Analysis of the Positive Feedback Effect of Estrogen on the Release of Gonadotropin in Women

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Synopsis

In order to elucidate the positive feedback mechanism of estrogen on gonadotropin release in women, the responses of plasma LH and FSH to the constant infusion of estradiol-17β for a prolonged period were studied. The infusion was initiated on various days of the follicular phase and maintained for 36-66 hr at a constant rate of 500 or 1,000 μg/24 hr. When the stimulus of estradiol was sustained for more than 30 hr in the women of the middle or late follicular phase, a positive feedback effect to elicit gonadotropin surges was observed during the maintenance of the infusion. In contrast, the stimulus of estrogen was ineffective in the early follicular phase, even if sustained for a longer period up to 66 hr. Gonadotropin levels, also, increased after the end of infusion. The magnitude of the responses, however, was much smaller, as compared to spontaneous preovulatory gonadotropin surges. In all cases, the effect of estradiol was greater for LH than for FSH. It is suggested that: 1) Preovulatory gonadotropin surges are triggered by estrogen increments rather than the withdrawal of the negative feedback effect of estrogen. 2) Low levels of estrogen for a certain period of the early follicular phase may play an important role in priming the control system which responds to the positive feedback effect of estrogen.

Based on the data obtained from animal experiments, it is a known fact that estrogen plays a key role in the preovulatory release of gonadotropin (Kurachi, 1965; Igarashi, 1967; Goding et al., 1969; Schwartz, 1969; Kamioka, 1970; Caligaris et al., 1971; Scaramuzzi et al., 1971; Yamaji et al., 1971). In monkeys, a precise interrelationship between the strength-duration characteristics of the estrogen stimulus and the LH responses was studied by Karsh et al. (1973). They demonstrated that plasma estrogen concentrations of 200–400 pg/ml maintained for 36 hr were effective in eliciting LH surges which were indistinguishable from those occurring spontaneously at mid-cycle. In human subjects, most of the previous studies on the regulation of gonadotropin release by gonadal steroids have employed single or repeated intravenous or intramuscular administration of the steroids which might result in unphysiological responses (Kurachi et al., 1966; Igarashi, 1967; Vande Wiele et al., 1970; Yen and Tsai, 1971; Taymor et al., 1972; Show et al., 1975).

Yen and Tsai (1972) showed that LH surges were induced in women after the termination of estradiol infusion which was sustained for 36 hr. In the preliminary studies (not published), we failed to demonstrate LH surges in response to a rise in circulatory estrogen in women who were administered estradiol at a constant rate for 48 hr during the early follicular phase. A withdrawal of estradiol from general

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circulation did not bring on significant LH release either. Our present experiments were designed to analyze the characteristics of the estrogen stimulus on the activation of the positive feedback system in women.

Materials and Methods

Subjects

Fifteen women having histories of normal menstrual cycles with a length of 27 to 33 days volunteered for the study. All subjects were hospitalized in a ward for 4 days. The experiments were initiated on various days of the cycle (days of 3, 4, 5, 6, 7, 10, 11, 12 and 13).

Infusion and blood collections

Two intravenous cannulae were inserted in the brachial and antecubital veins for iv infusion and blood collections, respectively. The cannula for sampling was connected via a three-way stopcock to iv infusion of 500 ml saline which contained 5,000 units of sodium heparin. Estradiol-17β was administered for 33-66 hr by the use of an infusion pump. Estradiol was dissolved in Ethanosol (Hartmann’s solution containing 5% of ethanol, supplied by Midoriuji Co.) The rate of infusion was 500 or 1,000 μg/150 ml/day. The former dose is equivalent to the production rate of estradiol during the pre-ovulatory follicular phase (Tsai and Yen, 1971).

After taking 3 control samples, 8 ml of blood samples were obtained at 3-hr intervals throughout the experiments. All the blood samples were analyzed for FSH and LH. Estradiol was measured in the selected samples; every other sample from 6 hr after the initiation to the end of infusion, and every sample at all other times of the experiment.

Radioimmunoassay

Plasma FSH and LH: FSH and LH concentrations in 100-μl aliquots of peripheral plasma were determined using a radioimmunoassay kit supplied by Daiiichi Isotope Co. The useful range of assay sensitivity is 2.0 to 500 mIU/ml. The intra-assay coefficients of variation (C.V.) for FSH and LH are 9.2% (N=21) and 8.5% (N=21), and the inter-assay C.V. 16% (N=21) and 17% (N=21), respectively. Gonadotropin values are expressed as mIU/ml 2nd IRP-hMG.

Estradiol: Estradiol concentrations were determined in 50- to 200-μl aliquots of peripheral plasma using a radioimmunoassay kit provided by Midoriuji Co. The intra- and inter-assay C.V. are 9.3% (N=19) and 20% (N=17), respectively and the sensitivity is 25 pg/ml.

