# Hypothalamic Luteinizing Hormone Releasing Hormone in Pregnant Rat

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## ABSTRACT

Changes in contents of hypothalamic luteinizing hormone-releasing hormone (LH-RH) were studied in midpregnant rats. The hypothalamus was dissected out on days 7, 9, 12 and 15 of pregnancy. LH-RH activity in the extracts was determined by a competitive radioimmunoassay method. The mean LH-RH content of the hypothalamus was the lowest on day 9, and gradually increased until day 15 ($p<0.05$). Midpregnancy surge of the serum estradiol levels is considered to inhibit through a negative feed-back the production of LH-RH in the hypothalamus.

*Key words: LH-RH; hypothalamus; rat; pregnancy*

## INTRODUCTION

The secretion of luteinizing hormone (LH) from pituitary in rat is mainly regulated by luteinizing hormone - releasing hormone (LH-RH) in the medial hypothalamus. The recent devolopment of highly sensitive radioimmunoassay methods has made it possible to measure changes of LH-RH levels in the serum as well as in the hypothalamus in experimental animals[1-7].

While LH plays an important role in maintaining early stages of pregnancy in the rat[8-11], little is known about the changes of LH-RH levels in the hypothalamus in pregnant rats. The present study, measurement of changes of LH-RH level in the hypothalamus in pregnant rats, was undertaken in view of the fact that midpregnancy surge is observed in the serum LH levels[12].

## MATERIALS and METHODS

Normally cycling female Sprague-Dawley rats, weighing 200-250 g,

were placed overnight in a cage with male rats of the same strain. The female rats were checked on the following morning for the presence of sperm plug. Those with a sperm plug were taken as day 1 of pregnancy.

The pregnant rats were sacrificed on days 7, 9, 12 and 15 of pregnancy. The hypothalamus was taken out as a block, limited anteriorly by 2 mm anterior to the margin of the optic chiasm, laterally by the lateral fissures and posteriorly by the posterior margin of the mammillary body, and with the thickness of about 5 mm from the basal surface of the hypothalamus.

Extraction of LH-RH from the blocks was carried out according to the method by Wheatone et al[2].. The specimens were homogenized in 2 ml of cold acidic ethanol (1:1, 0.2 N acetic acid and ethanol) and centrifuged at 1800×g. at 4°C for 20 min. The supernatant fluid was transferred into a 10-ml tube and dried at 40°C under the air stream.

LH-RH of the extracts were determined by competitive radioimmunoassay using $^{125}$I-labeled synthetic LH-RH as a standard according to the method previously reported[13]. Statistical calculations of the differences between mean values were carried out using the Student's t test.

## RESULTS

The presence of LH-RH in the hypothalamic extracts obtained in our study was assessed by radioimmunoassay. The hypothalamic extract was diluted two-, five- and ten-fold with 0.05 M phosphate buffered saline, pH 7.4 containing 0.1% BSA, and the inhibition of $^{125}$I-LH-RH binding to antibody was determined. The rate of inhibition with the diluted extracts was exactly the same as that with the diluted standard synthetic LH-RH as shown in Fig. 1.

The recovery rate of the LH-RH from the hypothalamic extract in our experiment was also studied by adding synthetic LH-RH to specimens of cerebral cortex and by extracting it in the same manner as hypothalamic extraction. A good recovery was observed between the LH-RH added and the LH-RH assayed ($r=0.90$) as shown in Fig. 2. Mean extraction rate, intra-assay variation and inter-assay variation were 92, 8.7 and 13.9%, respectively.

