Developmental changes in LH secretion by male pituitaries *in vitro*: from the infantile to adult period

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Abstract

The secretion of LH from the anterior pituitary of male rats was studied at different periods of postnatal development. According to an established classification we used rats 14 (infantile), 23 (juvenile), 45 (pubertal) and 90 (adult) days old. By using an *in vitro* incubation system, both basal and stimulated LH secretion were studied in the same gland. Age-related differences were observed in basal LH secretion, with juvenile and pubertal pituitaries showing higher secretion compared with infantile and adult pituitaries. However, the GnRH-induced secretory response was significantly higher in the infantile rats than in other ages. LH secretion was also studied in primary cultures from infantile or adult pituitaries. In 24 and 48 h cultures, infantile cells showed a significantly larger response to GnRH than that of adult cells. In the infantile pituitary LH-immunopositive cells showed differences in

size at different locations in the gland. At the periphery of the lobes the predominant cells were smaller and angular shaped, whereas in the center of the gland the majority of the cells were ovoid shaped. In the adult pituitary, the predominant LH-positive cells were ovoid in shape and larger in size. Furthermore, 10% more LH-positive cells were observed in infantile pituitaries. On the basis of these data we propose that at the infantile period the male rat pituitary has two populations of LH-secreting cells, one with adult secretory function and shape and a second with increased sensitivity to GnRH and with a morphology atypical of the adult cell. The results presented support the hypothesis that the infantile period is a transitional stage in the rat pituitary development.

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Introduction

It is well documented that the hypothalamus–pituitary–gonad axis is already established in the new-born male rat (Goldman *et al.* 1971, Swerdloff *et al.* 1971, Grady 1986, Negro-Vilar & Valenca 1988, Lalau *et al.* 1990). However, and from a developmental point of view, the rat is born with a reproductive system comparable to that observed in humans at 150 days of gestation life (Ojeda & Urbanski 1988).

In the male rat the serum concentration of luteinizing hormone (LH) fluctuates during postnatal development until adulthood is reached. Two peaks in LH serum concentrations have been observed: the first one starts in the second week, reaches its maximum at approximately 14 days of age and is followed by a rapid decline, whereas the second peak is present during the peripubertal period, between days 40 and 45 (Ojeda & Ramírez 1972, Döhler & Wuttke 1975, Chiappa & Fink 1977). In contrast to these observations, the hypothalamic content of gonadotropin-releasing hormone (GnRH) increases steeply between days 5 and 9, with a less marked increase rate in hypothalamic GnRH content afterwards, until the

adult stage is reached (Chiappa & Fink 1977). On the other hand, Ojeda *et al.* (1977) found that the infantile female rat is more sensitive to GnRH compared with the neonatal and juvenile rat.

Dalkin *et al.* (1981) observed a sharp peak in GnRH receptor density in the pituitaries of female infantile rats. However, during this development stage male pituitaries showed only a discrete increase in GnRH-binding capacity, and the largest receptor density was observed in 30-day-old rats (Dalkin *et al.* 1981). Also, it has been shown that infantile male pituitary gonadotrope cells are heterogeneous, with a significant population of small cells (Denef *et al.* 1978, Childs *et al.* 1981) as in the female pituitary (Denef *et al.* 1978), and also they exhibit an irregular shape and a high nucleo-cytoplasmic ratio (Childs *et al.* 1981). Naor & Childs (1981) observed that the smaller cells do not contain a measurable number of GnRH-binding sites and do not exhibit a GnRH-induced LH release. However, Denef & Andries (1983) showed an LH-stimulated secretion ten times higher in gonadotrope cells from 14-day-old male rats compared with those obtained from adult males, but still to a lesser extent than 14-day-old female rats. From these observations it can be

seen that male infantile LH gonadotrope cells share some morphological characteristics with infantile female cells. Although the higher LH secretion capacity by infantile female pituitaries is commonly accepted, the literature is still controversial about the functional pattern of LH secretion from male rat pituitaries at this development stage. The purpose of the present study was to compare the basal and GnRH-induced secretion of LH in pituitaries isolated from male rats at different stages of postnatal development, with emphasis on the infantile period.

Materials and Methods

Animals

Wistar male rats of different postnatal ages were used. The animals were bred in our colony and were maintained with free access to food and tap water. According to the classification proposed by Ojeda *et al.* (1980) we used rats 14 (infantile), 23 (juvenile), 45 (pubertal) and 90 (adult) days old. Fourteen-day-old animals were maintained with their mothers until used.

