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Morphological study of the rat pituitary follicle stimulating hormone cells after alternate day treatment with luteinizing hormone-releasing hormone (LHRH)

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Summary. The action of the luteinizing hormone-releasing hormone (LHRH) on the hypophyseal gonadotrophins has either an activatory or inhibitory effect depending on the doses administered or on the treatment followed. Both factors can induce a different response in the two hormones. In this work, the effect after the administration of 8 doses (40 μg/day) of LHRH at intervals of 48 hours, on the serum levels of follicle stimulating hormone (FSH), as well as the numerical density, distribution, intensity of staining and morphometrical parameters of the cells which react against the anti-FSH serum, are assessed. It has been found that with the treatment an increase of FSH serum levels, without modification in the number of immunoreactive cells, but a clear increase in the lightly stained cells, is produced. The distribution of the reactive cells, uniform in normal animals, shows a large numerical density in the dorsal and posterior hypophyseal areas in the treated animals. No change was observed in the nuclear and cellular areas between the different groups.

Key words: Anterior pituitary, FSH cells, Immunocytochemistry, LHRH treatment, Morphometry

Introduction

The existence of a single hypothalamic hormone which stimulates the gonadotrophic function, the LHRH, with an activatory action of both LH and FSH (Schally et al., 1971), is now disputed, as it is known that the two gonadotrophins respond differently to the LHRH (De Paolo, 1985; Culler and Negro Vilar, 1986). At the same time, the co-existence of both hormones in the same cell is accepted, and while in a few cells only one of the hormones is found (Childs et al., 1980), Inoue and Kurosumi (1984) showed that none of the pituitary cells reacted to anti-body against only one gonadotrophin. However, some cells are described as being «LH rich» while others are «FSH rich».

The LHRH presents a particular rhythm of pulsatile secretion of LHRH (Carmel et al., 1976; Levine and Ramirez, 1980) and its modification can induce the activation or inhibition of the hypophyseal function, or the preferential secretion of one of the hormones (Wise et al., 1979). This had led us to study the effect of high and repeated doses of LHRH on alternate days on the secretion of FSH and its action on hypophyseal cells stained with anti-FSH serum, with the aim of establishing the response to the treatment and, if any, the relation between the FSH serum levels and the morphological features of the FSH cells.

Materials and methods

Eighteen adult female Sprague Dawley rats, weighing between 250-300 g were used. Animals were housed on a day-light regime at a temperature 20 ±2° C and had free access to food and water. At the beginning of the experiment all the animals were in the preoestrous phase, confirmed by vaginal smears.

At 10.00 hours, on alternate days, over a period of 15 days, six of the animals were injected intra-muscularly (T) with 40 μg. of LHRH diluted in 0.1 ml of distilled water. The animals were sacrificed between 17.00 and 18.00 hours on the day of the last dose. Six female rats considered as controls (C), were injected with 100 μl of distilled water under the same conditions. The group of normal animals (N) was made up of six female rats sacrificed on the preoestrous afternoon without any previous treatment.

Animals were killed by decapitation, and the blood from trunk was collected in order to determine the serum

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levels of r-FSH by means of RIA, using NIH serum (Bethesda, Maryland), the threshold of analysis was 0.35 ng/ml.

The hypothalamus-hypophyseal block was carefully removed and fixed immediately in Bouin-Hollande solution and embedded in paraffin. Sagital sections were cut at 5 μ thickness through each entire gland and were mounted. Immunohistochemical staining was performed by the PAP method (Sternberger et al., 1970), using rabbit anti-FSH serum (Dako) (1:800).

For the study of the topographical cell distribution by light microscope (Carretero, 1984), the previously cut hemihypophysis were further divided into three sections (Fig. 1): medial, lateral and peripheral, determining in each four quadrants - two dorsals (anterior and posterior) and two ventrals (anterior and posterior). In each quadrant 28 different areas were photographed and studied, assessing on each the numerical density of the FSH cells (ND = number of reactive cells/ $3.66mm^2$).

Calculation of FSH cell surface area, as well as nuclear area was carried out with the use of a graphic table. For each animal, the parameters of at least 50 cells per group were plotted and the means of those data were calculated. Statistical analysis of these results was performed by using *t* test.

