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# Effects of cyclosporine on circulating levels of prolactin, LH, FSH, TSH and GH in chronic hyperprolactinemic male rats

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The effects of cyclosporine (CyA) on pituitary hormone secretion in animals with previously high plasma prolactin levels have been studied. Hyperprolactinemia was either induced in 30 day old male rats by the transplantation of one anterior pituitary gland from a litter mate donor or they were sham-operated to be used as controls. Both pituitary-grafted and sham-operated animals were injected s.c. with the vehicle or CyA (5 mg/kg weight per day) for 10 days, beginning 30 days after surgery. As expected, pituitary grafting markedly increased plasma prolactin levels as compared with the values found in control animals. Hyperprolactinemia was associated with reduced plasma LH and GH levels, increased plasma TSH levels and with no changes in circulating FSH levels. CyA administration to control animals increased plasma prolactin and TSH levels, decreased plasma levels of LH and did not modify circulating values of FSH and GH. Furthermore, CyA administration to pituitary-grafted animals decreased plasma prolactin and TSH levels, whereas plasma concentrations of GH and gonadotropins did not change. These data suggest that CyA differentially affect the release of pituitary hormones and that there is an interrelationship between previously high plasma prolactin levels and CyA to modulate pituitary hormone secretion.

Kew yords: Cyclosporine, Hyperprolactinemia, Prolactin, GH, TSH, LH, FSH.

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Prolactin is known to exert direct effects at the hypothalamic level to regulate its own secretion through the modulation of catecholamine, GABA or serotonin metabolisms (12, 24). Hyperprolactinemia, induced by pituitary grafting, disrupts the normal metabolism of these neurotransmitters (17, 18), leading to changes in the hypothalamic neuropeptides secretion, involved in the regulation of pituitary secretion (31). Most current literature deals with the effects of hyperprolactinemia on gonadotropin secretion (30). The existence of an interrelationship between prolactin and the other pituitary hormones, however, has been explored less (3, 4).

On the other hand, patients undergoing organ transplantation treated with cyclosporine (CyA) have been associated with neuroendocrine side effects (28), which include gonadal function impairment in both adult males (29) and females (15). The extension of CyA treatment to patients during childhood, showed an impaired growth and alterations in the gonadal functions as well. The mechanisms involved in these alterations, however, are not fully understood. CyA effects on the gonadal function have been previously shown to be age dependent (5).

Furthermore, the existence of an interrelationship between prolactin and CyA to regulate prolactin secretion from the in situ and ectopic pituitaries both *in vivo* (16) and *in vitro* (26) has been found. All these data have been obtained in animals treated with CyA from the day of surgery (16) to prevent ectopic pituitary rejection. The possible modulatory role of previously high plasma prolactin levels on the neuroendocrine effects of CyA, however, has been explored less.

This study was designed to analyze the effects of previously high plasma prolactin levels on the CyA effects on pituitary hormone secretion.

## Materials and Methods

Male rats of the Sprague-Dawley strain were used throughout the investigation. They were maintained in a room with controlled photoperiod (14 h L/10 h D; lights on from 06.00 to 20.00 h), and temperature (22 ± 2 °C) and with rat chow and water available *ad libitum*.

The studies were conducted in accordance with the principles and procedures outlined in the NIH guide for Care and Use of the Laboratory Animals.

*Hyperprolactinemia induction.*– At 30 days of age, half of the animals received a transplant under the right kidney capsule, of an anterior pituitary gland from a littermate donor (30). Age-matched male rats were sham-operated to be used as controls. Surgery was performed under tribromoethanol anaesthesia, 250 mg/kg, ip. (Merck).

*Cyclosporine administration.*– Cyclosporine was generously provided by Sandoz and was prepared as follows: 10 mg cyclosporine were dissolved in 0.5 ml absolute ethanol and further diluted with olive oil to a final concentration of 1 g/l. Both grafted and sham-operated rats were submitted to CyA (5 mg/kg b. w. per day, s.c.) or vehicle treatment for 10 days, beginning at 60 days of age. Animals were killed by decapitation at 70 days of age, 20 h after receiving the last injection of CyA or vehicle.

*Blood sampling.*– After decapitation, trunk blood was collected in tubes containing EDTA (60 g/l) and plasma was obtained after centrifugation for 15 min at

1,500 g at 4 °C. Plasmas were kept frozen at –20 °C until they were analyzed.

*Hormone determinations.*– Plasma hormone concentrations were measured by specific double antibody radioimmunoassay systems using material kindly supplied by the National Hormone and Pituitary Program (NHPP, Rockville, MD). Hormonal values were expressed in terms of NIADD rat PRL RP-3, GH RP-2, TSH RP-3, LH-RP3 and FSH RP-2 reference preparation for prolactin, growth hormone (GH), thyrotropin (TSH), luteinizing hormone (LH) and follicle stimulating hormone (FSH), respectively. The assay sensitivity was 5, 2, 40, 0.5 or 20 pg/tube for prolactin, GH, TSH, LH and FSH, respectively. Samples were analyzed within the same assay to avoid interassay variations. The intra-assay coefficient of variation was 7.2 %, 8.3 %, 6,8 %, 7.4 % and 9.4 % for prolactin, GH, TSH, LH and FSH, respectively.

