The Acute Stimulatory Effect of Estrogen on Gonadotropin Release in Gonadotropin-Releasing Hormone-primed Women

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Abstract

In order to prove the acute stimulatory effects of estrogen on pituitary gonadotropin release, we have performed the present experiments in 8 women with a hypergonadotropic state due to surgical castration or primary ovarian failure. They received gonadotropin releasing hormone (Gn-RH) for 12–21 h at the constant rate of 20 μg/h. In 5 of the women, estradiol-17β was concomitantly administered at the rate of 20 μg/h from 6 h after the start of Gn–RH infusion. Blood samples were collected frequently throughout the experiments for the analysis of LH, FSH and estradiol.

In response to the sole stimulation of Gn–RH, remarkable and prompt rises in LH (313.5%), but to a lesser degree in FSH (194.2%), were observed within the initial 3 h, and their high levels were maintained throughout the experimental period. However, the additional administration of estradiol brought on a further sudden rise in both gonadotropins levels: 178.3% for LH and 163.5% for FSH within 2 h. These high levels were sustained during estradiol infusions. In 2 of them, blood samples were obtained for several hours after cessation of estradiol infusion. The circulating gonadotropin level dropped precipitously close to the baseline level within 3 h after estradiol infusions.

Our data indicate that estrogen has an acute and strong augmentative effect on Gn–RH induced gonadotropin release in addition to its conventional negative and positive feedback effects.
monkeys. Nakai et al. (1978) observed that the administration of estradiol to ovariecto-
mized rhesus monkeys bearing hypothalamic
lesions which had abolished endogenous
LH-RH and estradiol production, resulted
in a decrease in circulating LH and FSH,
followed by unambiguous discharge of these
hormones. Delemarre et al. (1983) under-
took pulsatile LH-RH administration in a
nine-year-old prepubertal patient with 45 X
gonadal dysgenesis for three weeks. Follow-
ing the administration of 2.5 µg/day of
ethinyl-estradiol (E-E) in addition to LH-
RH, they recognized a remarkable increase
in LH resulting in a LH surge three days
after the beginning of E-E administration,
while the FSH level showed a suppression
that depended upon the E-E dose.

In the present study, we describe direct
evidence of the positive effects of estradiol
on LH and FSH at the pituitary level in
humans and will analyze the effects of
estradiol on pituitary responsiveness to Gn-
RH in detail.

Materials and Methods

Subjects
Six castrated women (ranging in age from 35
to 40) who had undergone hysterectomy with
bilateral salpingo-oophorectomy for uterine fib-
roma or carcinoma one month or more before
this study and two hypogonadal women (age 22
and 29, respectively) who were diagnosed by the
laboratory data and pathologic examinations,
participated in this study. These subjects were
informed about the nature of this study, and
consent was obtained.

Methods

Experiment 1: Three castrated women were
hospitalized, and an intravenous cannula (Argyle
18G) was inserted into an antecubital vein. The
cannula was connected via a L-type three-way
stopcock to the infusion line of 500 ml of saline
which contained 1000 units of sodium heparin
as an anticoagulant. The infusion rate was
maintained at 10 ml/h to keep the cannula open
in order to draw off blood samples. After collect-
ing the baseline blood samples, a smaller
 cannula (Argyle 22G) was inserted into another
antecubital vein, and Gn-RH (Tanabe Pharma-
ceutical Co., Japan) infusion was started at the
rate of 20 µg/6 ml/h, using a constant infusion
pump (Termo Co., Japan) for 12 h. Blood
samples were collected at the rate of 10 ml every
30 minutes for the first 2 h of the infusion and
every hour thereafter throughout the infusion.
After immediate centrifugation, the plasma was
separated and stored at -20°C until assay.

Experiment 2: Three castrated and two
hypogonadal women were hospitalized. 6 hours
after the start of Gn-RH infusion for 13–21 h,
estradiol-17β infusion was added for 7–8 h at
the rate of 20 µg/6 ml/h through the cannula for
Gn-RH infusion. Estradiol-17β was dissolved in
Ethanosol (Hartman's solution with 5 % ethanol:
Midorijuji Co., Japan). The blood
samples were collected, and the plasma was
stored as described above.