Results

Serum levels of FSH. There was an increase of the serum levels of FSH following treatment with LHRH (2.943 ±0.871 ng/ml), in relation to those of normal and control animals, which were undetectable in the analysis.

Morphology and distribution. Cells reactive against anti-FSH serum in normal females showed a great variation of shape. They frequently send cytoplasmic processes towards the vascular spaces (Figs. 2 and 3). The distribution of peroxidase immunoreactive material in the cytoplasm was homogeneous, although with different intensity of staining; it was not possible to establish a strict division of the cells according to this standard. The nucleus was usually excentrically placed. The intra-hypophyseal distribution of FSH cells of this group was uniform in the different quadrants and sections, with no significant differences among them (Table 1).

FSH cells in *female controls* (Figs. 4 and 5) showed the same morphological characteristics as the nomal animals, but the total number of stained cells (Table 2) was lower ($P<0.05$) than in normal females (Table 1). The medial section of this group of animals had a larger ND ($P<0.05$) than the lateral and peripheral sections, with no differences in the latter two. There was no difference between the different quadrants.

Although, in the samples from *LHRH treated females* strongly stained cells were seen, lightly stained cells were more abundant. *Light cells* (Figs. 6 and 7), in which hardly any peroxidase immunoreactive material remained, and which were generally concentrated in the cell periphery, were characteristic of this group. Though significant differences did not exist in the sectional distribution (Table 3), a greater cellular density of dorsal and posterior areas ($P<0.05$) than of ventral and anterior ones, was appreciable in the medial and lateral sections. The ND of this group was the same as that of the normal animals, but greater ($P<0.05$) than the controls.

Morphometric Analysis. No significant differences were found in either the cellular area (N = 123.891 ±33.829 μm^2, C = 131.039 ±46.428 μm^2 and T = 126.683 ±27.281 μm^2), in the nuclear area (N = 23.671 ±5.605 μm^2, C = 25.129 ±7.805 μm^2 and T = 22.021 ±5.101 μm^2) or in the nucleo-cytoplasmic ratio (N = 0.253 ±0.087, C = 0.269 ±0.125 and T = 0.233±0.076) of the three groups.

Table 1. Numerical density of FSH cells, Normal females.

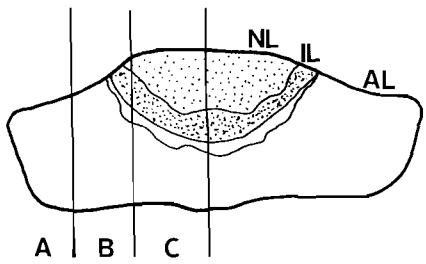
QUADRANTS	MED. SECT. χ	σ	LAT. SECT. χ	σ	PERIPH. SECT. χ	σ	AV. VOL. χ	σ
DORSAL	4.767	± 0,375	4.607	± 0,107	4.750	± 0.000	4.708	± 0,055
VENTRAL	4.995	± 0,178	5,089	± 0,088	4.374	± 0,839	4.821	± 0,349
ANTERIOR	4.606	± 0,214	4,839	± 0,339	4.142	± 0,607	4.529	± 0,266
POSTERIOR	5.160	± 0,107	4.857	± 0,142	4.982	± 0,232	4.999	± 0,054
AV. DENSITY	4.833	± 0,315	4.848	± 0,260	4.562	± 0,622	4.764	± 0,453

Table 2. Numerical density of FSH cells. Control females.

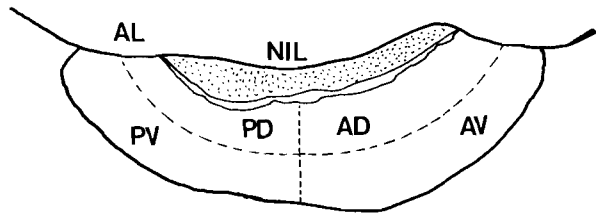
QUADRANTS	MED. SECT. χ	σ	LAT. SECT. χ	σ	PERIPH. SECT. χ	σ	AV. VOL. χ	σ
DORSAL	4.589	± 0,125	4.088	± 0,466	3.410	± 0.303	4.029	± 0,143
VENTRAL	5.412	± 0,587	3,678	± 0,500	3.785	± 0,143	4.291	± 0,835
ANTERIOR	4.644	± 0,180	3,856	± 0,678	3.410	± 0,107	4.107	± 0,601
POSTERIOR	5.357	± 0,642	3.910	± 0,268	3.347	± 0,267	4.213	± 0,600
AV. DENSITY	5.000	± 0,591	3.883	± 0,516	3.597	± 0,302	4.160	± 0,776

Table 3. Numerical density of FSH cells, LHRH treated females.