Hormone secretion experiments

The pituitary glands were removed and the anterior lobes were separated from the neurointermediate lobe and cut in half using a razor blade. The hemipituitary anterior glands of 23-, 45- and 90-day-old rats or the whole glands from 14-day-old rats were incubated for two 30 min periods for equilibrium. The incubation medium (medium 199 (Gibco, New York, NY, USA) plus 10 mM Hepes (Sigma Chemical Co., St Louis, MO, USA) and 4.7 mM sodium bicarbonate, pH 7.4, at 37 °C) was placed in a syringe (3 ml size and closed at the bottom, placed in a water bath) and gassed with 95% O₂/5% CO₂ through a thin needle (30 C 2 J, Becton Dickinson, Mexico City, Mexico). After these two periods the medium was discarded and replaced by fresh, and thereafter the glands were incubated for four consecutive periods of 20 min each, changing the medium in each period. The incubation volumes were 1 ml/pituitary for 23-, 45- and 60-day-old rats, and 0.75 ml/pituitary for 14-day-old rats. To examine the effect of GnRH on hormone release, the tissue was incubated for an initial basal period without GnRH, followed by three consecutive 20 min periods in the presence of the stimulus. Three different concentrations (0.01, 1 and 100 nM) of GnRH (Relefact, Hoechst, Frankfurt, Germany) were tested. The medium was collected at the end of each 20 min incubation period and replaced with new medium containing the same GnRH dose. Finally, the incubation media were centrifuged at 3200 g/15 min and the supernatants were stored at -20 °C until required for determination of LH by RIA. At the end of the experiment the glands were homogenized with 0.05% NaOH for protein

content determination using the method of Bradford (1976). To test the diffusion capacity through the pituitary tissue of 14- and 90-day-old rats, the uptake of inulin was determined. The experiments were carried out using the same system described for the hormone secretion experiments, and the specific uptake of [³H]inulin (1 µCi/ml; specific activity 2.6 Ci/mmol, Amersham International, Amersham, Bucks, UK) was measured according to the method of Hernández *et al.* (1993). The β emission was measured in a scintillation counter (Beckman, Palo Alto, CA, USA). Corrections for background and quenching were made. The [³H]inulin uptake by the 14- and 90-day-old rat pituitaries shows that the 14-day-old pituitary achieved a plateau in the uptake of inulin between 7.5 and 10 min of incubation and the 90-day-old hemipituitary reached a plateau after 12.5 min of incubation. The results indicate a small delay (2.5–5 min) in adult hemipituitary [³H]inulin uptake when compared with the infantile gland. Also observed was a higher total uptake of inulin by the infantile pituitary tissue than by the adult tissue, which exhibits a larger interstitial space.

Cell culture

Anterior pituitaries from 14- and 90-day-old rats were used. Primary cultures of dissociated cells were prepared with minor modifications of a previously described procedure (Mendoza *et al.* 1995). Briefly, the glands were dissected, minced and treated with 0.25% trypsin (1:250; Gibco) in a Ca²⁺- and Mg²⁺-free balanced salt solution for 10 min. The cell dissociation was accomplished by passing the tissue fragments through a Pasteur pipette. Viability was always better than 95%. Aliquots of 10⁵ cells/well were seeded in multiwell-plates (four wells, 11.3 mm well diameter; Nunc, Roskilde, Denmark) containing 1 ml culture medium composed of medium 199 (Gibco), 10 mM Hepes (Sigma) and 4.7 mM sodium bicarbonate, supplemented with 10% (v/v) fetal calf serum (Boehringer Mannheim GmbH, Mannheim, Germany) and 1% antibiotics (penicillin 10 000 U/ml, streptomycin 10 000 µg/ml; Gibco). Cell cultures were maintained in standard conditions and after 24 or 48 h were washed and incubated with 1 ml serum-free medium for 60 min. At the end of this period the medium was collected and replaced with fresh medium containing 100 nM GnRH and the cells incubated for another 60 min. At the end of this period the medium was collected and stored at -20 °C until assayed for LH.

