Possible Interactions of Cyclosporine and Hyperprolactinemia Modulating the Episodic Secretion of Prolactin (44052)

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Abstract. **The interrelationship between the effects of prolactin and cyclosporine (CsA) appears to be very complex and until now poorly understood. The aim of the present work was to analyze whether chronic treatment with CsA could modify the episodic secretion of prolactin in male rats and whether the presence of an ectopic pituitary could counteract the effects of the drug on the pulsatile secretion pattern of this hormone. At 30 days of age, male rats were implanted with one anterior pituitary under the kidney capsule or where sham-operated. Both pituitary-grafted and sham-operated rats were injected sc for 30 days with the vehicle or CsA (5 mg/kg/day), beginning on the day of surgery. Pituitary grafting and/or CsA administration changed the pulsatile secretion pattern of prolactin. In pituitary-grafted male rats, mean serum prolactin levels, absolute pulse amplitude, and half-life of the hormone increased, while the pulse frequency decreased, compared with the values found in sham-operated rats. CsA administration to sham-operated rats increased the relative amplitude of prolactin peaks and diminished the half-life of the hormone, compared with rats of the same group treated with vehicle. However, CsA treatment in pituitary grafted rats led to lower mean serum prolactin levels and absolute amplitude, while the frequency, duration, and relative amplitude of prolactin pulses were not modified. Plasma prolactin levels did not change in control animals, whereas a reduction in circulating values of the hormone was found in pituitary grafted animals. These data suggest that CsA modifies the pulsatile secretory pattern of prolactin in pituitary-grafted male rats. The different effects observed in the control and pituitary-grafted animals might be due to a direct effect of the drug on the ectopic lactotrophs that are submitted to local regulatory influences different from those of the *in situ* pituitary which are submitted to the regulatory influence of the hypothalamus.**

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Cyclosporine (CsA) is a potent immunosuppressive drug, widely used to improve the survival of transplanted organs. This drug has been studied mainly in adults, while only scant evidence of its effects during childhood or the prepubertal ages is available (1). Treatment with cyclosporine can lead to unwanted effects, which include alterations in the function of the hypothalamic-hypophyseal-gonadal axis (2, 3), although different neuroendocrine effects have been reported depending on the age of the patient and/or experimental animals (1). The best known mechanism for CsA effects involves the antagonism at the prolactin receptor level (4, 5) and/or the modification of the activity of second messengers in the lymphocyte. The latter mechanism was confirmed by reports in which this antagonism was not observed in either mammary tissue (6) or the Nb-2 cell line (7). However, antagonism between prolactin and cyclosporine modulating hypothalamic activity has been demonstrated (8), suggesting that CsA might exert

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prolactin-mediated effects on the neuroendocrine system.

Prolactin as well as other hypophyseal hormones are secreted following an episodic pattern (9, 10) which is influenced by the stage of the estrous cycle (11) in female rats. The pattern of prolactin and LH pulses was changed by CsA administration in female rats, suggesting the existence of an interrelationship between prolactin and CsA in modulating gonadal function (12). Prolactin pulsatility was also changed by high circulating levels of the hormone (10, 13). There are only few studies on the effect of CsA on prolactin secretion in male rats, and all of them were performed using single measurements of the hormone (3, 14).

Previous studies by our group have demonstrated that administration of CsA to peripubertal female rats, for 8 days, prevented the augmentation in plasma prolactin levels that occurred following an ectopic graft of a litter-mate pituitary gland and increased prolactin in non-grafted rats (8). However, in transplant patients treatment with CsA is given for years. This treatment is followed by changes in circulating prolactin, which may explain the impaired gonadal function observed in those patients (15). Therefore, it was considered important to elucidate whether chronic treatment with the immunosuppressor may affect prolactin secretion and thus exert prolactin-mediated effects on the neuroendocrine and/or immune system in male rats as was previously demonstrated in female rats (8).