Hormonal analysis

Plasma LH and FSH: Plasma LH and
FSH levels were measured in duplicate using a
radioimmunoassay kit (Daichi Isotope Co., Japan)
and were expressed as mIU/ml 2nd IRP-hMG.
The average intraassay coefficients of variation
(CVs) of LH and ESH were 8.5% and 9.2%
(n= 21), and the average interassay CVs were
17% and 16% (n= 21) respectively. The limit
of sensitivity of each assay was 2.0 mIU/ml.

Plasma estradiol: Plasma estradiol levels
were also measured using a radioimmunoassay
kit (Midorijuji Co., Japan). The average intra-
and interassay CVs were 9.3% (n=31) and 20%
(n=17) respectively. The limit of sensitivity of
the assay was 12.5 pg/ml.

Results

EFFECTS OF Gn-RH INFUSION AT THE
RATE OF 20 µG/H INTO CASTRATED
WOMEN (EXPERIMENT 1)

The results of Gn-RH infusion at the
rate of 20 µg/h into a castrated woman
(case 1) are shown in Fig. 1. The average
baseline levels of plasma LH and FSH of
this patient were 112.0 ± 5.0 (mean ± SE)
Fig. 1. Plasma LH and FSH levels under Gn-RH 20 μg/h infusion in a castrated woman (case 1).

Table 1. Summary of plasma LH and FSH levels under Gn-RH 20 μg/h infusion with and without estradiol-17β infusion in the eight women in experiment 1 and 2.

<table>
<thead>
<tr>
<th></th>
<th>Baseline Levels</th>
<th>Response to Gn-RH</th>
<th>Response to Gn-RH and estradiol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LH</td>
<td>FSH</td>
<td>LH</td>
</tr>
<tr>
<td>case 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>112.0±5.0</td>
<td>108.3±3.5</td>
<td>421.0±9.4</td>
</tr>
<tr>
<td>case 2</td>
<td>84.5±5.5</td>
<td>59.5±3.5</td>
<td>212.5±5.8</td>
</tr>
<tr>
<td>case 3</td>
<td>44.3±2.7</td>
<td>63.3±2.0</td>
<td>138.7±4.2</td>
</tr>
<tr>
<td>case 4</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>56.2±2.4</td>
<td>50.5±1.8</td>
<td>88.3±3.9</td>
</tr>
<tr>
<td>case 5</td>
<td>64.8±6.4</td>
<td>53.7±3.0</td>
<td>144.3±10.0</td>
</tr>
<tr>
<td>case 6</td>
<td>73.5±1.5</td>
<td>90.7±2.5</td>
<td>231.3±11.0</td>
</tr>
<tr>
<td>case 7</td>
<td>126.7±5.4</td>
<td>108.7±7.8</td>
<td>405.0±30.4</td>
</tr>
<tr>
<td>case 8</td>
<td>71.0±6.0</td>
<td>122.0±2.0</td>
<td>335.9±11.8</td>
</tr>
</tbody>
</table>

mean ± S.E
mIU/ml and 108.3 ± 3.5 mIU/ml, respectively. After the start of the Gn-RH infusion, an immediate rise in the LH level was observed, reaching a maximum of 449 mIU/ml within 3 h; no remarkable change was recognized thereafter. The FSH level also rose throughout the Gn-RH infusion, but to a lesser degree. The average levels of LH and FSH in response to Gn-RH infusion were 421.0 ± 9.4 mIU/ml and 272.3 ± 18.5 mIU/ml, respectively, which were significantly (p < 0.05) higher than the baseline level.

The other two women (case 2 and 3) showed the same significant response of gonadotropins to the Gn-RH infusion, and the results are summarized in Table 1.

From these control studies in experiment 1, it was found that there is no significant difference between the gonadotropin levels in response to Gn-RH infusion in the first and second 6 h periods. As compared with the baseline levels, the average rate of increase in LH and FSH in response to the Gn-RH infusion in the castrated women was 313.5% and 194.2%, respectively.