Immunocytochemistry for LH

Pituitaries of 14- and 90-day-old rats were removed and fixed for 60 min in 4% paraformaldehyde (Sigma) in Dulbecco's phosphate-buffered saline (DPBS, Gibco) at room temperature. After this period the glands were placed in 10% sucrose (Merck, Darmstadt, Germany) in

PBS and maintained at 4 °C. Sagittal sections of 8–15 µm were cut with a cryo-microtome (IEC minotome, Needham HTS, MA, USA) and placed in 0.2% Triton X-100 in tris-buffered saline for 15 min. The sections were incubated at 4 °C overnight with guinea-pig primary antiserum against rat β -LH (1:5000), kindly provided by Dr Parlow through the National Hormone and Pituitary Program of the NIDDK (National Institute of Diabetes, Digestive and Kidney Diseases, Bethesda, MD, USA). Thereafter, the tissues were stained by the technique of alkaline phosphatase (ABC kit, Vector Labs, Burlingame, CA, USA) with Fast Red chromogen (BioGenex, San Ramón, CA, USA) and counterstained with Gill's hematoxylin. Posterior pituitary tissue was the negative control. The single labeled sections were viewed on a confocal laser scanning microscope (Bio-Rad MRC-600, Richmond, CA, USA) using a 2 ×, 4 × and 60 × oil immersion objective lens on an epifluorescence Nikon microscope (Nikon, Yokohama, Japan). For each observation, six to ten consecutive scanning fields were projected onto the two dimensional plane. The images were stored on 1000 rewritable optical disk cartridges and photographed on T-Max 100 film.

RIA

The LH concentration in the media was determined by double-antibody RIA employing the protocol and reagents generously supplied by the National Hormone and Pituitary Program (NIDDK, and the University of Maryland School of Medicine). The standard was NIDDK rat LH-RP2 with intra- and inter-assay coefficients of variation of 5.5 and 6.9% respectively.

Data analysis

All values are expressed as mean $\pm$ S.E.M. Statistical analyses of the anterior pituitary incubation experiments were carried out using the Wilcoxon test for differences between incubation periods and by ANOVA followed by Student–Newman–Keuls multiple comparisons test for differences between age groups. Student's *t*-test was carried out for differences between LH secretion by 14- and 90-day-old pituitary cells in culture. Statistical analysis of LH-positive cell diameters was performed using Kruskal–Wallis nonparametric ANOVA followed by Dunn's multiple comparison test (GraphPadInStat 1.14 1990, San Diego, CA, USA).

Results

LH secretion from anterior pituitaries glands

Figure 1 shows basal and stimulated LH secretion by anterior pituitaries from adult (90-day-old) rats. With 10^{-7} M GnRH, the highest concentration tested, a clear

Figure 1 LH secretion of hemipituitaries from 90-day-old male rats stimulated with different GnRH concentrations. Values are means $\pm$ S.E.M., $n=6$. Comparisons with respect to basal secretion of each gland; * $P<0.05$, Wilcoxon nonparametric test.

response is observed during the first incubation period, with further increases in LH secretion during the second and third periods of incubation. A GnRH concentration of 10^{-9} M induced an increase in LH secretion, but the effect was observed only in the third period ($P<0.05$). No response was observed with the lower GnRH concentration used (10^{-11} M). Figure 2 presents the results of LH secretion obtained from the anterior pituitaries of 45-day-old rats (pubertal rats). These glands responded to 10^{-7} M GnRH, which is very similar to that of the adult glands. However, with 10^{-9} M GnRH a significant increase in LH secretion was observed after the first incubation period. Again, 10^{-11} M GnRH did not elicit

Figure 2 LH secretion of hemipituitaries from 45-day-old male rats stimulated with different GnRH concentrations. Values are means $\pm$ S.E.M., $n=10$. Comparisons with respect to basal secretion of each gland; ** $P<0.01$, * $P<0.05$, Wilcoxon nonparametric test.

Figure 3 LH secretion of hemipituitaries from 23-day-old male rats stimulated with different GnRH concentrations. Values are means $\pm$ S.E.M., $n=10$. Comparisons with respect to basal secretion of each gland; * $P<0.05$, Wilcoxon nonparametric test.