The present study was designed to evaluate possible neuroendocrine effects of chronic treatment with CsA, beginning during prepuberty in male rats. Specifically, we have attempted to answer the following questions: (i) whether chronic treatment with CsA can modify the pulsatile prolactin secretion in male rats and (ii) whether the presence of an ectopic pituitary could counteract the effects of the drug on the pulsatile secretion of prolactin.

Materials and Methods

Animals. Male rats of the Sprague-Dawley strain were used. They were maintained in a room with controlled photoperiod (14 hr light/10 hr darkness; lights on from 07:00 to 21:00), and temperature (22 ± 2°C), with rat chow and water available *ad libitum*.

Induction of Hyperprolactinemia and Cyclosporine Administration. Hyperprolactinemia was induced in 30-day-old male rats by implanting one pituitary gland of a brood sister rat under the kidney capsule of a recipient rat, following the technique of Mena *et al.* (16) as modified by Tresguerres and Esquifino (17). Host rats were anaesthetized with 2.5% tribromoethanol (1 ml/100 g body wt). Rats of the same age were sham-operated to serve as controls. These animals weighed 250–280 g at the time of the bleeding period.

Cyclosporine was generously delivered by Sandoz (Basel, Switzerland, and Barcelona, Spain). Fifty milligrams of CsA were dissolved in 0.5 ml of absolute ethanol and were further diluted with olive oil to a final dilution of 1 g/l. Both pituitary-grafted and sham-operated rats were subcutaneously injected with CsA (5 mg/kg body wt/day) or vehicle for 30 days, beginning on the day of surgery, as in previous studies from our laboratory (8).

Cannula Implantation. Twenty-eight days after surgery and 40 hr before the day of the experiment, animals were anaesthetized with tribromoethanol and atrial cannulas were implanted through the external jugular vein according to procedures used in previous studies (10–11, 13).

Experimental Design and Blood Sampling. Eight animals per group were used in this study. On the day of experiment, conscious and freely moving rats from each group (sham-operated treated with CsA or vehicle and pituitary-grafted rats with the same treatments) were continuously infused with 0.9% saline (0.5 ml/hr) for 4 hr, beginning at 9:30 hr. One hour after the beginning of the intravenous infusion of saline and 15 min after the administration of 350 IU of heparin, the rats were bled continuously through a peristaltic pump at a flow rate of 50 μl, every 7 min. Blood samples were collected in Hamilton microliter syringes every 7 min for 3 hr from 10:30 to 13:30 hr. The samples were collected into assay tubes kept on ice and containing phosphate buffer (0.01 *M*) with 0.1% gelatin. Hematocrits remained stable with this bleeding protocol (43%–38%). Samples were centrifuged at 1500*g* for 15 min at 4°C, and the serum was kept frozen at −20°C until analyzed.

Prolactin radioimmunoassay. Prolactin concentrations in all series of samples from each rat were determined by a specific double-antibody radioimmunoassay. The reagents were kindly supplied by the National Hormone and Pituitary Program (NHPP, Rockville, MD). Prolactin values are expressed in terms of rat NIADD PRL RP-3 reference preparation. The sensitivity of the assay was 5 pg/tube. To analyze the variability of the assay, a series of plasma of not less than 10 replicates at four different concentrations of prolactin standard curve were run. At the level of 1.5 ng/ml the coefficient of variation (CV, $n = 10$) was 9.2%, at 6.25 ng/ml it was 6.3%, at 12.5 ng/ml 5.9%, and at 25 ng/ml 4.8%. Samples were analyzed 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 (18) and reviewed by Richard *et al.* (19) 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 all hormone values that comprised the ascending and descending phases of each potential pulse. The pulse was defined when this CV was three times higher than 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 analysed 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 minimizes both false positive and negative errors (20).

