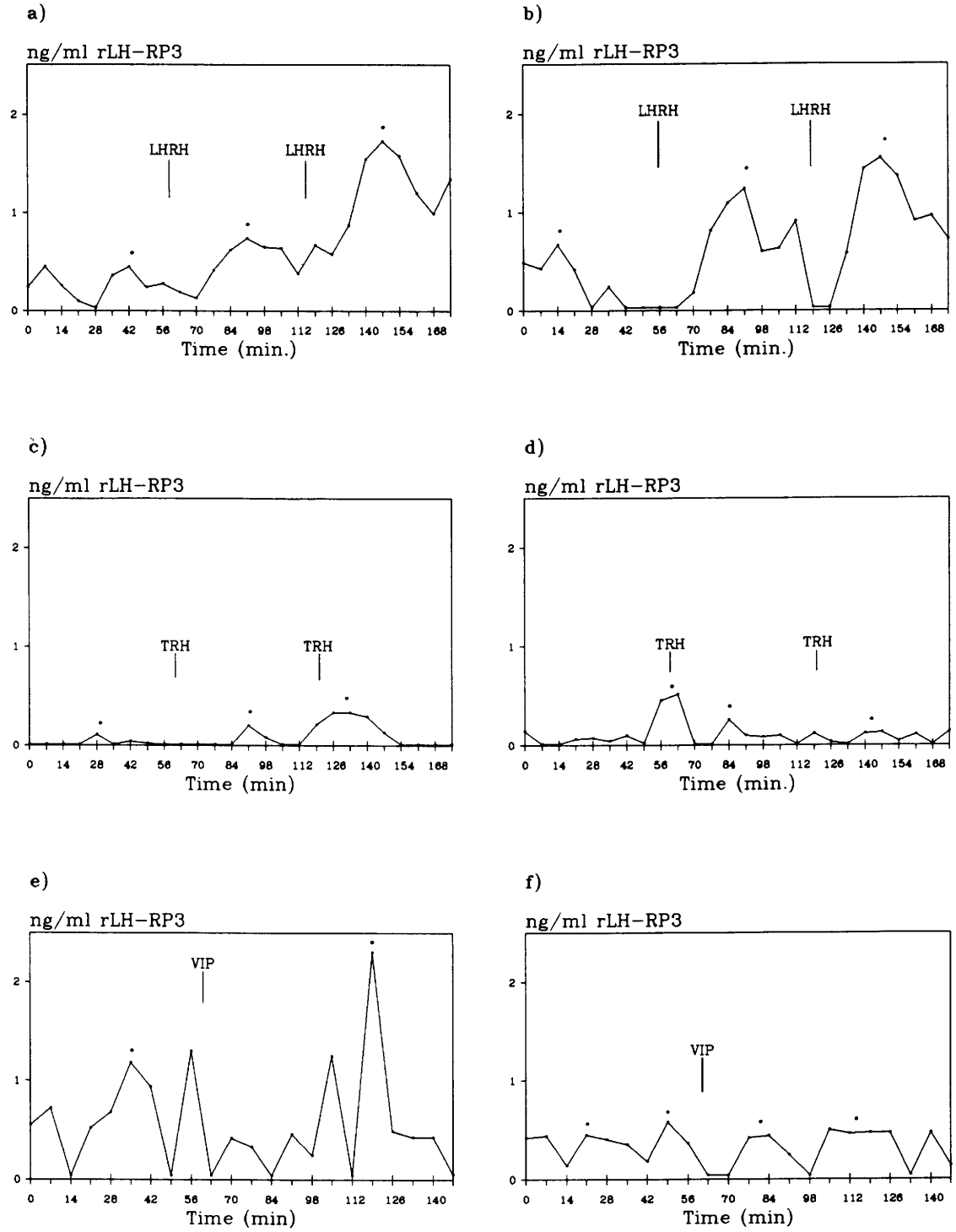

Figure 2. Individual pulsatile LH patterns. (a) Sham-operated rat with two pulses of LHRH administration; (b) pituitary-grafted rat with two pulses of LHRH administration; (c) sham-operated rat with two pulses of TRH administration; (d) pituitary-grafted rat with two pulses of TRH administration; (e) sham-operated rat with one pulse of VIP administration; (f) pituitary-grafted rat with one pulse of VIP administration. Asterisks indicate the LH peaks during the period studied.