any significant stimulation. The LH secretion from 23-day-old rats (juvenile) is illustrated in Fig. 3. As shown for the pituitaries from pubertal rats, an increase in LH secretion was obtained with both 10^{-7} and 10^{-9} M GnRH, and the increment in LH secretion was statistically significant after the first 20 min stimulation period. When comparing the total amount of GnRH-induced LH release above basal secretion, the stimulated LH secretion was slightly less in juvenile pituitaries than in the pubertal glands (Fig. 4). In contrast, the stimulated LH secretion obtained with pituitaries from 14-day-old rats (infantile) is significantly higher than the secretion from pituitaries of 90- and 23-day-old rats and similar to that of 45-day-old rat pituitaries (Fig. 5). The higher response to GnRH was observed during the first period of stimulation. Figure 4 is a summary of the increase in LH secretion during the three stimulation periods with the three GnRH concentrations tested on the anterior pituitaries from infantile, juvenile, pubertal and adult male rats. The highest stimulated LH secretion was obtained with infantile pituitaries in the presence of 10^{-7} M GnRH. Furthermore, infantile glands stimulated with 10^{-9} M GnRH released LH in concentrations similar to those obtained with juvenile, pubertal and adult pituitaries in response to a 100-times higher dose of GnRH. Another remarkable difference in LH secretion between infantile and both pubertal and juvenile anterior pituitaries was the rate of basal secretion (Table 1). Basal LH secretion observed for infantile anterior pituitaries was 67.3 and 61.3% less than those obtained from pubertal and juvenile glands respectively.

Anterior pituitary cell culture

The GnRH-stimulated secretion of LH from cultured anterior pituitary cells from 14- and 90-day-old rats also

Figure 4 Increase in LH secretion after GnRH stimulation of 14-, 23-, 45- and 90-day-old male rat pituitaries. Data were obtained from the experiments shown in Figs 1–3 and 5, and were the cumulative amount of the increment in LH secretion at different GnRH concentrations obtained in each 20 min period over its basal secretion. Values are means $\pm$ S.E.M. Comparisons between groups: (a) 14 days vs 23 days, (b) 14 days vs 45 days, (c) 14 days vs 90 days; * $P<0.05$, ** $P<0.01$, *** $P<0.001$; two-way ANOVA–Tukey–Kramer test.

Figure 5 LH secretion of pituitaries from 14-day-old male rats stimulated with different GnRH concentrations. Values are means $\pm$ S.E.M., $n=6$. Comparisons with respect to basal secretion of each gland; * $P<0.05$, Wilcoxon nonparametric test.

showed significant differences, similar to that observed in the whole gland at those ages (Fig. 6). The increase over basal LH secretion induced by 10^{-7} M GnRH at 24 and 48 h of culture of infantile cells was $515 \pm 69\%$ and $565 \pm 79\%$ respectively. Under the same experimental conditions the adult cells showed an increase in the LH secretion of $74 \pm 15\%$ at 24 h of culture, and $135 \pm 21\%$ at 48 h of culture. The difference between infantile and adult cells in the GnRH-induced release of LH was highly

Table 1 *In vitro* basal LH secretion (ng/ml per mg protein $\pm$ S.E.M.) by pituitaries from male rats at different postnatal ages. The LH concentration corresponds to the spontaneous LH secretion during a 20 min period

Rat ages (days)			
14	23	45	90
134 $\pm$ 20 ^{***a,***b}	345 $\pm$ 44	409 $\pm$ 55	214 $\pm$ 25 ^{***c}

Statistical differences were analyzed by ANOVA followed by Tukey–Kramer test. ^{***} $P < 0.001$, ^{**} $P < 0.01$; $n = 17$. ^a14 days vs 23 days, ^b14 days vs 45 days, ^c90 days vs 45 days.

significant ($P < 0.001$, unpaired Student's *t*-test). From these data it is clear that in the two culture periods studied, the response to GnRH is significantly larger in cells from 14-day-old rats than in those from 90-day-old rats.

Immunocytochemistry for LH

The immunolocalization of LH-positive cells in the anterior pituitary of infantile rats showed that these cells are localized mainly at the periphery of the gland and beneath the intermediate lobe, with a highly immunofluorescent band of cells at the anterior face of the gland (Fig. 7A). In the adult gland the LH-positive cells were observed at the periphery of the gland and concentrated in the anterior–ventral area (Fig. 7B). However, when examined individually (Fig. 8), LH-positive cells in pituitaries from 14- and 90-day-old rats showed differences in size, with a higher proportion of smaller cells in the infantile pituitary (Fig. 8A) than in the adult (Fig. 8C). When the diameters of LH-positive cells from infantile

glands were measured an uneven distribution was observed. Cells with an average 9 μ m diameter were seen at the periphery of the lateral faces of the gland, whereas in the anterior area most of the cells averaged 11 μ m. The median cell size obtained over the entire gland of adult rats was 11.5 μ m. The diameter of the LH-positive cells in the periphery of the lobes of the infantile pituitary was statistically different from that of the cells from the center of the gland, and from adult cells (Table 2). We also found 10% more LH-positive cells in the infantile glands than in the adult pituitaries.

