IN VITRO RESPONSIVENESS OF MALE RAT PITUITARIES OF DIFFERENT AGES TO LH-RELEASING HORMONE (LH-RH)

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1. Introduction

Recently LH-RH was isolated from porcine hypothalami and this hormone was shown to stimulate in rats, the release of luteinizing hormone (LH) *in vivo* as well as *in vitro* [1]. The structure of this material was proposed to be pyro-Glu–His–Trp–Ser–Tyr–Gly–Leu–Arg–Pro–Gly-NH_2 [2], and could be confirmed by the synthesis of a decapeptide having physiological and biological properties indistinguishable from those of natural porcine LH-RH [3, 4]. Most recently synthetic LH-RH was shown to stimulate the release of LH in man [5–8] and experimental animals [9, 10].

Little is known, however, about the mechanisms that control the stimulation of LH release by LH-RH. This knowledge is of importance for the understanding of the pre-ovulatory LH surge and the onset of puberty. We were therefore prompted to study the *in vitro* response of pituitaries of male rats of different ages to synthetic LH-RH.

2. Materials and methods

Male rats of the Sprague Dawley strain (Mus Rattus AG, Brunnthal, GFR) were used throughout the experiment. The animals were divided into groups according to their ages, which were 0, 10, 20, 30, 40 and 60 days, and served as pituitary donors for the *in vitro* studies. Rats in the 0 day group were between 3 and 24 hr resp., after parturition. The posterior lobes of the pituitaries were removed and the anterior pituitary cut in half. Seven halves were transferred asseptically 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 pituitaries were treated in the same manner and used as controls. Incubation was carried out at 37° under an atmosphere of 95% air and 5% CO_2. The glands were pre-incubated for 3 hr. Incubation was continued with new medium containing 0.5 μg LH-RH per ml and per pituitary for 4 hr. No LH-RH was added to the media of the control flasks. Four incubation flasks were set up for the experimental as well as the control groups. After incubation the media were stored at −25°. The pituitaries were washed twice with buffer (0.01 M phosphate, 0.15 M NaCl, 0.1% NaN_3) and snap frozen in 1 ml of the same buffer.

Medium and tissue were assayed for LH by radioimunoassay as described by Niswender et al. [11]. The separation of bound and free tracer was modified by using a solid-phase second antibody (Dako, anti-rabbit. N.V. Organon, Oss, Holland). Pituitaries were homogenized before LH-assays were performed. Protein determination was done according to Lowry et al. [12].

3. Results and discussion

Data on the *in vitro* LH release of pituitaries removed from rats of different ages are summarized in fig. 1. Pituitaries of neonatal rats (0 days) showed a basal secretion of LH into the medium, which was significantly lower than the release of 10 through 60 day old pituitaries. LH levels recorded for 10 day old pituitaries were higher than for 20 days ($P < 0.001$),

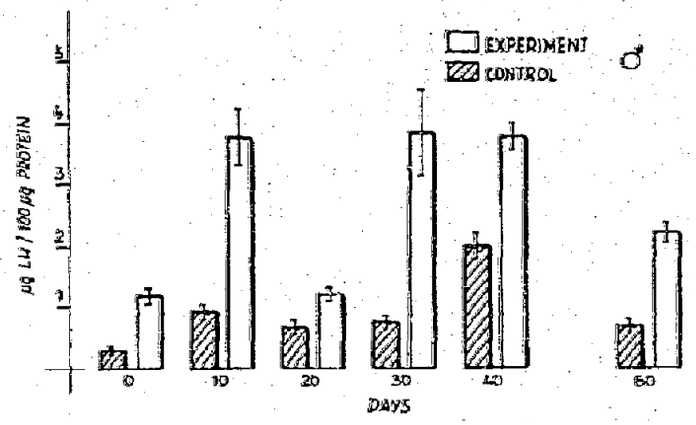

Fig. 1. *In vitro* LH release of pituitaries of male rats at various ages. Stimulation of LH release over the controls was by 0.5 μg LH-RH per ml and per pituitary. Means ± SD in terms of NIAMD-Rat LH-RP-1 per 100 μg of pituitary protein.

but LH basal concentrations at 10, 30 and 60 days, resp. were statistically not different from each other. The maximal LH concentration was found in the incubation media of 40 day old pituitaries.

