Self-Priming Effect of LH-RH on Pituitary Gonadotropins in Hyperprolactinemic Women

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Abstract

To investigate how various concentrations of serum prolactin (PRL) influence the priming effect of luteinizing hormone releasing hormone (LH-RH) on the pituitary gland, 24 women with various blood PRL concentrations received intravenous injections of 100 μ g of synthetic LH-RH twice at an interval of 60 minutes and their serum LH and follicle-stimulating hormone (FSH) were measured and analysed. In the follicular phase with a normal PRL concentration (PRL < 20 ng/ml, n=6), marked first peaks of the two hormones following the first LH-RH stimulation and enhanced second peaks after the second LH-RH administration were observed, indicating a typical priming effect of LH-RH on gonadotropins, though the second response of FSH was more moderate than that of LH. In hyperprolactinemia, in which the serum PRL concentration was higher than 70 ng/ml (n=13), the basal concentration of gonadotropins was not significantly changed but the priming effect of LH-RH on LH and FSH was significantly decreased ($p < 0.01$). No marked second peaks of LH and FSH were observed, suggesting an inhibitory effect of hyperprolactinemia on the second release of LH and FSH. In contrast, this effect was restored in a group of women whose serum PRL concentration was between 30 and 50 ng/ml (n=5). Furthermore, enhanced second peaks of both LH and FSH were noted after successful bromocriptine therapy reduced hyperprolactinemia (PRL > 70 ng/ml) to less than 25 ng/ml (n=5). Statistical analysis of the first and second peak ratio (A_1/A_2 ratio) of LH and serum PRL concentration revealed that these two factors correlated well with each other ($r = 0.9500$). These data suggest that the priming effect of LH-RH can be utilized for the evaluation of the pituitary function of secreting gonadotropin in hyperprolactinemia.

Hypothalamic luteinizing hormone releasing hormone (LH-RH) acting on an anterior pituitary gland sensitized with ovarian steroid hormones may exert a priming effect on the pituitary gonadotrophs so that further

exposure to the hormone is followed by a marked increase in response (Padmanabhan 1982). Continuous infusion of LH-RH or intermittent stimulation with this decapeptide has been reported to induce similar first and second LH release in various species including the human female (Aiyer *et*

al., 1974; Hoff *et al.*, 1977; Lin *et al.*, 1982). The second increased release of LH indicates that repeated stimulation with LH-RH could assist in examining both the release and synthesis of the pituitary function.

The purpose of this study was to investigate how various levels of serum prolactin (PRL) influence the priming effect of LH-RH on the pituitary gland by stimulating women with LH-RH intermittently at 60 minute intervals, so that all subjects can be tested within a day for hypothalamic disorders.

Subjects and Methods

Subjects

Twenty-four women aged from 21 to 39 years were divided into three groups according to their serum PRL levels at 10 a.m.. The limits of the PRL concentrations in these three groups were (1) less than 20 ng/ml ($n=6$), (2) between 30 and 50 ng/ml ($n=5$), (3) more than 70 ng/ml ($n=13$), respectively. All the women received intravenous injections of 100 μ g of synthetic LH-RH twice at an interval of 60 minutes on 7–10th day of the menstrual cycle or after the cessation of uterine bleeding induced by estrogen and/or progesterone injection intramuscularly. Blood samples were collected at 0, 30, 90, 120, 180 minutes after the first LH-RH stimulation. Five of 13 women with hyperprolactinemia were subjected to this 2 step LH-RH administration test after their serum PRL concentrations were reduced to less than 25 ng/ml by bromocriptine therapy (2.5–50 mg/day, p.o.). All sera collected at 9:00–12:00 hr in the morning were kept at -20°C until assayed.