Pulsatile prolactin secretion pattern was characterized by the mean hormone levels, absolute and relative amplitudes of the peaks, their frequency, pulse duration, and the half-life of the hormone. 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 observed during the bleeding period. 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-h period, and the average for the experimental group from the individual means. The half-life of the hormone was calculated by the tangent of the descending portion of the peak. The program calculates the arithmetic mean of the tangent values of the descending portion of all peaks during the bleeding period and this value is given as half-life.

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 depending on the number of groups. The results were considered significant at $P < 0.05$. All values represent the mean ± SEM.

Results

Prolactin secretion in animals from all experimental groups was pulsatile, and a representative profile from one rat of each experimental group can be seen in Figure 1.

As expected, pituitary grafting significantly increased the mean levels of prolactin ($P < 0.001$), compared with sham-operated rats (Table I). CsA administration significantly decreased mean values of prolactin in pituitary-grafted rats ($P < 0.01$) but not in sham-operated animals (Table I).

The absolute amplitude of the prolactin peaks was significantly increased in pituitary-grafted compared with sham-operated rats treated with vehicle ($P < 0.001$, Table I). CsA administration decreased the absolute pulse amplitude of the hormone in pituitary-grafted ($P < 0.05$) but not in sham-operated animals (Table I).

In pituitary-grafted rats mean half-life of prolactin was longer than in sham-operated animals ($P < 0.05$, Table I). Administration of CsA decreased this parameter in sham-operated but not in pituitary-grafted rats ($P < 0.001$, Table I).

Pulse duration was not altered by the presence of ectopic pituitaries (Table I). Also, administration of CsA did not change the duration of prolactin pulses either in sham-operated or in pituitary-grafted animals (Table I).

The number of prolactin peaks was significantly decreased in pituitary-grafted male rats ($P < 0.05$, Table I) compared with sham-operated controls. Administration of CsA did not change the frequency of the prolactin pulses in either sham-operated or pituitary-grafted animals (Table I).

The relative amplitude of the prolactin pulses was not altered by the presence of an ectopic pituitary (Table I), but the treatment with CsA increased this parameter in sham-operated rats ($P < 0.05$, Table I).

Plasma prolactin levels increased in pituitary grafted animals ($P < 0.001$, Table I) as compared to the values found in the control group. CsA administration did not modify plasma prolactin levels in control animals but decreased them in pituitary grafted rats ($P < 0.01$, Table I).

Discussion

These results provide a detailed characterization of pulsatile secretory patterns of prolactin in normal and pituitary-grafted adult male rats treated with CsA and/or vehicle. Analysis of the prolactin pulsatile pattern in vehicle-treated male rats indicated that the occurrence of prolactin peaks was markedly irregular, thus resembling our previous findings in females (11, 12). This irregular pattern may be due to the existence of two pulse generators. One of them may originate in the hypothalamus, and depends on the dopaminergic activity (21), and the other is located within the pituitary and depends on the inherent pulsatile property of the lactotrophs (22). The lower number of prolactin peaks observed in male compared with female rats in diestrus may reflect the role of estrogen in generating prolactin pulses in females (11). The results obtained in this study are similar to those described by Lopez *et al.* (9), and the differences observed may be due to the use of different computer programs to calculate the parameters that define the pulsatile profile of prolactin and to the differences observed for plasma prolactin levels (over 1 ng/ml vs over 2 ng/ml in Ref. 9).

