EFFECTS OF OESTROGEN ON SERUM LEVELS OF LH AND FSH

By
Sven Johan Nillius and Leif Wide

ABSTRACT

The acute effects on the serum levels of LH and FSH after the administration of some oestrogen preparations were investigated in oligo-amenorrhoeic women, in postmenopausal women and in men. Immuno-reactive LH and immunoreactive FSH in the serum were assayed by a radioimmunosorbert technique.

The diurnal rhythm in the secretion of the gonadotrophins was first studied. No significant circadian variation in either LH or FSH levels in the serum of hospitalized women or healthy men was found with this radioimmunoassay procedure.

The intravenous or intramuscular administration of 17β-oestradiol in doses from 5 μg to 5 mg resulted in a decrease in the serum levels of LH in 14 of 15 women studied and in the serum levels of FSH in 10 of 12 women. This can be explained by a negative feedback effect on the hypothalamic-pituitary system during the period of observation. The decrease in the serum gonadotrophin levels was as a rule more pronounced for LH than for FSH. No positive feedback effect on the hypothalamic-pituitary system following oestrogen treatment was observed. In men insignificant and variable changes in the serum gonadotrophin levels were found. This could possibly be explained by the low oestrogen doses used.

A reciprocal influence between the gonads and the hypophysis was proposed by Moore & Price (1932). The interrelationship between the gonadal hormones and the hypothalamic-pituitary system has since then been studied by many investigators. Most previous investigations were handicapped by insensitive and/or nonspecific methods used for the assay of gonadotrophins. During recent years radioimmunological techniques have been used for the assay of these hormones and it is now possible to immunoassay quantitatively both the...
luteinizing hormone (LH) and the follicle-stimulating hormone (FSH) in small samples of blood or urine. This has given new possibilities for the study of the hypothalamic-pituitary-ovarian interrelationship and has intensified research in this field.

The aim of the present investigation was to study the acute effect of the serum levels of LH and FSH after administration of some oestrogen preparations to oligo-amennorrhoeic women, postmenopausal women and to healthy men. A study of a possible circadian rhythm in the secretion of the gonadotrophins was also included.

MATERIAL AND METHODS

Subjects

A total of 56 women and men volunteered for this study.

Seventeen postmenopausal women with an age range of 50–74 years were investigated. All these women were in good general health. They were hospitalized for surgical treatment of some minor local disorder.

Eleven women, aged 20–44, were investigated in the diurnal study. They were patients in the hospital recovering from various diseases such as a slight salpingitis. The investigations were made at different phases of the menstrual cycle except at midcycle.

Six women 20–23 years old with menstrual disorders were given 17β-oestradiol intravenously. Four of these had secondary amenorrhoea of 9–18 months duration. Two women (cases YS and GF) had oligomenorrhoea. Case YS was studied on day 56 of a 60 day cycle and case GF on day 22 of a 42 day cycle.

The male volunteers, aged 24–38, consisted of 22 healthy men working at the hospital.

Samples

Venous blood samples were collected at known times. They were obtained by venopuncture but sometimes by means of a teflon cannula placed into a vein in the forearm. In the studies lasting for more than one day, the venopunctures were made at about the same time each day.

The blood samples were allowed to clot in the refrigerator where they were kept for less than 48 h. After centrifugation the serum was drawn off and stored frozen at −15°C until assayed.

Administration of oestrogens

1) 17β-oestradiol benzoate (Ovex B®, Leo), in a dose of 1 or 5 mg was administered intramuscularly to six postmenopausal women.

2 a) 17β-oestradiol (kindly supplied by Schering AG, Berlin) in a dose of 80, 200 or 1000 µg, dissolved in propylene glycol was administered intravenously to three postmenopausal women (cases AA, ME and HS).

2 b) 17β-oestradiol (Sigma) in a dose of 5 or 10 µg, dissolved in 96% ethanol and diluted with saline was administered intravenously to two women with secondary amenorrhoea (cases ML and MW) and to one woman with oligomenorrhoea (case YS).

2 c) 17β-oestradiol (Sigma) in a dose of 50 or 100 µg, dissolved in propylene glycol

584
Radioimmunoassay for LH and FSH

Immunoreactive LH and FSH in the serum were determined by a radioimmuno-sorbent technique (Wide & Porath 1966) with the modifications recently described in detail (Wide 1969). For this method the coefficient of variation calculated from repeated assays of serum samples giving an inhibition of 40 to 60 per cent was between 7 and 11 per cent. The results were expressed in ng per ml using highly purified human pituitary LH and FSH preparations as provisional laboratory standard preparations. The LH preparation (Roos SE 26) had a biological and immunological activity of 16 000 IU (2nd IRP-HMG) per mg and the FSH preparation (Roos) 14 000 IU (2nd IRP-MG) per mg (Roos 1968).

