IN VITRO STIMULATION OF LH RELEASE BY LH-RH IN FEMALE RAT PITUITARIES OF DIFFERENT AGES

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1. Introduction

Several recent reports showed that synthetic luteinizing hormone-releasing hormone (LH-RH) stimulated the release of luteinizing hormone (LH) in man [1–4] and experimental animals [5, 6]. Recently synthetic LH-RH has become available [7, 8] and was also reported to stimulate LH release *in vitro* [9]. Pituitary responsiveness to LH-RH in intact female and male rats of different ages was reported previously [10, 11].

Recently we could show varying degrees of responsiveness of male rat pituitaries at different ages to LH-RH *in vitro* [12]. The present experiments were designed to study the *in vitro* response of female rat pituitaries at different ages to LH-RH in order to obtain more information on pituitary regulatory function at pubertal ages.

2. Materials and methods

Female rats of the Sprague Dawley strain (Mus Rattus AG, Brunnthal, GFR) were divided into groups according to their ages which were 0, 10, 20, 30, 40 and 60 days, and served as pituitary donors. The posterior lobe was removed and discarded. Seven halves of the adenohypophyses were placed into 25 ml Erlenmeyer flasks containing 2 ml of sterile medium which consisted of 9 parts Medium 199 (Biocult Labs., Glasgow) and 1 part fetal bovine serum (Reheis Chemical Comp., Chicago). Penicillin (50 U/ml) and streptomycin (50 U/ml) were added to the media to reduce bacterial contamination. The opposite halves of the anterior lobes of the pituitaries were used as controls. After 3 hr of pre-incubation time at 37° under an atmosphere of 95% air and 5% CO_2, incubation was continued for another 4 hr with new medium which contained 0.5 μg LH-RH per ml and per pituitary. The control flasks did not contain LH-RH. Four incubation flasks were set up for each group. Media were stored at −25° after incubation. Pituitaries were washed twice with buffer (0.01 M phosphate, 0.15 M NaCl, 0.1% NaN_3), snap frozen in 1 ml of buffer and homogenized before LH estimations were performed. The procedure was described in detail previously [12].

Medium and tissue were assayed for LH by radioimmunoassay as described by Niswender et al. [13]. Protein was determined according to Lowry et al. [14].

3. Results and discussion

Basal LH secretion of pituitaries of female rats at different ages incubated without LH-RH increased progressively until 20 days (fig. 1). At this age a sharp maximum of unstimulated LH release was observed, and a sharp decrease at 30 days was recorded. LH release of unstimulated pituitaries at 10 days was significantly higher than that of 0 day old glands ($P < 0.005$). Pituitaries of 20 day old animals secreted significantly higher LH levels than adenohypophyses of 10 day old rats ($P < 0.001$). Levels of 30 day old controls were significantly lower than 20 day old glands ($P < 0.001$). Basal LH levels of 0, 30, 40 and 60 day old glands were statistically not different from each other.

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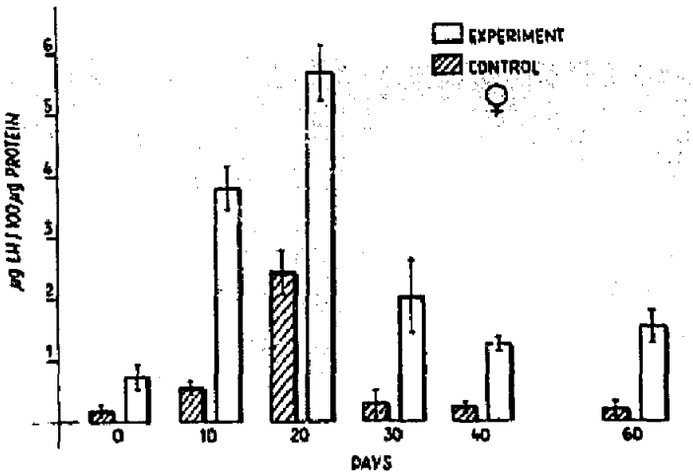

Fig. 1. LH release of female rat pituitaries at various ages. LH release was stimulated by 0.5 µg LH-RH per ml and per adenohypophyses. Means ± SD in terms of NIAMD-Rat LH-RP-1 per 100 µg of pituitary protein.

At all ages studied a significant stimulation of LH release was observed for adenohypophyses incubated in the presence of LH-RH and the patterns of LH levels of control and stimulated groups were similar (fig. 1). The highest LH levels in the LH-RH treated groups were recorded at 20 days. LH concentrations released by 40 and 60 day old glands were statistically not different from each other. LH levels of all other age groups were statistically distinguishable from each other.

Similar pattern of LH concentrations was exhibited in the glands themselves and the highest LH levels in both control and stimulated groups were at 20 days (fig. 2). However, the minimal percentage of LH release over the controls was observed in the 20 day old group of animals. This suggests that the availability of LH in the gland is not the only control over the release of the hormone, and it is striking that the highest percentage of LH release over the controls was recorded in the 0 day old pituitaries. Pituitary LH levels of this report are higher than reported earlier [10]. This discrepancy may be explained by the fact that different material was employed for the LH estimation in the present paper. The use of different standards, antisera and methodologies are known to alter absolute values obtained by radioimmunoassay systems [15, 16]. The pattern observed for female glands is quite different from the data obtained in the experiment with male pituitaries [12] and this may be an explanation for the different mechanisms that determine the onset of puberty in male and female rats, resp. But steroids may modulate LH-RH stimulated LH release as was shown by us in acutely ovariectomized rats (our own unpublished data). Further studies are necessary to explain LH-RH actions in more detail.

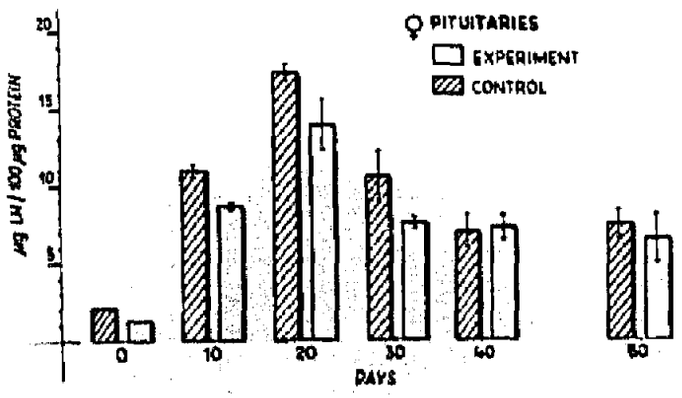

Fig. 2. LH concentrations in pituitaries of male rats at various ages used in experiments described in fig. 1. Values are expressed in terms of NIAMD-Rat LH-RP-1 ± SD per 100 µg of pituitary protein.

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