NOTE

Quick Release of FSH Stimulated by Repeated Applications of Synthetic Luteinizing Hormone-Releasing Hormone (LH-RH) in Immature Male Rats

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Synopsis

Serum FSH and LH levels were estimated by radioimmunoassay following single subcutaneous administration, and repeated subcutaneous administration at various intervals in immature male rats. Single injection of LH-RH induced slight increase in FSH levels in comparison with considerable increase in LH. By two successive injections, the release of FSH was markedly stimulated when the time intervals were 15, 30, and 60 minutes, and rapidly elicited to release after the second injection. The greatest response was observed by two injections at 60 minutes interval. Moreover, the elevation of serum FSH level after the third injection was particularly remarkable, and the response was much stronger than that to the second injection. On the other hand, serum LH levels following the repeated injections were quite similar to that of FSH. And both FSH and LH were linearly responded to doses of LH-RH repeatedly administered.

These results indicated that the repeated quick administrations of LH-RH under appropriate intervals caused the acute high release of FSH, which was quite similar to LH release, and that these acute release of FSH and LH showed the dose-response relationship.

It is well known that LH-RH stimulates the release of FSH in the rat and other mammalian species in addition to LH from anterior pituitary gland. A single injection of LH-RH was markedly effective in stimulating LH release, but it was difficult to evoke strong FSH release in this condition (Arimura et al., 1972b; Bowers et al., 1972; Debeljuk et al., 1972b; Yen et al., 1973). A considerably high serum FSH level was obtained in vivo only by the prolonged exposure (i.e. intravenous infusion) to LH-RH (Arimura et al., 1972b; Debeljuk et al., 1973). On the other hand, Aiyer et al. (1973) indicated that, by a brief exposure in advance to LH-RH, LH secretion capacity of anterior pituitary gland was changed, and that serum LH level was rapidly responded against a further stimulus by succeeding administration of LH-RH.

In connection with this finding, the present experiments were conducted to explore in detail the possibility to produce quickly a considerable elevation of serum FSH level in the immature male rats, by injecting subcutaneously repeated doses of LH-RH at various time intervals. At the same time, serum LH level was also estimated.

Materials and Methods

Animals

Immature male rats (25 day old) of Sprague-Dawley strain (SD-SLC, Shizuoka, Japan) were used.
throughout experiments since they responded relatively well to LH-RH in terms of FSH release (Debeljuk et al., 1972a).

**LH-RH**

LH-RH (the decapeptide) was prepared by classical stepwise elongation method followed by purification with partition chromatography on Sephadex G-25 (Solvent system: n-butanol: 0.1 M ammonium acetate=1:1).

**Blood collection**

Blood was taken from the external jugular vein under ether anesthesia at various time intervals following the final injection of LH-RH. Blood sampling per one group was carried out in 5-7 rats. Serum was separated and kept at −20°C until assayed for LH and FSH.

**LH-RH administration**

Experimental animals were received subcutaneous injection of 0.5 μg LH-RH except in the case of plotting dose-response curve. Throughout the experiments, a dose for single injection of LH-RH was dissolved in 0.5 ml saline.

i) single injection

Blood samples were taken at 0, 15, 30, 60, 120, 180, 240, and 360 minutes after the injection.

ii) double injection

The time intervals between two injections of LH-RH were 15, 30, 60, 120, and 240 minutes. Samples of blood were collected at 15, 30, 60, 120, and 180 minutes after the second injection.

A series of dose-response curves of LH and FSH by double injection were plotted at 60 minutes interval between two injections.

Blood samples were taken 30 minutes after the second injection.

iii) triple injection

The time interval between the first and the second injection was 60 minutes, and that between the second and the third was 30 minutes. Blood samples were obtained at 15, 30, 60, 120, and 180 minutes after the final injection.

**Hormone assay**

Serum LH and FSH levels were estimated by radioimmunoassay. Serum LH was determined by the method of Niswender et al. (1968). The results were expressed in terms of NIH-LH-S-17. Serum FSH was measured by use of NIAMD-RAT-FSH-RIA kit as described by Daane and Parlow (1971). The values were expressed in terms of NIAMD-RAT-FSH-RP-1. Each sample was assayed in duplicate.