Discussion

This study shows that the infantile LH gonadotropes from male rats are overall more responsive to GnRH stimulation than in later periods of postnatal development. The male infantile LH cells secrete LH in amounts similar to those of juvenile, pubertal and adult pituitaries, but with a GnRH stimulus 100 times smaller. By employing a similar incubation system, an earlier study reported comparable results (Spona & Luger 1973), although the differences between infantile glands and those obtained at other ages were more modest, even when the GnRH dose was considerably higher. On the contrary, Dullaart (1977) obtained a pattern of LH release characterized by a steady increase with age. Differences in methodologies could underlie the discrepancy between Dullaart's data and our own results. Dullaart incubated six hemipituitaries in each vial, for basal and stimulated release, and the amount of LH obtained was the sum of the LH secreted by the six glands during a 2 h incubation period. In our study, we used a pituitary in each vial, and the basal and the stimulated LH secretion were obtained from the same gland, and we obtained the LH secreted into the incubation medium during a 20 min period. Furthermore, our experience was that some pituitaries could not stabilize, showing a spontaneous LH secretion considerably higher, masking the increase in LH release from the responsive pituitaries induced by GnRH. The elevated stimulated LH secretion observed in the present study by infantile male pituitaries is similar to that observed in the infantile female rat *in vivo* in response to the administration of a bolus of GnRH (Ojeda *et al.* 1977) and also *in vitro* (Spona & Luger 1973, Dullaart 1977). The enhanced GnRH response observed in female rats is in part due to an increase in the population of gonadotrope cells (Denef *et al.* 1978). Furthermore, according to Dalkin *et al.* (1981) female rats exhibit a peak in GnRH receptor density around the 14th day that could explain the higher LH serum concentration and the hypersensitivity to GnRH observed in the infantile period. However, male rats exhibit a different pattern in pituitary GnRH receptors during postnatal development, with GnRH receptors increasing in number steadily at 5 days to reach a maximum level at 30 days of age (Dalkin *et al.* 1981). However, our results showed that the anterior

Figure 6 Basal and GnRH-stimulated LH secretion of infantile or adult pituitary cells in 24 and 48 h of culture. The cells were washed and incubated for 60 min to achieve basal LH secretion and at the end of this period the medium was collected and another 60 min period was performed with 10^{-7} M GnRH. Statistical differences between infantile and adult secretion at each culture period were analyzed by ANOVA followed by Tukey–Kramer test; ^{***} $P < 0.001$.

Figure 7 Immunolocalization of LH-positive cells in 14-day-old (A) and 90-day-old (B) rat pituitaries. The cells were labeled with an antibody against LH β and developed with ABC alkaline phosphatase and Fast Red as substrate. The posterior lobe was the negative control. The immunostaining of the 14-day-old gland was localized mainly at the anterior face and at the periphery of the gland, and the 90-day-old gland shows numerous staining at the anterior ventral area. The tissue sections shown in the photomicrographs correspond to the middle of the glands. Bar=250 μ m.

Figure 8 Morphological characteristics of LH-positive cells from 14- and 90-day-old anterior rat pituitaries. Panels A and B show the different types of LH-positive cells found in 14-day-old glands. Panel C shows a typical adult LH-positive cell. The cells were labeled with an antibody against LH β and stained with ABC alkaline phosphatase and Fast Red as substrate. Bar=5 μ m.

pituitaries from juvenile rats did not exhibit a stimulated LH secretion greater than that found during the infantile period, suggesting that the gonadotropin secretion is negatively modulated at the juvenile period. The lower density of GnRH receptors observed in the infantile male pituitary

Table 2 LH-positive cells diameters from 14- and 90-day-old male rat pituitaries. The LH-positive cells are different in size according to their location in the infantile gland (14 days old). Adult LH-positive cells do not exhibit regional size differences

Rat age (days)	Localization in the gland	Diameter (μ m)		
		Median	Mean	S.E.M.
14	Lobules	9.0***	9.2	0.1
	Center	11.1	11.4	0.2
90	Whole	11.5	11.60	0.1

Statistical differences were observed between 14-day-old pituitary lobules with respect to 14-day-old center and 90-day-old pituitary (Kruskal–Wallis test followed by Dunn's test). *** $P < 0.001$; $n = 300$ cells.

compared with female pituitaries (Dalkin *et al.* 1981) could explain the moderate serum LH concentration in infantile males compared with that observed in infantile female rats (Ojeda & Ramirez 1972, Döhler & Wuttke 1975, Chiappa & Fink 1977). However, we believe that the higher capacity of the infantile pituitary to secrete LH under GnRH stimulation could be a functional characteristic and linked to the morphology of the LH-positive cells present in the pituitary at this stage of development.