Changes in hypothalamic LH-RH contents of pregnant rats from day 7 to 15 were determined with the hypothalamic extract by radioimmunoassay and are shown in Fig. 3. The mean LH-RH content in the hypothalamus was the lowest on day 9 and then gradually increased up to day 15, so far studied. The mean content of LH-RH in the hypothala-

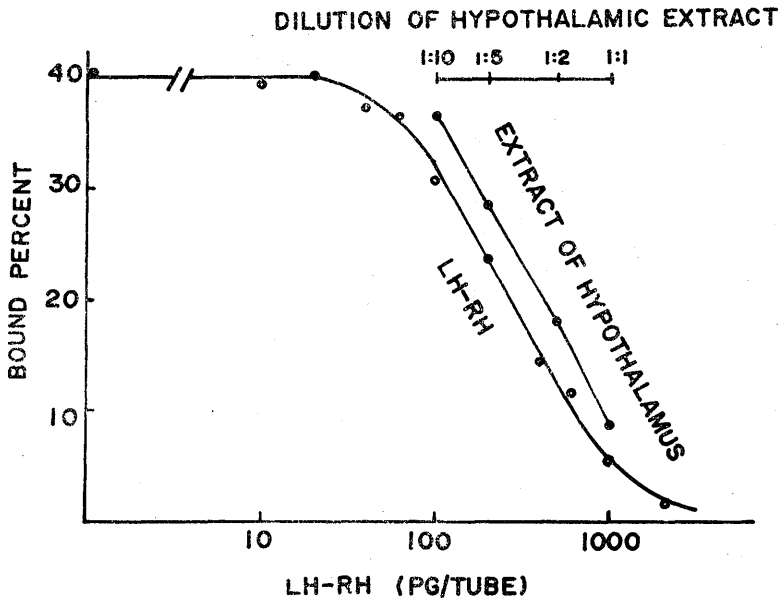


Fig. 1 Binding inhibition of $^{125}$I - LH-RH to the antibody with varying concentration LH-RH. Hypothalamic extracts from female rats and the synthetic LH-RH were assayed under tha same condition.

mus on day 15 was significantly higher than that on day 9 ($p<0.05$).

## DISCUSSION

The serum LH in pregnant rats is known to change, but the levels are high at day 9, and low at day 15 of pregnancy[12]. On the contrary, the LH-RH levels in the hypothalamus of rats were low at the 9th day of pregnancy, and gradually increased until day 15 from the present study. It is interesting to note that the two hormones, the hypothalamic LH-RH and serum LH, change in opposite directions as pregnancy progresses. Oshima et al[6]. reported that in normally cycling rats the hypothalamic LH-RH level is increased on the day of estrous while the serum LH level is decreased.

There have been some evidence that serum estradiol ($E_2$) levels inversely affect the LH-RH levels in the hypothalamus. Piacsek and Meitus[14] and Ajika et al[15]. using bioassay reported that estradiol benzoate ($E_2B$) injection in ovariectomized female rats caused a decrease of the LH-RH level in the hypothalamus. Oshima et al[6]. reported that in the hypothalamus of normally cycling rats the lowest levels of LH-RH