It is likely that, under these experimental conditions with these large dose Gn-RH infusions, the hypothalamus will have minimal or no influence on pituitary gonadotropin secretion, because the pituitary gland has continued to respond to the large dose of exogenous Gn-RH, in spite of endogenous Gn-RH fluctuation.

Based on this information, subsequent experiments were designed and performed.

**EFFECTS OF ESTRADIOL-17β INFUSION AT THE RATE OF 20 µG/H DURING THE Gn-RH INFUSION INTO CASTRATED WOMEN AND HYPOGONADAL WOMEN. (EXPERIMENT 2)**

![Fig. 2. Plasma LH, FSH and estradiol levels under Gn-RH 20 µg/h infusion with additional-17β 20 µg/h infusion in a castrated woman (case 4).](image-url)
Fig. 2 shows the effects of additional estradiol-17β infusion at the rate of 20 µg/h Gn-RH infusion into a castrated woman (case 4). Fig 3 shows the effects of the same infusions in a hypogonadal woman (case 7). The doses of estradiol are equivalent to the hourly production rates of estradiol in the late follicular phase, according to our preliminary experiments.

In Fig. 2, the average baseline levels of plasma LH and FSH are given as 56.2 ± 2.4 mIU/ml and 50.5 ± 1.8 mIU/ml, respectively. As shown in Fig. 1, the immediate and remarkable rise in LH and less remarkable rise in FSH were observed within 2 h after the start of the Gn-RH infusion, and there were minimal or no fluctuations noted in these levels thereafter. The average control levels of plasma LH and FSH in response to Gn-RH were 88.3 ± 3.9 mIU/ml and 52.6 ± 2.1 mIU/ml, respectively. Compared with these, rises in LH and FSH were recognized at 2 h after the start of the additional estradiol-17β infusion and these high levels of gonadotropins were sustained for 6–7 h throughout the infusion. The average levels of plasma LH and FSH, 206.4 ± 2.4 mIU/ml and 101.0 ± 2.9 mIU/ml, respectively, in response to the estradiol-17β infusion were significantly higher than the control levels. The manner of LH and FSH response to estradiol-17β was almost the same as LH and FSH response to Gn-RH because of the acute and remarkable response. Plasma
estradiol sustained an increase in its levels gradually after the start of the estradiol infusion and reached 202 pg/ml within 2 h after the start of infusion. The same significant response of gonadotropins to the Gn-RH and estradiol-17β infusion in case 5 and 6 is shown in Table 1.

A short summary of the above is as follows. The additional estradiol-17β infusion induced an acute and significant increase in both gonadotropins in this experiment. The rate of increase of LH and FSH induced by estradiol-17β infusion was 178.3% and 163.5% on the average, respectively, as compared with the control gonadotropin levels. In the same way, the rate of increase of LH and FSH induced by Gn-RH infusion was 233.2% and 136.8% on the average, respectively, as compared with the baseline gonadotropin levels.

According to these experimental results, it is possible to say that estradiol-17β exerted a similarly strong influence on gonadotropin release and was similar to gonadotropin release as Gn-RH in castrated women.

These results led to further experiments to demonstrate the effects of estradiol-17β in hypogonadal women (case 7 and 8), and the results are shown in Table 1 and Fig. 3.

Fig. 3 shows, as previously mentioned, that the additional estradiol-17β infusion elicited a secondary increase in LH and FSH. The average baseline level of LH and FSH was 126.7±5.4 mIU/ml and 108.7±7.8 mIU/ml, respectively, and the average level of LH and FSH in response to estradiol-17β, 555.1±33.9 mIU/ml and 226.6±17.1 mIU/ml, was significantly higher than the control levels, 405.0±30.4 mIU/ml and 166.7±12.7 mIU/ml, which were in response to the Gn-RH infusion. Moreover, at 3 h after the cessation of the estradiol-17β infusion, an acute and significant decrease in both gonadotropins was observed, and it reached the baseline value below the control. The plasma estradiol level was 16 pg/ml at the time.