*Statistical analysis.*– Statistical analysis of results was performed by using the Student's *t* test or one way analysis of variance (ANOVA) followed by Duncans's multiple range test. The results were considered significant at $p < 0.05$. All values represent the mean ± SEM.

## Results

Pituitary-grafting significantly increased plasma levels of prolactin and TSH (table I), while plasma GH and LH levels significantly decreased as compared to controls. No changes in plasma FSH levels were found.

CyA administration to sham-operated rats significantly increased plasma prolactin and TSH levels (table I) as compared to rats from the same group treated with vehicle. Besides, plasma LH levels significantly decreased after CyA treatment. Plasma levels of GH or FSH did not change.

Plasma prolactin levels decreased in pituitary-grafted rats after CyA treatment (table I), although hyperprolactinemia persisted. Furthermore, CyA treatment to animals of this group blocked the increase of TSH values found in vehicle treated rats of same group. CyA treatment to pituitary-grafted rats did not further modify plasma levels of GH, LH and FSH.

TABLE I. *Plasma levels (ng/ml) of prolactin, TSH, GH, LH and FSH, in adult male rats bearing an ectopic pituitary from day 30 of life.*

Age matched sham-operated rats were used as controls. On day 60 of life half of the animals of each experimental group were submitted to the administration of cyclosporine (5 mg/Kg/day) for 10 days beginning on day 60 of life. The other half were injected with the vehicle, using the same experimental design. Values are expressed as mean+SEM.

| Group | rPRL-RP-3 | rTSH-RP-3 | rGH-RP-2 | rLH-RP-3 | rFSH-RP-2 |
|---|---|---|---|---|---|
| sham-Veh. | 0.98 ± 0.13 | 6.55 ± 0.78 | 26.99 ± 3.62 | 6.43 ± 0.59 | 14.15 ± 2.36 |
| sham-CyA | 1.78 ± 0,15** | 9.44 ± 2.44 | 26.57 ± 6.42 | 3.42 ± 1.54* | 12.77 ± 2.57 |
| PG-Veh | 14.37 ± 2.13*** | 14.05 ± 2.29** | 20.17 ± 3.62* | 5.12 ± 0.28* | 12.24 ± 0.89 |
| PG-CyA | 5.98 ± 1.10## | 7.26 ± 1.66## | 21.11 ± 4.48 | 5.47 ± 0.26 | 11.13 ± 2.22 |

*P < 0.05, **P < 0.01, ***P<0.001 vs. sham-operated rats with vehicle administration. #P < 0. 05, ##P < 0.01 vs. Pituitary-grafted rats with vehicle administration.

## Discussion

The present results suggest that CyA modifies the secretory pattern of pituitary hormones differentially, as well as the existence of interactions between high plasma prolactin levels and CyA to modulate the release of pituitary hormones. A direct effect of the drug on the pituitary seems to be evident, but hypothalamic effects cannot be excluded if the data from control rats are considered.

Increased plasma prolactin levels in pituitary-grafted rats reported here, have been repeatedly observed in various studies (1, 14, 22, 23) where different sampling protocols were used. The ectopic origin of the increased prolactin levels is indicated by both *in vivo* (18) and *in vitro* (13) studies, and by histological ones showing the presence of a lymphocytic infiltration that resembles a reaction of graft-rejection (27). Activated lymphocytes release prolactin (7), which can contribute to the early increase in plasma prolactin levels found after grafting.

Hyperprolactinemia has been associated to changes in the secretory pattern of other pituitary hormones (3, 4, 21). Most of these investigations, however, analyzed the interrelationship between prolactin and gonadotropins (10, 25, 30). In this study, the existence of an inverse relationship between prolactin and LH, was shown, as previously described, by using the same experimental protocol (3, 30). The absence of changes in plasma FSH was in agreement with previous reports from our group in female rats, when the hormonal measurements were made at similar times after surgery (3, 30).

Hyperprolactinemia was associated to increased plasma TSH levels. This finding is contrary to previous works (2, 4). The discrepancies may be due to experimental protocol differences when measuring the circulating hormone values. Sex differences may also explain the differential effect of hyperprolactinemia on TSH secretion in male vs. female rats, when the existence of dimorphic regulatory mechanism for other pituitary hormones is considered (8). The discrepancies observed in previous studies (4, 11) may also be due to the time elapsed after surgery, when plasma TSH levels were measured. Furthermore, time dependent effects of hyperprolactinemia on the secretion of another pituitary hormone (FSH) has been reported (27) and other physiological functions seemed to be similarly affected (5). These effects were not dopamine dependent, considering that DA metabolism always increases in hypeprolactinemic states (16). This increase in DA metabolism has previously been found to be associated to decreased plasma TSH levels (4, 31).