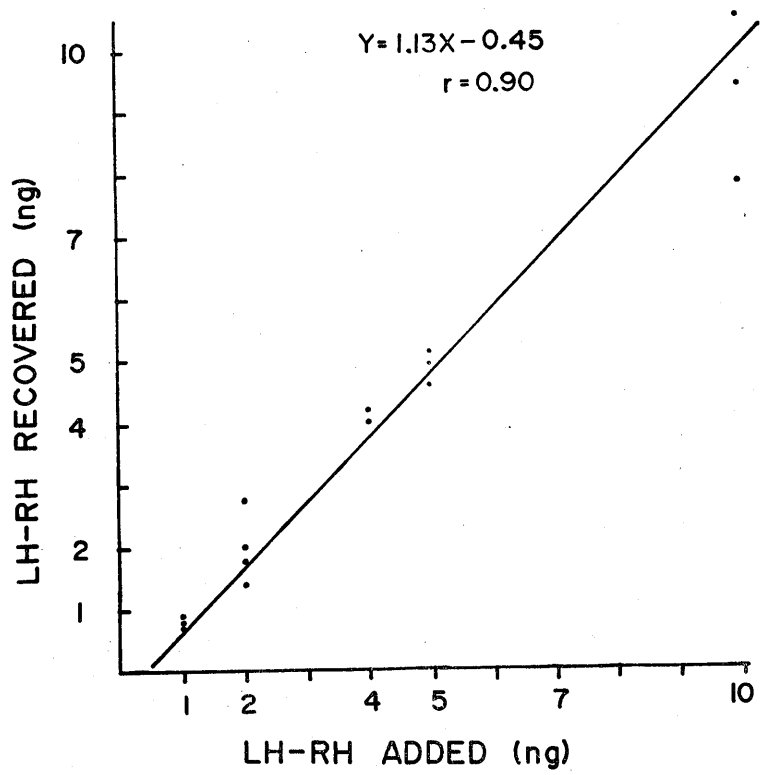


Fig. 2 Recoveries of synthetic LH-RH, which was added to and then extracted from the cerebral cortex. A good correlation was found between the LH-RH added and the LH-RH assayed (r=0.90).

were observed on the day preceding to estrous, when the $E_2$ levels were the highest in a cycle. Beattie[16] reported that the highest serum $E_2$ levels were observed on day 9 in pregnant rat. Our preliminary data indicate that in pregnant rats the serum $E_2$ levels on day 9 of pregnancy are higher than those on day 15 (Fujino and Kato, unpublished). Daily injection of 1 $\mu$g $E_2$ from day 7 to day 15 resulted in the increase of the LH-RH levels in the hypothalamus (Yamashita and Kato, unpublished). Factors other than estradiol, progesterone for instance, may also affect the hypothalamic LH-RH levels in pregnant rats.

The localization of LH-RH in the hypothalamus merits attention. Ramierz and Kordone[17] reported that 98% of LH-RH activities in the brain are found in the region including n. paraventricularis, n. arcuatus, n. ventromedialis, n. dorsomedialis and the median eminence of the hypothalamus. The preparations from the hypothalamus in our study

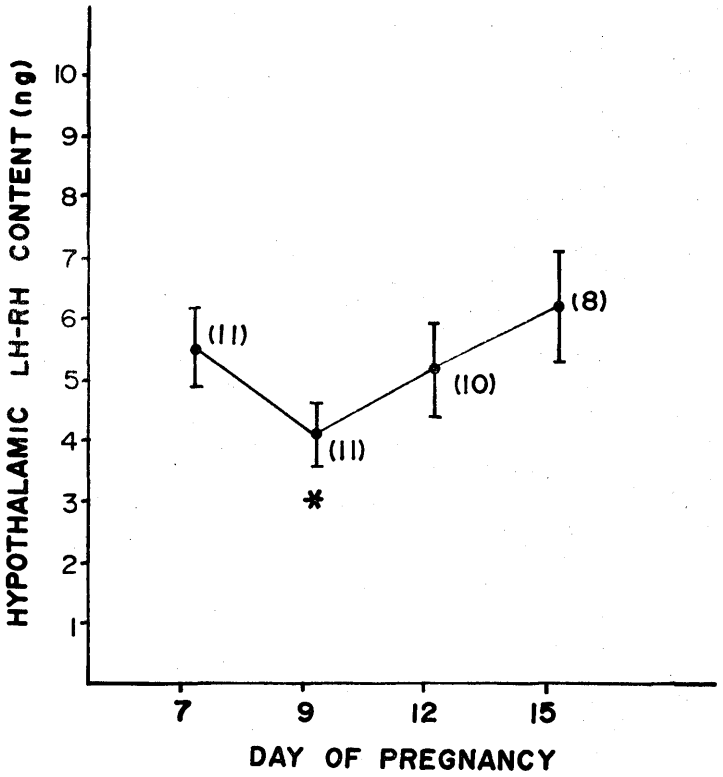


Fig. 3 Changes in hypothalamic LH-RH contents in midpregnant rats. Each value represents the mean ± S.E. The numbers in the parentheses represent the number of rats used for study.

included all these regions and thus are probably adequate for the evaluation of changes in the hypothalamic LH-RH levels.

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