Results

Effects of estradiol treatment at the infusion rate of 500 μg/day for 66 hr

Nine women were exposed for 66 hr to elevated circulatory estradiol which effected final increments of approximately 200–400 pg/ml. The patterns of FSH, LH and estradiol in the individuals are illustrated in Figs. 1, 2 and 3. In some cases, the treatment was associated with a slight reduction in FSH and LH levels within 6 hr after the onset of the infusion. However, since the control levels were low, the declines of FSH and LH were not prominent. The FSH and LH rise to the control levels or slightly above were observed during the late periods of the infusions in AO, EH and MN in which the infusions started by Day 6 of the cycle (Fig. 1).

In two subjects of Day 10 and 11, LH levels were gradually increased from 45–48 hr after the onset of estradiol treatment, reaching the maximal levels of 90–100 mIU/ml. In the subjects of the late follicular phase, the treatment of estradiol maintained for more than 30 hr elicited LH surges (Figs. 2 and 3) which were indistinguishable in either the magnitude and duration from those observed during the pre-ovulatory periods of normal menstrual cycles (Tamada et al., 1973a). Since the responses of FSH were much smaller than those of LH, even if it existed, the changes in FSH concentrations under the present experimental conditions preclude the satisfactory statistical analysis.

Effects of estradiol treatment at the infusion rate of 1,000 μg/day for 36, 48 and 66 hr

The patterns of circulatory gonadotropins and estradiol are illustrated in Figs. 4 and 5. In the subjects YK and MH, who were exposed to the estrogen stimulus for 36 hr in the early follicular phase, the levels of FSH and LH were increased slightly above the
control levels after the end of the infusion. This finding may be considered to be a rebound from the negative feedback action of estrogen. In subject MM, the infusion was initiated on Day 7 of the cycle and maintained for 48 hr (Fig. 4). Only a moderate rise in plasma LH occurred following the cessation of the infusion. In subject YS (Day 7 of the cycle), who received the estradiol treatment for 66 hr, LH rise over the control levels was observed during the late period of the infusion (Fig. 5). The increased levels of plasma LH, however, were much lower, as
compared to those observed during the preovulatory period of normal cycle.

In the subjects, CS and TW, since the control levels of estradiol were remarkably high, the infusion must have been started after the initiation of spontaneous preovulatory estrogen increase. Subject CS showed profound responses of FSH and LH after 42-hr maintenance of high levels of estrogen. In subject TW, estradiol was administered during the preovulatory period. As shown in Fig. 5, the mid-cycle surge of both LH and FSH was not immediately suppressed in spite of the continuous infusion of a large dose of estradiol.

Discussion

Karsh et al. (1973) reported that increments in circulatory estrogen which exceeded a threshold of approximately 100 pg/ml, if sustained around 36–42 hr, induced LH surges in the monkeys of the early follicular phase. In the present study, the similar positive feedback action of estrogen is demonstrated in women.

However, depending on the days of cycle when estrogen administration started, the duration of an effective stimulus was markedly different. When exogenous estro-
Fig. 4. Plasma LH, FSH and estradiol levels before, during and after the continuous infusion of estradiol (1,000 µg/24 hr) in the subjects of days 3 and 7. The striped areas designate plasma estradiol levels.

Fig. 5. Plasma LH, FSH and estradiol levels before and during the continuous infusion of estradiol (1,000 µg/24 hr) in the subjects of days 7, 10 and 13. The striped areas designate plasma estradiol levels.
gen whose dose was equivalent to the production rate of estrogen during the normal preovulatory follicular phase, was administered in the late follicular phase, the duration of the treatment required for the initiation of LH surges could be reduced to approximately 30 hr. The amplitude of LH release during the estrogen treatment appears to be larger as getting closer to the period of mid-cycle. In contrast, the stimulus of estrogen was ineffective in the early follicular phase, even if sustained for a longer period up to 66 hr. These results suggest that the low levels of circulatory estrogen during the early follicular phase may play an important role in priming the control system which responds to the positive feedback effect of estrogen, and that the duration of the stimulus required for priming the system may be more than several days.

In the earlier study, we found that preovulatory LH surges take place with an acute fall in circulatory estrogen and proposed that a decline of estrogen may play a role in triggering preovulatory LH surges (Tamada et al., 1973b). The same considerations have been made by others (Korenmann and Sherman, 1971; Mishell et al., 1971; Yen and Tsai, 1972). Our present data show that gonadotropin levels increase following the cessation of estrogen infusion. The magnitude of the responses, however, was much smaller, as compared to spontaneous preovulatory gonadotropin surges. This rebound phenomenon must be defined separately from the so-called "positive feedback effect of estrogen".

We observed that the estradiol treatment for more than 30 hr elicited vast LH surges during the maintenance of the elevated levels of estradiol. The responses of LH were indistinguishable, in either the magnitude or the duration from spontaneous preovulatory LH surges. These observations suggest that preovulatory LH surges are induced by the increments of estrogen rather than the withdrawal of the negative feedback effect of estrogen.

In the present study, the mid-cycle surges of FSH and LH were not suppressed by the estrogen treatment. This finding is supported by the data of Tsai and Yen (1972), who demonstrated that the negative feedback action of estradiol could not be effective, once the mid-cycle surges were triggered. Since the hormonal events in the human pituitary gland, i.e. the content or the rate of synthesis of gonadotropin, are not known, the question why a priming action of estrogen on hypothalamic-pituitary axis should be sustained for a long term remains to be solved.

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