QUADRANTS	MED. SECT. χ	σ	LAT. SECT. χ	σ	PERIPH. SECT. χ	σ	AV. VOL. χ	σ
DORSAL	4.749	± 0,321	5.196	± 0,321	5.133	± 0.348	5.026	± 0,131
VENTRAL	4.964	± 0,286	3,981	± 0,660	4.053	± 0,589	4.332	± 0,488
ANTERIOR	4.839	± 0,411	4,142	± 0,821	4.124	± 0,660	4.374	± 0,758
POSTERIOR	4.874	± 0,196	5.035	± 0,392	5.062	± 0,419	4.990	± 0,416
AV. DENSITY	4.856	± 0,325	4.588	± 0,783	4.593	± 0,725	4.679	± 0,656



-Fig. 1a-

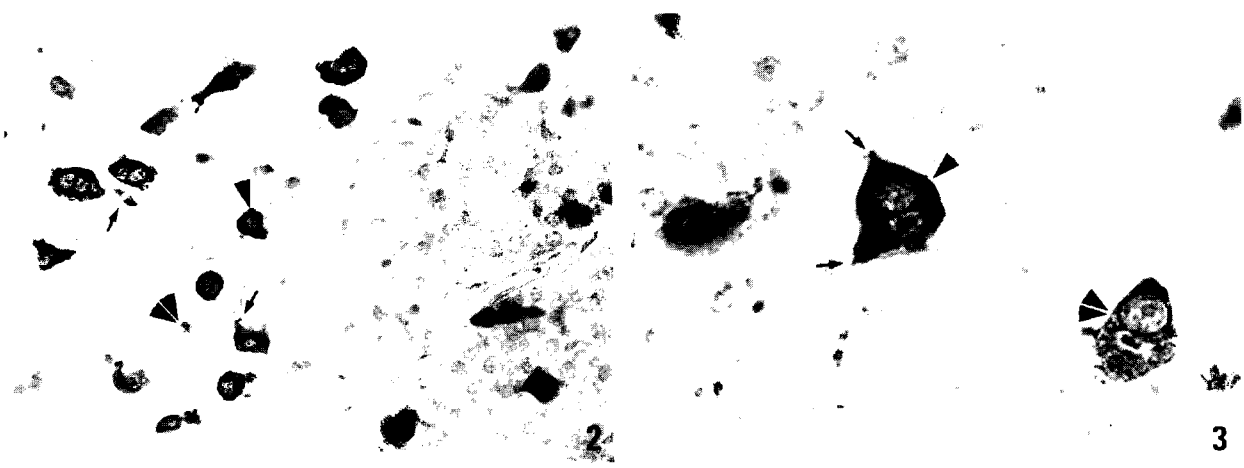


-Fig. 1b-

Fig. 1a. Schematic representation of a coronal cut through the hypophysis of the rat. Three sagital planes divide each hemihypophysis into three sections, (A) peripheric, (B) lateral and (C) medial. (AL Anterior-Lobe, (IL) Intermediate-lobe, (NL) Neural-lobe.

Fig. 1b. Sagital view of the hypophysis, which correspond to the lateral section, showing the division into four quadrants: (PD) postero dorsal, (PV) postero ventral, (AD) antero dorsal and (AV) antero ventral. (NIL) Neurointermediate-Lobe.