LH and FSH assays

Serum LH and FSH concentrations were determined by double antibody radioimmunoassays using reagents provided by the National Institute of Arthritis, Diabetes and Digestive and Kidney Disease. NIAMDD-hLH-I-1 (AFP-4345B) and NIAMMD-hFSH-I-1 (AFP-2844B) were used as reference preparations for LH and FSH, respectively, and their concentrations were expressed as ng per ml serum. The within- and between-assay coefficients of variation were 2.5% and 8.5% for LH, and 3.7% and 9.8% for FSH,

respectively. The limit of detection of the LH assay was 10 ng/ml while that of the FSH assay was 150 ng/ml.

$\Delta_1\Delta_2$ ratio

By defining the first peak of LH or FSH (maximum level before the second administration of LH-RH minus 0 min. level) as Δ_1 , and the second peak of the hormones (maximum level after the second administration of LH-RH minus 60 min. level) as Δ_2 , respectively, the $\Delta_1\Delta_2$ ratio was calculated by dividing Δ_2 values by Δ_1 values as indicated in Fig. 1. The correlation between the LH- $\Delta_1\Delta_2$ ratio and serum PRL level in hyperprolactinemia was also investigated.

Statistics

The data were processed to test the difference between group means for their statistical significance ($P<0.05$) by one-way analysis of variance and Student's test, or by correlation coefficients computed by least squares analysis.

- Δ_1 : Maximum level before the 2nd administration—0 min. level
- Δ_2 : Maximum level after the 2nd administration—60 min. level
- $\Delta_1\Delta_2$ ratio : Δ_2/Δ_1

Fig. 1. Schematic illustration of Δ_1 and Δ_2 of gonadotropin in two step administration of synthetic LH-RH at 60 minute interval (100% = 30 min. levels).

Fig. 2. Two peaks of LH in follicular phase of normal cycling women (n=6) after two step LH-RH administration (mean $\pm$ S.E.).

Fig. 3. Two peaks of FSH in follicular phase of normal cycling women (n=6) after two step LH-RH administration (mean $\pm$ S.E.).

Fig. 4. Serum LH levels in hyperprolactinemia in response to two step LH-RH stimulation (mean $\pm$ S.E.).

Hyperprolactinemia
"PRL > 70 ng/ml" (n=13)
Relative hyperprolactinemia
"30 < PRL < 50 ng/ml" (n=5)
After treatment with Bromocriptine
"PRL < 25 ng/ml" (n=5)

P < 0.01 A vs. B
P < 0.01 A vs. C
P < 0.01 D vs. E
P < 0.01 D vs. F
P < 0.01 G vs. H
P < 0.01 G vs. I

Fig. 5. Serum FSH levels in hyperprolactinemia in response to two step LH-RH stimulation (mean $\pm$ S.E.)

Results

In the follicular phase, normal control women with normal PRL levels (< 20 ng/ml) showed marked first peaks (Δ_1) of LH and FSH after the first LH-RH stimulation and enhanced second peaks after the second LH-RH administration (Figs. 2 and 3). The second response of FSH was more moderate than that of LH. Thirteen women with hyperprolactinemia whose serum PRL levels were more than 70 ng/ml showed a significantly less pronounced priming effect of LH-RH on LH and FSH without significant second peaks ($p < 0.01$), although the basal levels of these hormones were not apprecia-

bly different from those of normal women in the follicular phase (Figs. 4 and 5). In contrast, the priming effect of LH-RH was observed in the group with moderate hyperprolactinemia (30–50 ng PRL/ml). Furthermore, enhanced second peaks of both LH and FSH were noticed after successful bromocriptine therapy reduced hyperprolactinemia to less than 25 ng/ml. The correlation of the $\Delta_1\Delta_2$ ratio of serum LH and PRL concentrations is shown in Fig. 6. As shown in this figure, a significant correlation was observed between them, indicating that the priming effect of LH-RH on gonadotropin can be recovered as serum PRL is reduced to normal.

Fig. 6. Statistical analysis on co-relation between LH A_1/A_2 ratio and serum PRL levels in hyperprolactinemia.