levels, in agreement with previous reports of our group (15, 17) and from the literature (19) using different sampling protocols. Surprisingly, LHRH did not modify mean values of serum prolactin levels. This lack of effect is in agreement with that reported by Ceballos and McCann (20) but is in disagreement with previous findings of our laboratory (12). This discrepancy might be due to differences in the age at which hyperprolactinemia was induced (prepubertal in previous works and adult age in this study), to the use of Sprague-

Table I. Quantitative Parameters[a] of Pulsatile LH Secretion and Mean Values of Serum Prolactin Levels in Sham-Operated and Pituitary-Grafted Rats: Effects of LHRH, TRH, or VIP Administration

Group	PRL-RP-3 (ng/ml)	LH-RP-3 (ng/ml)	Absolute amplitude	Relative amplitude
Sham-operated				
Saline	1.23 ± 0.16	0.07 ± 0.01	0.16 ± 0.03	5.99 ± 0.89
LHRH	1.12 ± 0.20	0.82 ± 0.06[b]	0.93 ± 0.08[b]	12.6 ± 2.96[c]
TRH	3.34 ± 0.83[c]	0.19 ± 0.08	0.28 ± 0.08	7.92 ± 1.04
VIP	1.10 ± 0.44	0.40 ± 0.07[b]	0.74 ± 0.15[b]	19.2 ± 3.96[b]
Pituitary-grafted				
Saline	9.99 ± 1.74[d]	0.39 ± 0.05[d]	0.68 ± 0.17[e]	5.27 ± 1.27
LHRH	11.4 ± 2.35	0.56 ± 0.04[f]	0.73 ± 0.04	11.9 ± 2.59[f]
TRH	17.0 ± 2.93[f]	0.21 ± 0.04[f]	0.26 ± 0.04[f]	7.52 ± 0.99
VIP	30.9 ± 3.84[g]	0.34 ± 0.06	0.64 ± 0.16	13.5 ± 3.63[f]

Note. Values are expressed as mean ± SEM. The number of animals per group is eight. The relative pulse amplitude was calculated as the quotient between absolute pulse amplitude and preceding nadir value.
[a] Mean serum LH levels, absolute pulse amplitude, and relative pulse amplitude. [b,d]$P < 0.001$, [c,e]$P < 0.01$ versus sham-operated rats and saline administration; [f]$P < 0.05$, [g]$P < 0.001$ versus pituitary-grafted rats and saline administration.

Table II. Qualitative Parameters of Pulsatile LH Secretion in Sham-Operated and Pituitary-Grafted Rats Under LHRH, TRH, or VIP Administration

Group	Frequency (*n* pulses/3 hr)	Duration (min)
Sham-operated		
Saline	3.07 ± 0.24	34.72 ± 5.58
LHRH	3.33 ± 0.33	41.16 ± 1.77
TRH	3.66 ± 0.21	25.95 ± 2.61[a]
VIP	2.60 ± 0.40	35.32 ± 5.70
Pituitary-grafted		
Saline	3.00 ± 0.33	33.03 ± 4.22
LHRH	3.33 ± 0.23	41.64 ± 4.75
TRH	3.62 ± 0.32	32.33 ± 1.50
VIP	2.83 ± 0.60	26.44 ± 1.87

Note. Values are expressed as mean ± SEM. The number of animals per group is eight.
[a] $P < 0.05$ versus sham-operated rat with saline administration.

