A STUDY OF GONADOTROPIN RELEASE AND SYNTHESIS IN THE ANTERIOR PITUITARY GLAND — ON-OFF MECHANISM BY LH-RH — *

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ABSTRACT

To clarify the *on-off* mechanism of gonadotropin synthesis and its release in the anterior pituitary gland, the anterior pituitaries of the male rat were cultured *in vitro* through a leuteinizing hormone-releasing hormone (LH-RH) stimulation and a washing sequence for the analysis of cyclic nucleotide and gonadotropins. After the first stimulation of gonadotrophs with synthetic LH-RH for 20 minutes and the removal of LH-RH by washing, the alteration of the gonadotropin release and its content in the anterior pituitaries were investigated for several hours. The data indicate that the primary stimulation by LH-RH to the gonadotrophs has no continuous effect on the gonadotropin release and synthesis after washing and that LH-RH, primarily administered, does not change the responsiveness to the secondarily administered LH-RH. The experiments also showed that by the stimulation of LH-RH, adenosine cyclic 3', 5'-monophosphate (c-AMP) was accumulated in the anterior pituitary cells, but that such high accumulation did not continue after washing, and gonadotropins were released and synthesized by the administration of dibutyryl c-AMP and theophyllin. It is suggested that the continuous gonadotropin release and synthesis required the continuous LH-RH stimulation, which maintains an elevated c-AMP concentration in the rat anterior pituitary gland.

INTRODUCTION

It is well known that luteinizing hormone-releasing hormone (LH-RH)

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stimulates the release and synthesis of gonadotropins, however the exact mechanism of the hormonal action of LH-RH to the anterior pituitary gland still remains to be clarified.¹ As far as the hormone release is concerned, the first step of the metabolic sequence is triggered by the stimulation of LH-RH to the anterior pituitary and the end portion of the metabolism is the release of a luteinizing hormone (LH) and follicle-stimulating hormone (FSH) which are restored in the gland. The anterior pituitary cells are also capable to release a certain amount of gonadotropins for a certain period without any hypothalamic stimulation, then LH-RH has an *on-off* modification on the release of pituitary gonadotropins. The same concept might be applicable to the aspect of the gonadotropin synthesis by LH-RH. In this study the mode of action of *on-off* stimulation by LH-RH for both release and synthesis of pituitary gonadotropins was investigated in the *in vitro* culture of the rat anterior pituitary.

MATERIALS AND METHODS

Cultuer of the rat-hemipituitaries

Adult male rats (200–250 g) were killed by decapitation, and the anterior pituitary glands obtained were hemisected and weighed. The hemipituitaries were paired for the control and the treated flasks.

Three to 10 pituitary halves were incubated in 25 ml Erlenmeyer flasks containing 2 ml KRB (Krebs-Ringer bicarbonate bufffer, pH 7.3) or TC199 (tissue culture medium 199; Difco) in an atmosphere of 95%O₂-5%CO₂ in an incubator at 37°C with constant shaking at a speed of 60 cycles per minute. All flasks were preincubated for 30 to 40 minutes, and afterwards the medium was discarded and the new medium was added into each flask for further experiments.

After the first incubation with LH-RH, washing procedures were taken for the removal of LH-RH at some experiments. During the main experiment (Fig. 1), hemipituitaries were washed only one time by 10 ml of TC199 at 37°C, which revealed sufficient for the purpose (Fig. 2). On the supplementary experiment, hemipituitaries were washed four times by 4 ml of KRB.

Hormone and Chemicals

Synthetic LH-RH, a gift from Sankyo Pharm. Co., Ltd. was diluted with 0.01 M phosphate buffer solution (PBS) and used at the dosage of 12.5 ng/ml medium during the main experiment. For the control flask, only the vehicle (= PBS) was added. On the supplementary experimetns, a cerebral cortex extract was added to a final concentration of 10 mg per flask as the control.

Fig. 1 Protocol of the main experiment.

Fig. 2 LH-RH content in medium before and after washing by 10 ml of TC199.