**Results and Discussion**

Changes in serum FSH and LH levels after subcutaneous single and multiple injections of LH-RH to immature male rats, are given in Fig. 1–3.

Single administration of LH-RH in dose of 0.5 μg increased serum LH by approximately 10 fold based on the preinjection level, while an increase of serum FSH level was approximately 1.8 fold.

These results were similar to those obtained in vivo by the previous workers (Arimura et al., 1972b).

However, when injected subcutaneously two successive doses of LH-RH, not only LH release but also FSH release was stimulated rather quickly and easily (Fig. 1,2). As shown in Fig. 1, the second injection of LH-RH resulted in a considerable elevation of FSH when time intervals between the first and second injection were 15, 30, and 60 minutes. And large amounts of FSH was easily elicited to release within 15 minutes after the second injection. The maximum response of FSH release (6.8 fold increase over the preinjection level) was obtained under 60 minutes intervals.

When the intervals were 120 or 240 minutes, however, the response to the second injection was almost similar to that observed at single injection.

Furthermore, third injection was performed at the time when the maximum serum FSH concentration was observed after the second injection. Remarkable additional release of FSH was observed within 15 minutes and it reached maximum (approximately 9.1 fold increase over preinjection level) at 30 to 60 minutes after the third injection (Fig. 3).

So far a prolonged infusion was the only method to induce such a high FSH release by LH-RH, in vivo. However, also by repeated administrations at a relatively short time intervals as shown in the present study, large amounts of FSH was released. Thus it was possible to induce significantly greater release of FSH by quick injection, when anterior pituitary gland was previously exposed to LH-RH by re-
Fig. 1 and 2. Time course of serum FSH and LH release in immature male rat following two injections of LH-RH at varying time intervals (A; 15, B; 30, C; 60, D; 120, E; 180 minutes, respectively). LH and FSH levels following the single injection of LH-RH are illustrated by broken lines. Standard errors are represented by vertical lines.

peated administration under appropriate intervals.

On the other hand, the profiles of LH responses were illustrated in Fig. 2 and 3. Double stimulation at 15, 30, and 60 minutes intervals caused the significantly greater rise of serum LH level and the response was greatest (approximately 41 fold increase over preinjection level) under 60 minutes interval. The maximum serum LH concentration were in approximately the same range as a prolonged infusion data (Arimura et al., 1972b). However, when the intervals were 120 or 240 minutes, the response was slightly enhanced. Generally, the relationship between the LH response and the time interval were quite similar to that of FSH.

In female rats anesthetized with sodium pentobarbitone, the pituitary response on
Fig. 3. Time course of serum FSH and LH release following three injections of LH-RH, which are illustrated by the double solid lines. Time interval between the first and second injection is 60 minutes, and that between the second and third is 30 minutes. Broken line indicates the time course after a single injection, and solid line shows that after the second injection. Standard errors are represented by vertical lines.

the day of proestrus increased considerably by two LH-RH injections at one hour interval (Aiyer et al., 1973). Our results presented here, clarified the details of injection time intervals to release large amounts of FSH in addition to LH. Accordingly, our observations indicated conclusively that the repeated quick administrations of LH-RH under appropriate intervals could markedly stimulate the release of both LH and FSH at the same time.

Although the mechanism of quick response remains still unclear, it is conceivable that LH-RH itself significantly enhances the secretory capacities of LH and FSH of anterior pituitary gland in response to a further stimulus. In addition, in view of the results indicating that LH-RH can stimulate the synthesis of both LH and FSH in the pituitary gland (Redding and Schally, 1972), the strong responses by repeated administrations presented here, may reflex the stimulation of the syntheses of these hormones in addition to release. On the other hand, it might be possible to consider the enhancement of the sensitivity of anterior pituitary gland by sex steroids which were secreted by LH-RH administration through the elevation of serum LH level.

Finally, as shown in Fig. 4, both LH and FSH were linearly responded to the logarithmic scale of the total doses of LH-RH repeatedly administered. This relationship may be conveniently applicable to compare the proportion between LH and FSH releasing activities of LH-RH analogs with that of LH-RH itself.

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References


Fig. 4. Dose-response curves of LH-RH by double injections at 60 minutes time interval. Total doses of LH-RH administrated are plotted by the logarithmic scale. Standard errors are represented by vertical lines.