Plasma GH levels were decreased in this study 40 days after surgery, in agreement with previous ones (12). This inhibitory effect was not observed in female rats,which seems to support the existence of sex dependent regulatory mechanisms for GH secretion, as previously suggested (8). This circulating GH reduction might be due to the increased dopaminergic tone induced by hyperprolactinemia, as mentioned above. No changes in GH secretion were found, however, in hyperprolactinemic animals in other studies. The anesthesia effect in the obtention of blood samples and the rather small number of animals used in those reports could account for the differences observed.

CyA treatment to sham-operated rats modified pituitary hormone secretion specifically and differentially as shown by plasma prolactin and TSH concentrations increases, together with a reduction in plasma LH levels. CyA administration, however, did not modify plasma GH and FSH levels. CyA effects in sham-operated rats on plasma LH may be explained by a

drug hypothalamic action, reducing LHRH content as observed in female rats (13), while it confirms the hypothesis advanced by SIKKA *et al.* (29). Unexpectingly, however, CyA treatment did not modify plasma FSH levels in males, whereas it did in females, suggesting that sex differences may account for such effects.

The plasma prolactin reduction after CyA administration in pituitary-grafted rats could be explained through a direct effect of the drug on the ectopic gland (26) acting mainly on the lymphocytes within it (ESQUIFINO *et al.* unpublished observations). The drug effects tend to disappear in time, so that the reduction of previously high plasma prolactin levels was not as potent in time function, as in female rats (16). The drug effects in pituitary-grafted animals also indicated that high plasma prolactin levels interfere with CyA effects on the hypothalamic-pituitary axis similarly to other described neuromodulators (18). In control animals, however, the drug effects suggest the existence of a hypothalamic action site as in previous works (16). The differential drug effects in control vs. pituitary-grafted animals may be due to the presence of the ectopic gland in the latter group, which interferes in the normal regulatory mechanism operating at the hypothalamic pituitary axis.

After CyA treatment, prolactin and TSH values increased in pituitary-grafted rats and decreased in sham-operated ones. These data suggest that the drug may change the metabolism of various neuromodulators at the hypothalamus in control animals as shown in previous studies (15, 16, and ESQUIFINO, unpublished observations).

In conclusion, the present data suggest that an interrelationship between high plasma prolactin levels and CyA modulate pituitary hormone secretion, the main effects of which are observed on prolactin itself and on LH and TSH secretion patterns. CyA also differentially affect the secretory patterns of pituitary hormones.

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A. LAFUENTE, E. ÁLVAREZ-DEMANUEL, A. BLANCO, M. GARCÍA-BONACHO and A. I. ESQUIFINO. *Efectos de la ciclosporina sobre los niveles circulantes de prolactina, LH, FSH, TSH y GH en ratas macho hiperprolactinémicas.* J. Physiol. Biochem. (Rev. esp. Fisiol.), 52 (3), 161-166, 1996.

Se estudian las posibles modificaciones inducidas por la ciclosporina (CyA) sobre la secreción hormonal hipofisaria, en ratas macho adultas con niveles de prolactina elevados previamente. Se realiza el trasplante de la hipófisis de un hermano de camada bajo la cápsula renal o su operación simulada en los animales controles, en el día 30 de vida. A ambos grupos de animales se les administra s.c. vehículo o CyA (5 mg/Kg /día) durante 10 días, comenzando el tratamiento 30 días después de la operación quirúrgica. La presencia de una hipófisis ectópica incrementa los niveles plasmáticos de prolactina. La hiperprolactinemia está asociada con una disminución en los niveles plasmáticos de LH y GH, y con un incremento en la concentración plásmatica de TSH, no modificándose los niveles circulantes de FSH. Tras la administración de CyA en las ratas con operación simulada, aumentan los niveles plasmáticos de prolactina y TSH y disminuyen los de LH, no modificándose los de FSH y GH. En cambio, al administrar el inmunosupresor CyA a los animales con hipófisis ectópicas, se observa un descenso en la concentración plasmática de prolactina y TSH, mientras que permanecen inalterados los niveles de GH y gonadotropinas. Estos datos sugieren que la CyA afecta diferencialmente los patrones secretores de las hormonas hipofisarias y que los niveles de prolactina previamente elevados interfieren con los efectos de la CyA sobre los

mecanismos de secreción de las diferentes hormonas hipofisarias.

Palabras clave: Ciclosporina, Hiperprolactinemia, Prolactina, GH, TSH, LH, FSH.

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