In our laboratory serum levels of gonadotrophins within the following ranges were considered as normal \( P = 0.05 \):

<table>
<thead>
<tr>
<th></th>
<th>Women of fertile age</th>
<th>Postmenopausal women</th>
<th>Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH (ng/ml)</td>
<td>0.5–3.0</td>
<td>5.0–30</td>
<td>0.4–3.5</td>
</tr>
<tr>
<td>LH (ng/ml)</td>
<td>0.6–3.5</td>
<td>1.5–10</td>
<td>0.8–3.0</td>
</tr>
</tbody>
</table>

All sera were assayed in duplicate or triplicate at least twice in separate assays.

For the calculation of differences between mean values (paired observations) formulas based on the \( t \)-distribution were used (see standard textbooks in statistics).

RESULTS

Diurnal study

Diurnal variation in the gonadotrophin levels of the serum was studied in hospitalized women before and after the menopause and in healthy men. All the subjects had serum levels of LH and FSH within the normal range for their sex and age. Venous blood samples were taken at different time intervals. The results were expressed as the percentage change from a mean level. The mean percentage change and standard error of the mean for each point were plotted in Figs. 1–3.

In five postmenopausal women blood samples were taken every fourth hour during 24 h. The mean 24 h level for each individual was calculated from the
Diurnal study of the serum levels of LH and FSH in five postmenopausal women. The results are expressed as the average percentage change from the mean 24 hour serum level.

Fig. 1.

Diurnal study of the serum levels of LH and FSH in eleven women of fertile age. The results are expressed as the average percentage change from a mean serum level of three observations (8 a.m., 8 p.m. and 8 a.m.).

Fig. 2.

Diurnal study of the serum levels of LH and FSH in eleven men. The results are expressed as the average percentage change from a mean serum level of the four observations (9 a.m., 1 p.m., 5 p.m. and 9 p.m.).

Fig. 3.
first six observations. The percentage change from this mean level was then computed for each observation. No significant diurnal variation in either LH or FSH in serum was found (Fig. 1).

Eleven hospitalized women of fertile age were investigated, venous blood samples were taken at 8 a.m., 8 p.m. and 8 a.m. next morning. The mean level for each individual was calculated from the three observations. The percentage change from this mean level was then computed for each observation. There was no significant difference between the morning and the evening levels of the gonadotrophins in the serum (Fig. 2).

Fourteen men were studied while performing their normal routine work in the hospital. The first blood sample was taken at 9 a.m. and thereafter at every fourth hour during 12 h. The mean level for each individual was calculated from the four observations. The percentage change from this mean level was then computed for each observation. No significant variation in the FSH level in the serum was found (Fig. 3). A small increase in the LH level from 9 a.m. to 9 p.m. was noticed. However, this was not highly significant (t = 1, 9201; 0.05 > P > 0.01).

Treatment with 17β-oestradiol benzoate

17β-oestradiol benzoate was given as a single intramuscular injection to six postmenopausal women. A dose of 1 mg was administered to three women and the other three each received 5 mg of the oestrogen. Serum samples were obtained before treatment and 2, 4, 6 and 8 h after the injection. At the same time intervals blood samples were taken from a control group consisting of three postmenopausal women who received no treatment. The results were expressed as the percentage change from the initial level of the serum gonadotrophins.

The administration of 17β-oestradiol benzoate resulted in a decrease in the serum levels of LH in all six women (Fig. 4). The effect on the serum levels of FSH was also to produce a decrease but this was less pronounced.

Treatment with 17β-oestradiol

The effect on the serum concentrations of LH in three postmenopausal women after an intravenous injection of 80, 200 or 1000 µg 17β-oestradiol is shown in Fig. 5. Serum samples were obtained at frequent intervals for about three hours. During the first 60–90 min there was a gradual decrease in the serum concentrations of LH in the three women. The decreased LH level was sustained in two women during the remainder of the observation period. In the third woman the LH in serum returned to the initial level after 180 min. The six women with oligomenorrhoea or secondary amenorrhoea were given 5, 10, 50 or 100 µg 17β-oestradiol intravenously. In order to study the acute effect of the administered oestrogen, most of the blood samples were drawn
Effects of 17β-oestradiol benzoate on the serum gonadotrophin levels in six postmenopausal women. A control group of three postmenopausal women not treated is shown in the upper curves. One treated group consisting of three women received 1 mg (middle curves) and a second treated group 5 mg 17β-oestradiol benzoate intramuscularly (lower curves). Each point represents the mean percentage change from the initial level and the vertical lines the total range of the results.