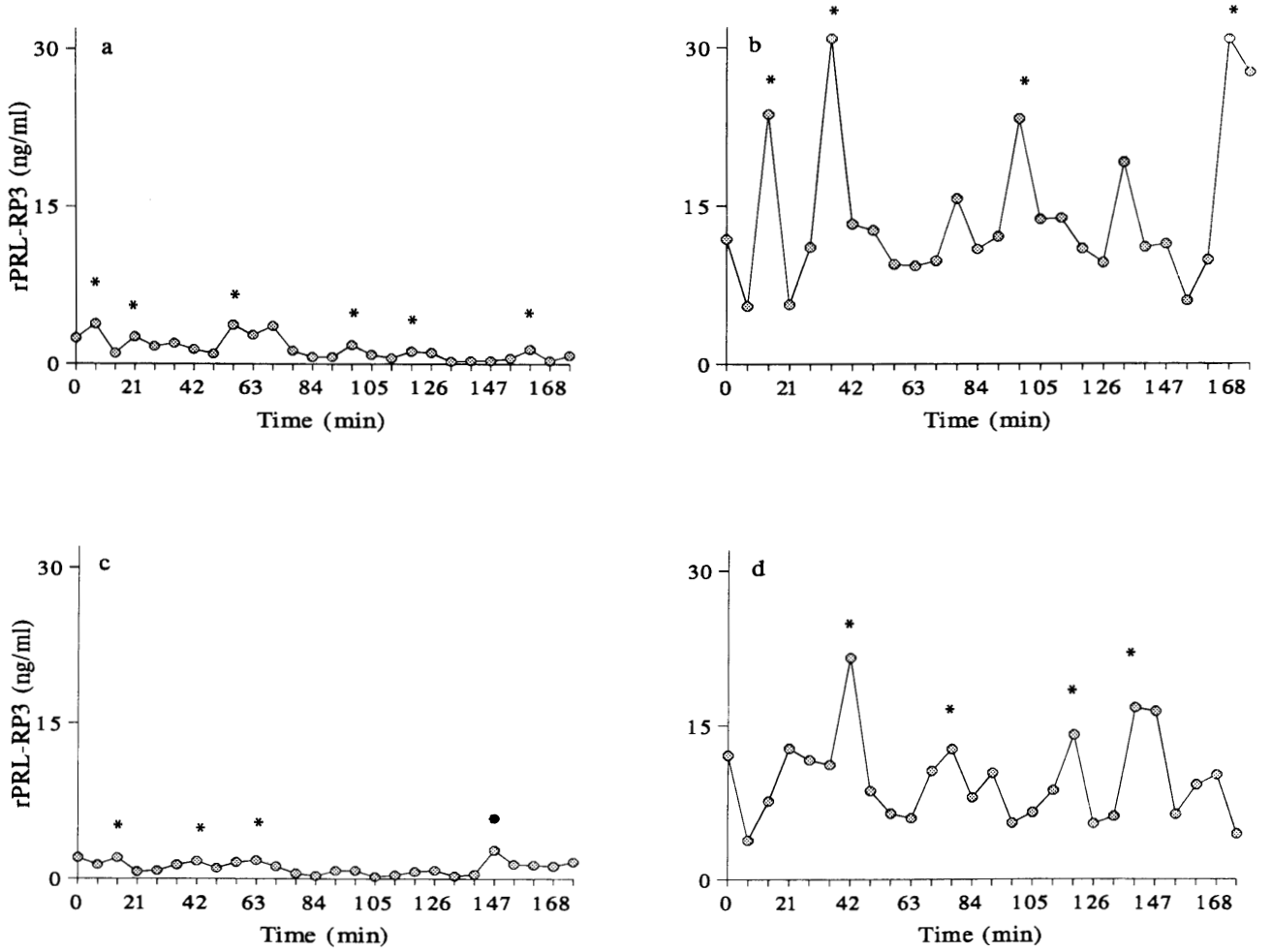

Figure 1. Individual pulsatile prolactin patterns in control rats (a); rats with pituitary grafts (b); CsA-treated rats (c); and CsA-treated pituitary-grafted rats (d). *A pulse.