Figs. 2-7. Anti-FSH reactive cells. Figs. 2 and 3 correspond to normal females. Most of the cells are strongly stained (one arrow head) being present some weakly stained cells (two arrow heads). Some of these cells show cytoplasmic processes (arrows). FSH cells in control females (Figs. 4 and 5) are similar to those seen in the normal group, although a great number of weakly stained cells (two arrow heads) could be pointed out. In Figs. 6 and 7 slightly stained cells, (three arrow heads) frequently with the immunoreactive material concentrated in the cytoplasmic marginal area, «light cells» are the top feature of the LHRH treated animals. Figs. 2, 4 and 7, × 960. Figs. 3, 5 and 7, × 2400



Discussion

The physiological action of LHRH on gonadotrophin secretion, maintained by its pulsatile liberation, can modify and provoke an inhibitory effect when the hypophysis is exposed continuously to the hypothalamic hormone and/or to high doses of LHRH or LHRH-superacting analogues (Schuiling et al., 1984). For the hypophysis to recover its functions after being subjected to the inhibitory influence of LHRH, a period of latency is necessary (Koiter et al., 1981a).

Althoug it is well known (Wise et al., 1979; Wildt et al., 1981) that depending on the dosage and rhythm of LHRH administration, hypophyseal LH and FSH secretion may differ, and that the development of the response to an acute dose of LHRH is different in both gonadotrophins, the results obtained in this work are very similar to those we have previously obtained for LH (Carbajo, 1987).

Following the treatment we have found a decrease of immunostained material in FSH cells, as we did in those stained with anti-LH serum (Carbajo, 1987). These results do not agree with those obtained by Römmler et al. (1978), who found that the population of secretory granules in gonadotrophs, after a large initial liberation, is recovered 120 minutes after the administration of an acute dose of LHRH. However, our results are closer to those obtained by Watanabe (1986) in the female rat, observing a pronounced depletion of immunoreactive material in LH cells 24 hours after LHRH continous or pulsatile exposure *in vitro*.

Considering that the hypophyseal and serum levels of the hormone may diverge, Koiter et al. (1981b) showed that after inducing an inhibition of the hypophyseal response by the continuous administration of LHRH, there was a great decrease of the pituitary hormonal store, but that, after discontinuation of the infusion period, the pituitary responsiveness to LHRH recovers more rapidly than the hypophyseal hormonal content. This leads us to assume that the recovery of the *pool of releasable hormones* is quicker than that of the *storage pool*.

The frequent finding of FSH cells in treated females, in which the stained material, with an irregular distribution and granular aspect, accumulates in the periphery of the cytoplasm, may correspond to the migration of secretory granules described as moving towards this zone by Lewis et al. (1985) after the administration of LHRH and to that described by Blake (1980), after the pre-ovulatory peak of LH in

the rat. This fact could also be connected to the peripheral localisation of the *pool of releasable hormones* proposed by Bremner and Paulsen (1974) and with the possible relation of the marginal granules near the plasmalemma with the most readily releasable form of LH (Lewis et al., 1984). Although there are no firm theoretical foundations which enable us to establish an exact topography of the dynamics of hormonal liberation within the gonadotrphic cells, taking together those data, with the decrease and redistribution of the immunoperoxidase reactive material showed in the treated animals, we can suggest that under the present experimental conditions, whilst the *central storage compartment* is disminished, the *peripheral releasing compartment* remains in good condition.

The area of gonadotrophic cells is modified under different experimental situations. This area increases as does hormonal secretion in castration (Childs et al., 1982) or diminishes when the hypophyseal function is supressed by long-term treatment with an LHRH superactive analogue (Dubé et al., 1987). In our experiment, the area of FSH cells in the treated animals is similar to that of normal and control animals, as previously found in LH cells of mice by Lewis et al. (1985), after stimulation of gonadotrophin secretion with LHRH.

Although in the control animals a decrease of the ND has been observed with a dominance of this in the medial section, for which we have no clear explanation, in the treated animals the ND is similar to that of the normal animals, with a greater ND in the dorsal and posterior areas at the expense of the medial and lateral sections. This modification, which is in contrast to the homogeneous distribution in normal animals, could be due to the existence of a functional regionalisation within the hypophysis, depending on the source of LHRH, as suggested by Li et al. (1984).

Given these results we can conclude that the administration on alternate days of high doses of LHRH has an activating effect on the release of FSH with a parallel decrease in the hypophyseal reactive material. At the same time, the existence of a hypophyseal redistribution of FSH cells, with a greater ND in the dorsal and posterior quadrants, should be considered. Further studies must be carried out to confirm the funtional significance of those data.

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