During the experiment for the study of the effect of "washing" on c-AMP content in the anterior pituitary gland (Fig. 11), a rat hypothalamic extract

(NIAMDD-HE-RP-1), diluted with distilled water, was used at the concentration of 4 mg HE per 50 μ l. N⁶, O²-dibutyryl cyclic AMP and theophylline were dissolved into the medium just before used.

Radioimmunoassay of gonadotropins and c-AMP

LH and FSH in the mediums and tissues were measured by a double antibody radioimmunoassay technique. Purified rat LH (NIAMDD-Rat LH-I-5) and FSH (NIAMDD-Rat FSH-I-5) were labelled respectively with 1 mCi of ¹²⁵I. LH and FSH values are expressed in terms of NIAMDD-Rat LH-RP-1 and NIAMDD-Rat FSH-RP-1.

The radioimmunoassay of c-AMP in the pituitaries were performed according to the method of Steiner and associates.² Approximately 7.5×10^{-9} M SCAMP-TME in 50% ethanol solution was iodinated with 1 mCi of ¹²⁵I by a slight modification of the method of Greenwood and associates.³ The pituitaries were homogenized with 0.5 ml of 5% (w/v) trichloroacetic acid (TCA) and centrifuged. The supernatant fluid was extracted three times with five volumes of petroleum ether (H₂O saturated) and the aqueous phase was heated at 90°C for three minutes, then lyophilized. The residue was diluted with 0.05 M Na acetate buffer (pH 6.2) and 50 μ l of aliquot was added to each assay tube. The tracer amounts of ³H-adenosine (0.07 to 0.1 μ Ci, specific activity 10 Ci/mM) were added to samples before homogenization and the recovery through the extraction was corrected.

Statistics

A paired test was used to determine the significance of differences between the treated and the control groups.

RESULTS

After the first stimulation of LH-RH to the anterior pituitaries for 20 minutes, the effect of the second stimulation was examined (Fig. 3 and Fig. 4). Two hours after washing, LH was significant ($p < 0.05$) released in the treated flasks by LH-RH. As for FSH, the releasing effect by LH-RH was also significant ($P < 0.02$). While LH contents of the gland after 4 hours were significantly increased in the treated flasks ($P < 0.02$), FSH contents showed no difference between the control and the treated pituitaries.

It was demonstrated that LH-RH, secondarily administered, had the gonadotropin releasing effect to the pituitary tissue to a certain extent. But there was no direct evidence whether LH-RH, primarily administered, changes the responsiveness of gonadotrophs to the second stimulation or not. To answer this question, gonadotropins, released during the second incubation with

Fig. 3 Effect of secondarily administered LH-RH on LH.

Fig. 4 Effect of secondarily administered LH-RH on FSH.

Fig. 5 Effect of preliminarily administered LH-RH on LH.

Fig. 6 Effect of preliminarily administered LH-RH on FSH.

LH-RH, were assayed with or without the primary stimulation (Fig. 5 and Fig. 6). There were no significant changes in the treated flasks as compared

Fig. 7 Effect of short stimulation of LH-RH on LH.

Fig. 8 Effect of short stimulation of LH-RH on FSH.

Fig. 9 Effect of LH-RH on cAMP content in AP. Cyclic AMP in AP after 4 hour incubation with LH-RH (15 ng/flask). Four pairs of flask were examined and each contained 2 ml of TC199. The vertical lines are mean $\pm$ S.E.

Fig. 10 LH and FSH released after incubation with dibutyryl c-AMP (10 mM) and theophylline (1 mM) or H.E. (4 mg). The vertical lines are mean $\pm$ S.E.

to the controls. Gonadotropin contents were also unchanged in the anterior pituitaries after 4 hour incubation both in the treated flasks and the controls.

After the anterior pituitary glands were exposed with LH-RH for a short time, there seems likely to exist a continuous effect of LH-RH in terms of the gonadotropin release (Dotted line in Fig. 3 and Fig. 4). To investigate this effect, the second incubation under the non-stimulated state was performed after the first incubation with LH-RH or only PBS (Fig. 7 and

Fig. 11 Cyclic AMP content in AP after treatment. Hemipituitaries were incubated for 10 minutes with 4 mg of H.E. or C.E., then washed. After removal they were incubated only in the medium. The values are the means of triplicate assays.