Pituitary-grafted rats also showed an irregular prolactin pattern and exhibited a decrease in the number of hormone peaks during the period studied. However, these peaks were of higher magnitude and longer duration than in control animals. The latter change explained the increase in the mean plasma prolactin levels in pituitary-grafted compared with control rats observed in this study, which agrees with other reports using single sampling to measure the circulating values of prolactin (16, 23). These changes may be explained

Table I. Study of sham-operated and pituitary-grafted adult male rats treated with CsA or vehicle

Group	Mean serum prolactin levels (ng/ml)	Absolute amplitude (ng/ml)	Relative amplitude (%)	Frequency (pulses/3 hr)	Duration (min)	Half-life (min)	Plasma prolactin levels at sacrifice (ng/ml)
Sham-operated							
Vehicle	0.96 ± 0.12	0.83 ± 0.13	3.72 ± 0.62	5.43 ± 0.37	24.85 ± 1.40	9.38 ± 0.55	3.02 ± 0.58
CsA	0.98 ± 0.13	0.86 ± 0.18	6.62 ± 1.07[a]	4.86 ± 0.77	24.15 ± 1.72	5.53 ± 0.32[b]	3.50 ± 0.61
Pituitary-grafted							
Vehicle	12.5 ± 1.03[b]	18.19 ± 2.79[b]	3.16 ± 0.59	3.83 ± 0.70[a]	28.33 ± 3.74	13.22 ± 1.45[a]	21.50 ± 2.50[b]
CsA	6.98 ± 0.99[c]	12.22 ± 1.38[d]	3.63 ± 0.96	3.43 ± 0.72	38.00 ± 5.70	15.90 ± 3.67	13.50 ± 1.5[c]

Note: CsA (5 mg/kg weight per day) or vehicle, during 30 days, beginning on day 30 of life. Mean serum prolactin levels as an arithmetic mean of all samples obtained during the bleeding period. The relative pulse amplitude was calculated as the quotient between absolute amplitude and preceeding nadir value. Values are expressed as mean ± SEM. Eight animals per group were used in this study.

[a] $p < 0.05$ vs. sham-operated rats treated with the vehicle.
[b] $p < 0.001$ vs. sham-operated rats treated with the vehicle.
[c] $p < 0.01$ vs. pituitary-grafted rats treated with the vehicle.
[d] $p < 0.05$ vs. pituitary-grafted rats treated with the vehicle.

by the inhibition of the hypothalamic pituitary axis in grafted rats due to the well-known effect of hyperprolactinemia of increasing dopaminergic activity (24).

Under these circumstances, most of the prolactin present in the circulation may mainly come from the ectopic gland, as was suggested earlier (8, 12, 25). Therefore, the pulsatile pattern of the hormone in pituitary-grafted animals may be due to the persistence of the inherent pulsatile activity of the lactotrophs in the ectopic gland (22). The increase in the number of lactotrophs in the ectopic gland may also account for the higher mean values of the hormone during the period under study, as the number of these cells immediately increased after pituitary grafting (26; Esquifino, unpublished observations).

CsA had no effects on the mean concentration of prolactin in sham-operated rats, a finding similar to our previous observations (3). This may be explained by the significant reduction in the mean half-life of the hormone observed in this study. Under these circumstances, prolactin disappears faster from the circulation in sham-operated rats treated with CsA than in animals of the same group but treated with the vehicle. These data suggest that CsA in male rats modifies the rate of metabolism of peripheral prolactin.

However, in pituitary-grafted animals CsA treatment significantly decreased the absolute amplitude and the mean values of the hormone. These data suggest that CsA blunts the increase in plasma prolactin levels observed in nontreated animals after pituitary grafting (8). This effect could be explained by a direct effect of the drug on the ectopic pituitary gland as was shown in *in vitro* studies (8, 27). The latter may be explained by considering that CsA is decreasing the activity of the lymphocytes, which are infiltrating the ectopic gland (Esquifino *et al.*, unpublished observations). These cells, when activated, can synthetize great amounts of prolactin, which may contribute to the increase in plasma levels of the hormone, observed immediately after pituitary grafting. The treatment with the immunosuppressor may reduce the secretion of prolactin by the activated lymphocytes. Also *in vitro* studies showed that CsA's effects on the ectopic pituitary disappeared over time (27), thus explaining the moderate increase in plasma prolactin levels observed in pituitary-grafted animals treated with CsA. These changes may be considered evidence that the cells of the remaining pituitary are functional and suggestive of regulation (Esquifino, unpublished observations). The effects are opposite to those observed in animals submitted to pituitary grafting during the adulthood (8); this suggests that the effects of CsA are dependent on the age of the animals under study and/or on the length of the treatment with the immunosuppressor (28).