during the first eight hours after administration. After this only daily samples were taken for a few days. The effect on the serum concentrations of the gonadotrophins after this intravenous administration of 17β-oestradiol is shown in Figs. 6 and 7. The serum LH decreased in all the cases except for case ML who was given the lowest dose of 17β-oestradiol, namely 5 µg. This decrease in the LH level started 1-2 h after the injection. In four of the six subjects the LH in the serum returned to the initial level after 24 h. A decrease in FSH in the serum was also found 1-2 h after the injection but only in three of the women. This decrease was maintained during less than 24 h. The FSH level after 24 h was above the initial level in four of the six subjects.

Treatment with ethinyloestradiol

Seven men received ethinyloestradiol orally each day for seven days. A group of three men were given 50 µg daily and the other four men received 100 µg daily. Venous blood samples were taken every second day during ten days. The same time schedule for blood sampling was used in three men who received no oestrogen treatment. The results were expressed as the percentage change from the initial level.
Effect on the serum concentration of LH in three postmenopausal women after intravenous administration of 80–1000 µg 17β-oestradiol.

Effects on the serum concentrations of LH and FSH after intravenous administration of 5–10 µg 17β-oestradiol to three women with amenorrhoea or oligomenorrhoea.
Effects on the serum concentrations of LH and FSH after intravenous administration of 50–100 µg 17β-oestradiol to three women with amenorrhoea or oligomenorrhoea.

This oestrogen treatment did not induce any major changes in the serum levels of the gonadotrophins (Fig. 8). There was, however, a similar pattern with a lowering in the LH level in both groups and in the FSH level in the group treated with 100 µg. This decrease during treatment was followed by a rise above the initial level four days after treatment. In the 50 µg treated group the effect on the FSH in the serum was very variable. The percentage standard deviations for the concentrations of LH and FSH in the three men not treated with oestrogen ranged from 8 to 13 during the ten days studied.

**DISCUSSION**

For the present study it was essential to investigate the possible circadian variation in the serum gonadotrophin levels by means of this particular radioimmunoassay procedure. The diurnal study failed to show any significant circadian variation in either the LH or the FSH levels in the serum in hospitalized women before and after the menopause or in the men. This agrees with most other studies made in men (Franchimont 1966; Odell et al. 1967; Peterson et al. 1968; Burger et al. 1969). However, Faiman & Ryan (1967) and Saxena et al. (1969) reported higher morning levels of FSH in the serum or plasma of men. The same was also found by the latter investigators with LH levels in
Fig. 8.

Effects on the LH and FSH in the serum of seven men during and after oral treatment with ethinyloestradiol. A control group of three men were not treated (upper curves). Three men received 50 µg (middle curves) and four men were given 100 µg ethinyloestradiol (lower curves) daily for seven days. Each point represents the mean percentage change from the initial value and the vertical lines the total range of the results.

the plasma of both men and women. Higher morning levels of the gonadotrophins in the serum during the follicular phase in menstruating women were reported by Midgley & Jaffe (1968) while Thomas et al. (1970) found no circadian variation in the plasma levels of LH around the time of ovulation. Preliminary results of a diurnal study of 23 healthy women investigated during the follicular or the luteal phase in a normal ovulatory menstrual cycle show no significant circadian variation (Nillius & Wide, to be published). The reason for these conflicting results is not known. It is possible that these can be ex-
plained by differences in the treatment of samples, differences in the radio-
immunological techniques or in the particular antigens and antisera used by
the different investigators.

The lack of circadian variation in the LH or FSH levels in the serum found
in the present study has facilitated the performance of the short term studies
of acute affects on the serum gonadotrophin levels after the administration of
oestrogens. This absence of circadian variation also indicates that the concentra-
tion of LH or FSH in a serum sample obtained at any time during the day
should be representative of the 24 h level of LH or FSH in the serum.

The intravenous or intramuscular administration of 17β-oestradiol in doses
from 5 µg to 5 mg resulted in a decrease in the serum levels of LH in 14 of
the 15 women studied. The only subject who did not show any decrease was
the women given the lowest dose. The serum levels of FSH decreased in 10 of
12 women. This can be explained by a negative feedback effect on the hypo-
thalamic-pituitary system during the period of observation. In no case did
stimulation of either LH or FSH secretion occur.