A short summary is as follows. The effects of the estradiol-17β infusion in hypogonadal women were almost the same as those in castrated women and indicated that estradiol had acute augmentative effects on pituitary responsiveness to Gn-RH also in hypogonadal women as well. The rate of the increase in LH and FSH induced by estradiol-17β infusion was 140.2% and 161.0% on the average, respectively, as compared with control gonadotropin levels. On the contrary, the rate of increase in LH and FSH induced by Gn-RH infusion was 395.5% and 159.3% on the average, respectively, as compared with the baseline gonadotropin levels. With regard to the rate of LH increase, Gn-RH exerted a stronger effect on LH discharges than did estradiol-17β in hypogonadal women. The precipitous decreases in both gonadotropin levels after the cessation of the estradiol-17β infusion reveal the existence of pituitary desensitization to Gn-RH during the decrease of estradiol-17β. No negative effects by estradiol-17β were recognized in our experimental series. We will refer to the analysis of this in the discussion.

**Discussion**

Recently, it has been established that estrogen has biphasic effects on pituitary gonadotropin release as negative and positive feedback effects. It is believed that, in most physiological circumstances, the main effect of estrogen is inhibitory, but in the preovulatory phase, the positive feedback effect of estrogen is recognized (Vande Wiele et al., 1970). Further investigations of the mechanism of the positive feedback action of estrogen in the hypothalamo-pituitary axis were undertaken using exogenous estrogen in rats (Legan and Karsch, 1975), in monkeys (Yamaji et al., 1971;
Karsh et al., 1973) and in humans (Tompson et al., 1974; Araki et al., 1978). Based on these experimental data, especially in rhesus monkeys, Knobil (1974) concluded that the development of the positive feedback, which played an important role in spontaneous preovulatory LH surges, was dependent not simply on a critical threshold concentration of circulating estradiol (approximately 100 pg/ml in peripheral plasma concentration), but also on an important time component (12–42 hours), and mentioned that the negative feedback was operative with subthreshold levels of estradiol or brief exposure periods. In addition to these observations, Jewelwitz et al., (1974) have proposed that spontaneous LH surge was blocked by the administration of an estrogen antiserum that neutralized the action of estrogen. From the fact mentioned above, it was clearly demonstrated that estrogen had biphasic effects on pituitary gonadotropin releases. However, these data could not clearly identify whether the site of action of the estrogen was the hypothalamus or the pituitary.

On the other hand, the availability of synthetic Gn-RH (Matsuo et al., 1971) for laboratory and clinical investigations gave rise to new possibilities for further detailed examinations of pituitary gonadotropin release, and the augmentation of pituitary responsiveness to Gn-RH by estrogen was recognized to exist in animals (Arimura and Schally, 1971) and in humans (Yen et al., 1972) during the high estrogenic phase of the menstrual cycle. As regards exogenous estrogen administration, many reports have been published to support the effects of estrogen on pituitary responsiveness to synthetic Gn-RH, and the authors agreed with the opinion that estrogen had biphasic effects on the pituitary responsiveness to Gn-RH, depending upon the dose and duration of administration of the estrogen (Libertun et al., 1974; Young and Jaffe, 1976), the early effect being inhibitory and the latter being stimulatory, but they could not all agree about the site of estrogen action (Yen and Lein, 1976; Aiyer et al., 1973). Considering the possibility of endogenous Gn-RH fluctuations by the administration of estrogen, we should carefully draw our conclusions about the effects of estrogen on the responsiveness to Gn-RH at the pituitary level.

Concerning the remaining problems, endogenous Gn-RH fluctuations in pituitary portal blood have become of world-wide interest. The development of radioimmunoassay for Gn-RH (Nett et al., 1973; Tamada et al., 1973) has made it possible to measure the concentrations of Gn-RH in peripheral plasma and to explain the physiological significances of endogenous Gn-RH. There are several reports on the peripheral plasma Gn-RH concentration in normal women (Jones et al., 1975) and in women with various endocrinological conditions (Rosemblum and Schlaff, 1976; Kawamura et al., 1980; Miyake et al., 1980). These authors have concluded that high levels of plasma Gn-RH were recognized at the midcycle in normal women, in women with secondary amenorrhea, Sheehan's syndrome and Turner's syndrome, and in postmenopausal women. These data suggest that the release of a greater amount of Gn-RH from the hypothalamus into the pituitary portal vessels plays an important part in pre-ovulatory gonadotropin discharge.