However, effects of the drug at hypothalamic level cannot be excluded. Changes in the metabolism of hypothalamic catecholamines and serotonin have been previously demonstrated (8), and these neurotransmitters are known to be involved in the regulation of prolactin (12, 29) and other hypophyseal hormone secretion (29). The effect of CsA increasing dopamine turnover at the hypothalamic level may counteract the effects of the drug on prolactin secretion in sham-operated animals. Further studies will allow us to better understand the interaction mechanisms between prolactin and cyclosporine regulating pituitary function and the rate of the secretion of the hormone as well.

In conclusion, CsA administration to male rats did not modify the pulsatile secretion pattern of prolactin in control animals but did increase its metabolism, perhaps by a direct effect of the drug on the kidney and/or the liver, although further studies need to be done to clarify the physiological relevance of these changes in prolactin metabolism. However, the differential effects of CsA on prolactin pulsatility in grafted animals may reflect a direct effect of the drug on the ectopic pituitary gland, although a hypothalamic site of action potentiating the effects of the hormone on catecholamine metabolism cannot be excluded. These data may give new insight into the effects of the drug on the rejection reaction that occurs after organ transplantation.

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1. Ingulli E, González R, Rajport D, Pomratz A, Sumrai N, Hong J, Butt KNH, Tejani A. Long-term outcomes of one haploidentical live related donor transplantation in children. Children Nephrology and Urology **9**:312–318, 1988–1989.
2. Davenport AT, Hodson CA. Effect of cyclosporin treatment on luteinizing hormone and prolactin. Life Sci **50**:1001–1006, 1992.
3. Villanua MA, Esquifino AI, Steger RW, Bartke A. Effects of treatment of normal and hyperprolactinemic rats with cyclosporine in vivo on the release of gonadotropins and prolactin from their pituitaries. Life Sci **50**:2037–2042, 1992.
4. Russell DH. New aspects of prolactin and immunity: A lymphocyte derived prolactin-like product and nuclear protein kinase c activation. Trends Pharmacol Sci **10**:40–44, 1989.
5. Bernard DJ, Maurizis JC, Sauvezie B, Bignon YJ, Chassagne J, Chollet P, Plagne R. Antagonism of prolactin binding by cyclosporine A on MCF breast tumour cell line. Anticancer Res **11**:2147–2152, 1991.
6. Mahajan PB, Ebner KE. Cyclosporin A and rabbit mammary prolactin receptors. Biochem Biophys Res Commun **133**:753–758, 1985.
7. Varma S, Ebner KE. The effect of cyclosporine A on the growth and prolactin binding to Nb-2 rat lymphoma cells. Biochem Biophys Res Commun **156**:233–239, 1988.
8. Esquifino AI, Moreno ML, Arce A, Agrasal C, Perez-Diaz J, Villanúa MA. Effects of cyclosporine at the hypothalamic-