Discussion

Pituitary response to exogenous LH-RH is varied in many species by the mode of stimulation with this peptide. It is well known that single stimulation with LH-RH induces secretory peaks of both LH and FSH 15–30 minutes after its administration either intravenously or intramuscularly. In contrast, continuous infusion of LH-RH (Koninckx *et al.*, 1976) to the anterior pituitary induces the first peaks of gonadotropins approximately 30–60 min. after the initiation of exposure, then the discharge of both LH and FSH gradually declines. If this infusion is continued, the discharge of LH and FSH again gradually increases and peaks 3–4 hr later. Similarly, intermittent stimulation of the pituitary with LH-RH at 60-minute intervals gives rise to an increase in LH and FSH initially, followed by more enhanced second peaks of the hormones (Aiyer *et al.*,

1974) in rats. Wildt *et al.*, (1981) demonstrated that the intermittent administration of LH-RH re-established gonadotropic secretion in rhesus monkeys bearing hypothalamic lesions which abolish endogenous LH-RH production. All these reports suggest that LH-RH not only stimulates the discharge of LH and FSH from the anterior pituitary, but also possibly primes this gland to enhance the responsiveness of the gonadotrophs to further exposure to LH-RH. LH-RH stimulation can be done at 60 to 120 minute intervals in human subjects, however all subjects were administered LH-RH at 60 minute intervals in the present study to complete this examination during the morning clinic. The exact mechanism of this priming effect is still unknown, but one possibility is that LH-RH may increase the synthesis of a releasable pool of LH so that the response to the second LH-RH stimulation is increased. Another hypothesis is that the first LH-RH may stimulate the

production of additional LH-RH receptors on the gonadotrophs with ovarian steroids which are synthesized and released by the first LH-RH (Padmanabhan *et al.*, 1982). Quite recently we demonstrated that the number of secretory granules in the gonadotrophs of the rat anterior pituitary observed electronmicroscopically were significantly decreased after the first administration, but the number increased to the control levels within 60 minutes before the second stimulation, and furthermore, protein synthesis inhibitor suppressed this primary effect in some species (Takahashi, 1986).

In the present study, hyperprolactinemia abolished the priming effect of LH-RH on the gonadotrophs presumably by decreasing the synthesis of the hormones. The reduction of the serum PRL concentration resulted in recovery of the priming effect. It is not clear how the abnormal dopaminergic mechanism acts on the mechanism of synthesis and release of gonadotropins and how normalization of the PRL concentration restored the priming effect, but it is likely that the cyclic center of the hypothalamo-pituitary axis is disordered by the hyperprolactinemic endocrine state, because basal concentrations of LH and FSH were not influenced by hyperprolactinemia and only the priming effect is suppressed by this endocrine state. By utilizing highly sensitive radioimmunoassay for LH-RH, we previously demonstrated that peripheral circulatory levels of endogenous LH-RH in matured women were extremely low (0.99 ± 0.14 pg/ml serum) (Makino *et al.*, 1980) and pulsatile alteration of this decapeptide in the subjects could not be detected. However, others reported the intermittent discharge of LH-RH into the portal vessel of the hypothalamo-pituitary system in primate (Carmel *et al.*, 1976, Knobil, 1980). Thus, pulsatile stimulation of endogenous LH-RH on the anterior pituitary is a key to maintaining normal pituitary functions of releasing and synthesizing LH and FSH. Valk *et*

al. (1980) reported that the administration of LH-RH to their patients with isolated gonadotropic deficiency in intermittent doses for 5 days induced a rapid pituitary response, suggesting that the mode of LH-RH secretion controls pubertal maturation.

Although further studies are required to elucidate the details of the mechanism by which the priming effect is exerted, the data indicate that two step stimulation by synthetic LH-RH can be applied to clinical evaluation of the pituitary function and this test is more meaningful than the conventional single LH-RH administration test.

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