More advanced studies have been carried out in animals to measure Gn-RH concentrations in pituitary portal blood (Carmel et al., 1976), and they have revealed considerable fluctuations in the concentration of Gn-RH in the portal blood as pulsatile and episodic secretions. These data showed clearly that hypothalamic Gn-RH played an important part in the pituitary gonadotropin release and the manner of the release.

Based on previous reports mentioned above, we have designed this experiment to investigate the positive effect of estrogen at
the pituitary level in humans and analyzed the results in detail. In the present study, in order to avoid the influence of endogenous estrogen, castrated women and hypogonadal women were the subjects of the observations. Based on our preliminary experiments (Konuma and Tamada, 1976), large physiological doses of synthetic Gn-RH (20 µg/h) were administered to these subjects to overcome the effects of endogenous Gn-RH fluctuations and under this Gn-RH administration, the circulating plasma concentration of Gn-RH was presumed to be sustained at a high level of 300 pg/ml or more. Under these experimental conditions, we intended to expose these subjects to large physiological doses of estradiol-17β and distinctly recognized the acute and significant increase in both gonadotropins 2 h after the start of the estradiol-17β infusion. Furthermore, we have succeeded in ascertaining that the manner of release of pituitary gonadotropins induced by the additional estradiol-17β infusion showed a similar pattern to that induced by Gn-RH infusion alone. Moreover, 3 h after the cessation of estradiol-17β infusion, the decrease in plasma estradiol caused both gonadotropins to be decreased to very low levels.

The summary of the results of estradiol-17β infusion is as follows. (1) Our results clearly revealed direct evidence of acute and strong augmentative effects of estradiol-17β in women on the release of both gonadotropins at the pituitary level. (2) Our results demonstrated evidence for pituitary desensitization to Gn-RH under falling estradiol levels. (3) Estradiol-17β infusion exerted a strong influence similar to Gn-RH on gonadotropin discharge at the pituitary level. (4) Estradiol-17β manifested striking similarities to Gn-RH in the manner of reaction of pituitary gonadotropins. We can estimate from these results that estradiol-17β plays a role in augmenting pituitary responsiveness to Gn-RH. (5) Because the augmentation of pituitary responsiveness to Gn-RH induced by estradiol-17β was recognized 2 h after the start of the infusion and the desensitization of it was also recognized 3 h after the cessation of the infusion, it is likely that estradiol-17β induced synthesis of proteins, probably Gn-RH receptor synthesis on gonadotroph cell membranes first of all and augments the pituitary responsiveness to Gn-RH in the process of the synthesis and release of gonadotropins. As we were able to find these striking phenomena not only in LH, but also in FSH, we have concluded that these phenomena are reflected in the relationship between ovarian estrogen and pituitary gonadotropin releases at preovulatory gonadotropin surges. Our data also strongly support previous reports that simple continuation of pulsatile Gn-RH administration can induce ovulatory menstrual cycles in women with secondary amenorrhea (Skarim et al., 1982) and in monkeys bearing hypothalamic lesions.

We have failed to recognize the negative effect of estradiol in our present experiment, so we can suppose that estradiol does not have a negative effect at the pituitary level in vivo under these strong influences of hypothalamic Gn-RH.

Finally, we have concluded in agreement with the results of Labrie et al., (1978), that three important factors (1) increased Gn-RH secretion or pulse rate induced by circulating estrogen (levels and duration), (2) increased gonadotropin responsiveness to Gn-RH induced by estrogen (levels), and (3) increased gonadotropin responsiveness to Gn-RH induced by Gn-RH itself, should contribute to the preovulatory gonadotropin surges in normal females, and we are of the opinion that the first two factors are more important and effective in preovulatory gonadotropin surges.
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