It was not surprising that the basal secretion of LH was different among the ages studied. In the earlier work of Spona & Luger (1973), a higher basal LH secretion by pubertal pituitaries was obtained, similar to that reported herein. However, they observed a low basal secretion by juvenile pituitaries, which is at odds with our results. We believe that hypothalamic GnRH could be implicated in the higher basal LH secretion observed in juvenile and pubertal pituitaries, since at these development periods the hypothalamus shows bursts of GnRH secretion that could stimulate LH synthesis and lead to elevated secretion of LH (Ojeda & Urbanski 1988).

Our data show a clear difference between infantile and adult stimulated LH secretion, this being significantly larger in the infantile pituitaries, possibly suggesting a hypersensitivity to GnRH at this developmental stage. Likewise, a different time course of GnRH-stimulated LH secretion was observed. With infantile pituitaries, the highest secretion of LH in response to GnRH was obtained during the first period of incubation, in contrast to adult pituitary where a step-wise increase in LH was observed during the three incubation periods. Taking into account that the location of most of the LH-positive cells is near the periphery and at the anterior–ventral area (Nakane 1968, 1970), where the glands were cut into two parts, and a delay of only 2.5–5 min in the diffusion rates between adult and infantile glands, it is unlikely that a diffusion barrier could be responsible for the LH secretion differences observed. In accord with Denef & Andries (1983) the results reported herein for primary cell cultures

from infantile and adult pituitaries clearly show greater GnRH-induced LH secretion in the infantile cells. This difference was more evident at 24 than 48 h of culture because of an increase in the secretory response by the adult pituitary cells at 48 h of culture. Loughlin *et al.* (1984) observed that at 24 h postdispersion, the LH secretion reflects more accurately the hormonal environment and pituitary LH content found at the time that the animals were killed, but 24 h later the hormonal concentration of the culture medium seems to be the main factor influencing LH secretion. The increase in the stimulated LH secretion observed in adult pituitary cells at 48 h of culture could therefore reflect the activity of hormone steroids present in the fetal calf serum used in the culture medium.

Moreover, the infantile and adult pituitaries exhibit different proportions of LH-positive cells. LH-positive cells are more abundant (+10%) in the pituitaries from 14-day-old rats. This result agrees with those of Denef *et al.* (1978) and Childs *et al.* (1981). Furthermore, a significant proportion of the infantile LH-positive cells are angular shaped, with long processes facing the sinusoids, whereas most adult LH-positive cells are ovoid shaped (Childs *et al.* 1981, Inoue & Hagino 1984). However, according to Inoue & Hagino (1984) the infantile follicle-stimulating hormone (FSH)-positive cells are similar in shape to adult gonadotrope cells. In the present study we observed a regional distribution of the small LH-positive cells, with a great proportion of them being localized at the periphery of the lobules. However, according to Nakane (1970) adult pituitaries also show regional differences in the localization of mono-hormonal gonadotrope cells. He observed that in general the gonadotropic cells situated at the periphery of the gland contained both FSH and LH whereas those at the center tended to contain only one of these hormones. Data from our group have shown that infantile pituitary cells, cultured in defined medium containing fibroblast growth factor, can maintain their level of GnRH-induced LH secretion, whereas in adult male pituitary cells this growth factor cannot induce the same response (Cruz-Soto *et al.* 1996). The morphological and functional data obtained in gonadotrope cells from infantile male rats support the hypothesis that two different populations of LH-secreting cells are present in the pituitary at this period. These different populations could result from dissimilar stages of development of the same population, or arise from different cell precursors.

A number of previous works have shown the importance of serum LH concentrations for early postnatal development in the male rat (Chemes *et al.* 1979, Molenaar *et al.* 1986, Teerds *et al.* 1989). According to Teerds *et al.* (1989) the rate of development of the new Leydig cells during the postnatal period strongly depends on LH levels. Furthermore, the participation of LH has also been involved in the regulation of Sertoli cell differentiation (Chemes *et al.* 1979). Thus, the larger GnRH-

stimulated LH secretion observed in infantile rat pituitaries compared with that in the adult could be important to testis development.

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