A significant increase of LH release over the controls was observed in all age groups, when LH-RH was added to the incubation media. However, similar patterns of LH levels from 0 to 60 days were recorded for control and experimental groups (fig. 1). LH concentrations at 10, 30 and 40 days were not statistically different from each other, and in these three age groups the highest LH concentrations were assayed. At 0 and 20 days statistically indistinguishable LH levels were recorded. On the other hand, the percentage of increase in LH after LH-RH as compared with control levels were similar at days 0, 10, 30 and 60. Minimal responses to LH-RH over the controls were recorded for 20 and 40 day old pituitaries.

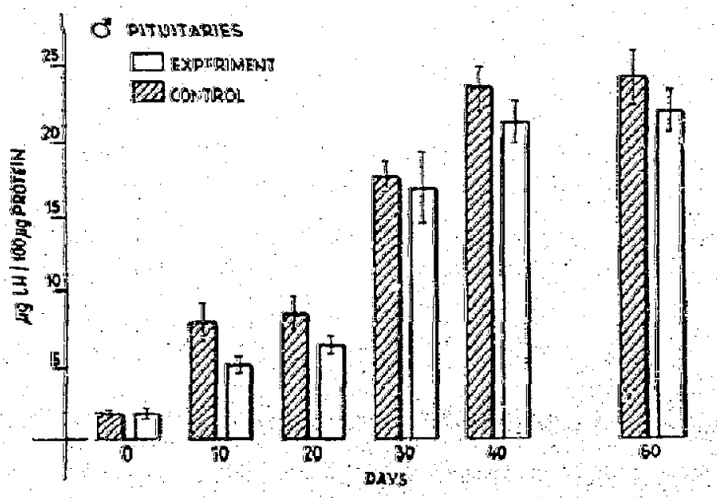

Fig. 2. LH concentrations in pituitaries of male rats at various ages used for experiments described in fig. 1. Values are expressed in terms of NIAMD-Rat LH-RP-1 ± SD per 100 μg of pituitary protein.

Basal pituitary LH concentrations were significantly lower in neonatal rats than concentrations at any other age studied (fig. 2). The basal levels of 10 and 20 day old pituitaries were not statistically different from each other. The highest pituitary LH levels were recorded at 40 and 60 days, and they were statistically indistinguishable from each other. LH concentrations of pituitaries incubated in media containing LH-RH showed similar patterns as those found in the control groups (fig. 2). However, only 10 and 20 day old pituitaries of the control groups showed higher LH concentrations than the LH-RH treated glands ($P < 0.025$). At all other ages no evidence of significant LH depletion after LH-RH was noted. This is not in accordance with the data of fig. 1 but this discrepancy may be explained by the relative large amounts of LH stored in the pituitaries (fig. 2).

Our data presented in figs. 1 and 2 suggest that the sensitivity of the pituitary gland to hypothalamic hormones is quite different at all ages tested, but it is clearly evident that sensitivity is not necessarily determined by the pituitary content of the gonadotrophin. The present investigation also suggests that one of the mechanisms which influence the onset of puberty may be the pituitary responsiveness to hypothalamic releasing neurohormones. Previous experiments on the dependence of LH release upon LH-RH doses showed that the LH-RH concentration used in the present report was in the flat portion of the dose response curve (unpublished data).

Recently a maximal response to LH-RH was reported during prepubertal age (between 30 and 45 days) in male rats *in vivo* [13], but the earliest age studied was 15 days.

Our data for the LH contents of the pituitaries are higher than those observed earlier [13]. However, this discrepancy may be explained by the fact that different material and a modified methodology was employed for the radioimmunoassay in the present study. These factors are known to influence the absolute values obtained by radioimmunoassay systems [15, 16]. The

experimental design of our *in vitro* study was to exclude possible interference by other endocrine glands. However, some of the steroids secreted in varying amounts by the testicular Leydig cells at pubertal ages could be bound by pituitary receptors and then in turn alter the LH release stimulated by LH-RH in our *in vitro* system. High steroid levels present in the fetal blood circulation during gestation may still exert an influence at zero and 10 days after parturition. Testosterone was shown to influence LH-RH stimulated LH release *in vivo* [14]. LH-RH stimulated LH release could also be modulated by sex steroids *in vitro* (our own unpublished data). At present this possibility seems to be the most reasonable explanation for our findings, although further work is needed to investigate this important problem.

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