This agrees with similar studies by Franchimont (1966) and Odell et al.
(1967, 1968) in postmenopausal women. The assumption by Funnel et al. (1951)
that oestrogens given to menopausal women stimulate the release of LH from
the pituitary gland could not be confirmed. The decrease was as a rule more
pronounced in the serum LH levels than in the serum FSH levels. This was
also found in the studies of Franchimont (1966) and Odell et al. (1968). The
observation that LH is more responsive than FSH to the inhibitory action of
oestrogens is supported by studies in the rat (Parlow 1964).

The initial half-time of disappearance of labelled LH or of endogenous LH
after surgical hypophysectomy has been reported to be between 21 and 69 min
(Kohler et al. 1968; Schalch et al. 1968; Yen et al. 1968). In the present study
the fall in the serum LH immediately after the intravenous injection of
oestradiol into the three postmenopausal women corresponds to a half-time
disappearance of between 120 and 180 min. It is therefore likely that the doses
of oestrogen administered did not fully block the release of LH from the
pituitary gland, the result being a diminished rate of production and/or secre-
tion of LH.

The studies in women before the menopause were restricted to young women
with amenorrhoea or oligomenorrhoea. These women lack the typical cyclical
variation in the serum gonadotrophin levels seen during the normal menstrual
cycle and should therefore be suitable for studies of this kind. Furthermore
intravenous oestrogen injections were suggested by Kupperman et al. (1958) as
treatment for women who do not ovulate. The oestrogen was assumed to release
LH and subsequently induce ovulation. This was supported by McArthur et al.
(1961) who found an increase in the biological LH activity in the urine after
intravenous administration of Premarin (conjugated oestrogens equine) in the
of the follicular phase of normally menstruating women. However, in the five patients in this study the intravenous administration of $17\beta$-oestradiol in doses from 10–100 $\mu$g brought about a decrease in the serum level of LH.

In a study on the interrelationship between oestradiol and LH in the plasma during the human menstrual cycle Corker et al. (1969) found that the peak in plasma oestradiol occurred on the day before the peak in plasma LH. This was recently confirmed by Dufau et al. (1970). Thus, there seems to be a relationship between oestradiol and LH at midcycle but it is not as yet known whether the trigger mechanism for the release of LH is the rise or the fall in blood oestradiol.

In a recent study Goding et al. (1969), using a radioimmunoassay for ovine LH, found a typical oestrous peak of LH secretion in anoestrous sheep after intramuscular or intravenous administration of $17\beta$-oestradiol. The latent period of the oestrogen stimulus was reported to be approximately nine hours. In the present investigation the blood samples were mainly drawn during the first eight hours after the oestrogen administration. Thus, a later rise cannot be excluded. In further investigations of this kind it seems essential to extend the period of observation with frequent blood sampling during the first 24–48 hours.

It is possible that the insignificant and variable changes in the serum levels of LH or FSH in the men depend on the oestrogen doses used. It may be that a daily dose of 50–100 $\mu$g ethinyl oestradiol is just on the borderline causing a significant effect on the serum gonadotrophin levels in men. The rise in the serum gonadotrophin levels observed in most men after discontinuation of the oestrogen treatment is interesting and might suggest a rebound effect. However, this has to be further studied. With much higher doses (1.5 mg ethinyl oestradiol or 15 mg diethylstilboestrol daily) a decrease in the serum gonadotrophin levels both during and after therapy has been reported (Odell et al. 1966, 1968; Peterson et al. 1968). After the administration of very high doses of oestrogen (30–90 mg diethylstilboestrol daily) to men with prostatic cancer, decreases in the plasma levels of LH were found by Alder et al. (1968).

The interrelationship between oestrogens and gonadotrophins needs further study with different doses of various natural oestrogens, proper timing of the oestrogen administration and careful selection of suitable subjects. The limited number of volunteers for studies of this kind and the great individual variation makes it difficult to get enough subjects in certain well-defined endocrine situations to submit the results to statistical treatment. The conclusions are therefore drawn from studies on a few subjects and the results as a rule will be unsuitable for statistical analysis. However, when the results from different investigators are combined they may lead to a better knowledge of the hypothalamo-hypophyseal-ovarian interrelationship.
ACKNOWLEDGMENTS

This study was supported by the Swedish Medical Research Council. A Ford Foundation postdoctoral fellowship was gratefully received by one of us (S. J. N.).

We are indebted to Dr. Paul Roos at the Institute of Biochemistry, Uppsala, for supplying the highly purified LH and FSH preparations.

REFERENCES


Parlow A. F.: Endocrinology 75 (1964) 1.


Rothchild I.: Endocrinology 50 (1952) 583.


Received on March 6th, 1970.

594