pituitary axis in pituitary-grafted young female rats. J Endocrinol **144:**159–164, 1995.
9. Lopez FJ, Dominguez JR, Sanchez-Franco F, Negro-Vilar A. Role of dopamine and vasoactive intestinal peptide in the control of pulsatile prolactin secretion. Endocrinology **123:**740–746, 1989.
10. Lafuente A, Marcó J, Esquifino AI. Effects of hyperprolactinemia on prolactin and LH pulsatile pattern in female rats. Rev Esp Fisiol **48:**291–296, 1992.
11. Lafuente A, Marcó J, Esquifino AI. Pulsatile prolactin secretory patterns throughout the oestrous cycle in the rat. J Endocrinol **137:**43–47, 1993.
12. Esquifino AI, Marcó J, Lafuente A. Cyclosporine modifies the pulsatile secretory patterns of prolactin and luteinizing hormone in normal and pituitary-grafted female rats. Neuroendocrinology **60:**581–588, 1994.
13. Lafuente A, Marcó J, Esquifino AI. Physiological roles of thyrotrophin releasing hormone and vasoactive intestinal peptide on the pulsatile secretory patterns of prolactin in pituitary grafted female rats. J Endocrinol **142:**581–586, 1994.
14. Sikka SC, Bashim C, Coy DC, Koyle MA, Swerdloff RS, Refjer J. Effect of cyclosporine on the hypothalamic-pituitary-gonadal axis in the male rat: Mechanism of action. Endocrinology **123:**1069–1074, 1988.
15. Ramirez G, Narvarte J, Bittle PA, Ayers-Chastain C, Dean SE. Cyclosporine-induced alterations in the hypothalamic hypophyseal gonadal axis in transplant patients. Nephron **58:**27–32, 1991.
16. Mena F, Haiweg H, Grosvenor CE. Effect of ectopic pituitary glands upon prolactin concentration in the in situ pituitary of lactating rats. Endocrinology **83:**1359–1362, 1968.
17. Tresguerres JAF, Esquifino AI. Dissociation in the regulation of luteinizing hormone and follicle-stimulating hormone in a hyperprolactinaemic rat model: Interrelationships between gonadotrophin and prolactin control. J Endocrinol **90:**41–51, 1981.
18. Van Cauter E. Diurnal and ultradian rhythms in human endocrine function a minireview. Horm Res **34:**45–53, 1990.
19. Richard JL, Bringer J, Daurès JP, Parer-Richard C, Jaffiol C, Mirouze J. Méthodes d'analyse de la pulsatilité hormonale. Ann Endocrinol (Paris) **51:**181–193, 1990.
20. Van Cauter E. Estimating false-positive and false negative errors in analyses of hormonal pulsatility. Am J Physiol **254:** E786–E794, 1988.
21. Pohl CR, Weiner RI, Smith MS. Concordance between LH and prolactin pulses in ovariectomized rats in the presence or absence of dopamine inhibition. Endocrinology **123:**1591–1597, 1988.
22. Shin SF, Reifel CW. Adenohypophysis has an inherent property for pulsatile prolactin secretion. Neuroendocrinology **32:**139–144, 1988.
23. Bartke A, Smith MS, Michael SD, Peron FG, Dalterio S. Effect of experimentally-induced chronic hyperprolactinemia on testosterone and gonadotropin levels in male rats and mice. Endocrinology **100:**182–186, 1977.
24. Cramer OM, Parker CR, Porter JB. Secretion of dopamine into hypophyseal portal blood by rats bearing prolactin-secreting tumors or ectopic pituitary glands. Endocrinology **105:**636–640, 1979.
25. Everett JW. Luteotropic function of autografts of the rats hypophysis. Endocrinology **54:**685–690, 1954.
26. Iturriza FC, Rubio MC, Gómez Dum CLA, Zieher LM. Catecholamine metabolizing enzyme and synthesis of dopamine in normal and grafted pituitary pars distalis. Neuroendocrinology **37:**371–375, 1983.
27. Moreno ML1, Villanúa MA, Arce A, Esquifino AI. Cyclosporine effects on In Vitro responsiveness of anterior pituitary hormone release to dopamine and thyrotropin-releasing hormone in young female rats. J Neuroendocrinol **7:**765–774, 1995.
28. Arce A. Papel de la prolactina y la inervacion autonomica en los efectos de la ciclosporina sobre la respuesta immunologivca de los ganglios linfaticos submaxilares. Doctoral Thesis (Directed by Esquifino AI), Universidad Autonoma de Madrid, July 1994.
29. Tuomisto J, Manisto P. Neurotransmitter regulation of anterior pituitary hormones. Pharmacol Rev **37:**249–332, 1985.