Fig. 8). As a result, LH-RH, preliminarily administered for a short time, did not affect the subsequent gonadotropin release and synthesis after washing.

After a short stimulation or a 4 hour stimulation of the anterior pituitaries by LH-RH, c-AMP was accumulated intracellularly (Fig. 9). After washing the high accumulation diminished (Fig. 11). By the continuous stimulation of c-AMP to the anterior pituitary tissue, gonadotropin were released in the medium (Fig. 10).

DISCUSSION

Moyle et al. revealed that the steroidogenesis, once triggered by LH, continued for one hour even without LH.⁴ Interestingly in our study, the primary stimulation of LH-RH did not have any continuous effect to the gonadotropin synthesis and release after a washing. In general the steroid hormone is immediately released into blood circulation after syntheses in the tissue, whereas the considerable amount of the peptide hormone is restored in the endocrine tissue before it is released into the blood. Therefore, the mechanism of release of steroid cannot be applied to that of gonadotropins, and the releasing and synthetic mechanisms must be discussed as another phenomenon. After viewing the differences between these two hormones, the discrepancy between Moyle's result on steroid and ours on gonadotropins seems likely.

Moyle suggested that LH is probably bound to the Leydig cell tumor even after washing. In contrast, LH-RH seems to be easily dissociated by washing in our experiment. However both LH and LH-RH are continually required to keep the elevated levels of c-AMP to maintain the hormone release.

In LH synthesis, it was also demonstrated that LH-RH was continually required to maintain the elevated levels of c-AMP. Although there were no statistically significant changes at the content of FSH in the pituitaries after 4 hour incubation (Fig. 4), the increasing tendency of FSH concentration in the treated anterior pituitaries was observed, and it may be suggested that the same conclusion could be applied to the synthesis of FSH.

It was reported that the primarily administered LH-RH changes the responsiveness to the secondarily administered LH-RH *in vivo*.⁵ Of interest in our *in vitro* study, the primarily stimulated anterior pituitaries did not show any altered responsiveness to the secondarily administered LH-RH. It is not always capable to translate some mechanism of *in vivo* phenomenon to similar effect in *in vitro*. The pulsatile release of gonadotropin *in vivo* was reported by several investigators.^{6,7} This phenomenon can be induced by the continuous stimulation of the hypothalamic LH-RH or by the cyclic stimulation of this hormone. If the latter is true, such intermittent stimulations of LH-RH might be essential to maintain the gonadotrophs awakened for the release of gonadotropins. In contrast, the first stimulation period in our *in vitro* study was for 20 minutes following the 2 hour incubation. This could be an explanation in which the responsiveness of the pituitary to the secondary stimulation was not enhanced nor depressed, after the relatively short time stimulation (20 min.) of the pituitary gland by LH-RH.

Cyclic nucleotides have been suggested as the second messenger of many hormones.⁸ Some investigators reported c-GMP as the second messenger of LH-RH,^{9,10} whereas c-AMP is regarded as the second messenger by many other researchers.^{11,12} In our study the incubation with dibutyryl c-AMP showed the similar effect of the incubation with LH-RH. The incubation with LH-RH elevated the intracellular c-AMP content, and by washing the elevated c-AMP was decreased to control level. The data obtained here indicate that c-AMP could be a second messenger of LH-RH. However the whole metabolic pathway from c-AMP synthesis to the release of gonadotropins should be further studied.

In conclusion, in our *in vitro* study, the continuous LH-RH stimulation, which also maintains the continuous intracellular accumulation of c-AMP, is required for the anterior pituitary to maintain the gonadotropin release and synthesis at the stimulated levels. The priming of LH-RH on the male rat pituitary gland for 20 minutes in the *in vitro* culture showed no significant effect on subsequent gonadotropin release, and the priming stimulation did not alter the responsiveness of the gonadotrophs to the secondary stimulation by LH-RH.

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