Dawley rats instead of Wistar in previous studies, and to differences in the length of the dark period (10 hr in this study and 12 hr in previous studies) (12). TRH effects on prolactin secretion were blunted in pituitary-grafted female rats (1.7 in pituitary-grafted versus 2.7 times in control animals after TRH administration) as described earlier (21).

VIP administration in sham-operated rats did not change serum prolactin levels, although a marked increase in the circulating values of the hormone was observed in pituitary-grafted female rats. These effects might be explained by taking into account the existence of VIP receptors in tumorous pituitary lactotrophs (22). The ectopic gland might act as a localized tumor, or VIP might act directly on the pituitary cells as has been demonstrated *in vitro* (23, 24). The higher number of lactotrophs present in pituitary-grafted rats (*in situ* plus ectopic lactotrophs) might account for an increased response of prolactin to VIP.

Hyperprolactinemia is associated with changes in the pulsatile secretion pattern of LH. The changes in the absolute amplitude of LH pulses in pituitary-grafted animals without modifications in any other parameters that characterize its pulsatile secretion support the increase in the mean serum levels of LH during the bleeding period, as reported earlier (15). Chronic increases in circulating LH levels have been associated with alterations of ovarian function (25) in humans. A desensitization of LH receptors in the ovary may be the mechanism involved in this effect (26).

LHRH administration was followed by an increase in the release of LH in sham-operated rats, which was blunted by the presence of high circulating prolactin values, in agreement with previous studies in lactating postpartum rats both *in vivo* (27) and *in vitro* (28). Modulation by prolactin of LH responses to LHRH might be explained by effects of the hormone in the disposability of LH to be released from the gonadotrophs, as was suggested earlier (29). It is possible that prolactin could influence the tonic regulatory center of the hypothalamus to modulate the episodic release of LH (30) or exert a paracrine effect on the gonadotrophs in the pituitary (18).

TRH did not modify LH pulsatile pattern in sham-operated rats, while in pituitary-grafted rats a decrease in absolute amplitude of LH peaks and mean serum LH levels was observed. This differential effect in control and pituitary-grafted rats suggests the existence of a modulatory role of high prolactin levels for TRH effects on LH pulsatility. Data obtained in sham-operated rats are in agreement with those reported by Deis and Alonso (31), who have described serum LH not significantly modified by TRH in doses ranging between 0.25 and 1 μg/rat. However, in pituitary-grafted animals an inhibitory role for TRH on LH pul-

satility emerge. A direct effect of TRH on the pituitary is suggested since secretion of TRH by the hypothalamus was not changed in pituitary-grafted rats (32). It is possible that the higher serum prolactin levels observed after TRH administration in pituitary-grafted rats might interfere with TRH effects on the pulsatile center at the hypothalamus to modify LH pulsatility (9).

On the other hand, VIP administration increased mean serum values of LH and the absolute and relative amplitude of LH peaks in sham-operated rats. This stimulatory effect of VIP on LH secretion observed in the control group is in agreement with previous works (7) and is confirmed by the stimulatory effect of VIP on GnRH secretion from the hypothalamus both *in vivo* (8) and *in vitro* (33) and by the fact that anti-VIP administration is followed by inhibitory effects on LH secretion (34). However, controversial data regarding the inhibitory effect of VIP on LH secretion were also obtained (35). The use of different experimental approaches could explain the variation in effects of VIP on LH pulsatile secretory pattern and might suggest that the previous endocrine status of the animal in which VIP is administered modulates VIP's effects on LH secretion. The latter is confirmed by the results obtained in this study for pituitary-grafted animals, with no modifications of LH pulsatility, only in the relative pulse amplitude. These data have been observed for other hormones, such as growth hormone or thyrotropin (21). All these data suggest that prolactin might modulate LH pulsatility by modifying the effects of the different neuromodulators at the pulsatile center of the hypothalamus.

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