MODULATION OF PITUITARY RESPONSES TO SYNTHETIC LH-RH BY GONADAL STEROIDS IN WOMEN WITH SECONDARY AMENORRHOEA

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ABSTRACT

LH-RH stimulation tests were performed on two successive days in 25 women with secondary amenorrhea of probable hypothalamic origin by intravenous injection of 150 µg of synthetic LH-RH. The patients were selected by exclusion of definite pituitary or ovarian disease and in the absence of clinical or laboratory evidence of androgen excess. Five women received successive LH-RH stimulation tests only without administration of steroid hormone. Twenty women were treated with gonadal steroids in addition to the double LH-RH stimulation test by intramuscular injection of the steroid 4 h after the first LH-RH injection. Of these 20, 9 received oestradiol (Oe₂), 5 received progesterone (P) and 6 received Oe₂ + P. Gonadotrophin responses to LH-RH in successive tests without steroid were not significantly different. Oe₂ or P alone each produced suppression of LH and FSH responses to LH-RH in the second test as compared to the first. Combined Oe₂ + P produced augmentation or suppression of the second gonadotrophin responses depending on the dose of Oe₂ administered. The results demonstrate a direct effect of oestrogen or progesterone alone and in combination on the pituitary responsiveness to LH-RH. The effect of the combined steroids can be modified by variations in the oestrogen:progesterone ratio.

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Gonadotrophin secretion in the normal human female is regulated by the positive and negative feedback effects of ovarian steroids (Vande Wiele et al. 1970; Franchimont 1971). Whether this effect is mediated at the hypothalamic or pituitary level, or both, remains ill-defined. We have previously shown (Thompson et al. 1973) in a small group of patients that both oestrogen and progesterone can exert a direct inhibitory effect on the response of the pituitary gland to luteinizing hormone releasing hormone (LH-RH) in women with secondary amenorrhoea. We have now extended our studies on the possible regulatory effect of sex steroids on the pituitary gland using different doses of oestrogen and progesterone and using combined oestrogen and progesterone in some patients. This report presents the results of these investigations.

**M A T E R I A L S A N D M E T H O D S**

*Patient selection*

A total of 25 patients with secondary amenorrhoea of presumed hypothalamic origin has been included in this study. The amenorrhoea was of 6 months minimum duration and studies performed prior to admission routinely included 24 h urine assay of 17-ketosteroids and 17-hydroxycorticoids, X-ray of the sella turcica, serum gonadotrophins, oestrogen, testosterone and tests of thyroid function. The patients were hospitalized for the LH-RH phase of the investigation and for further studies as indicated.

The diagnosis of hypothalamic amenorrhoea in the 25 patients was made primarily by the absence of clinical or laboratory evidence of androgen excess, by the exclusion of definite pituitary or ovarian disease, and by the association of the onset of amenorrhoea with factors such as emotional disturbances, discontinuation of oral contraceptives, use of tranquillizers and weight loss with dietary deficiency. Serum gonadotrophins, oestrogens and testosterone were all in the low or normal range in these patients in multiple samples. In the design of the study, each patient served as her own control in relation to the modulation of the pituitary response to LH-RH observed after administration of steroid.

**LH-RH stimulation**

Synthetic LH-RH used in this study was generously supplied by Dr. R. Guillemin. Each patient received an LH-RH stimulation test on two successive days by administration of 150 μg of synthetic LH-RH intravenously. During each LH-RH stimulation test, baseline gonadotrophin values were obtained at 15 min intervals for 1 h prior to injection of LH-RH, followed by 10 min sampling for 60 min and 15 min sampling for 2 h thereafter. Oestrogen (Oe₂) or progesterone (P) or Oe₂ + P when given, was administered by a single intramuscular injection 4 h after the initial LH-RH injection. Oe₂ was given as oestradiol benzoate (Progynon®, Schering) and progesterone as aqueous progesterone or progesterone in oil.

**Hormone assays**

Serum LH and FSH were measured by specific radioimmunoassay (RIA) as previously described in detail (Aono et al. 1967; Taymor et al. 1968). Results are expressed as equivalents of the Second International Reference Preparation of Human
Menopausal Gonadotrophin (2nd IRP-HMG) in milli-International Units (mIU) per ml of serum. One $\mu$g of LER-907 is equivalent to 210 mIU of 2nd IRP-HMG in the LH assay and to 40 mIU of 2nd IRP-HMG in the FSH assay. The within-assay coefficient of variation (CV) for six duplicate tubes in each assay was 5-10% and the between-assay CV was 5-9%. Serum oestrogens (Oe) and progesterone (P) were measured by RIA as previously described (Hotchkiss et al. 1971; Sanyal et al. 1974). All samples from each subject were measured in a single assay of the respective hormone.

Statistical analysis

The RIA data were analyzed with a computer programme prepared by Dr. E. Y. Lee as previously described (Sanyal et al. 1974). Gonadotrophin responses were calculated as the arithmetic mean of the gonadotrophin levels at 7 points from 20-90 min after LH-RH administration minus the arithmetic mean of the baseline level. Differences between the mean gonadotrophin responses before and after administration of steroid were assessed for significance by Student's $t$-test.

LH response to LH-RH before and after steroid administration. $E_2$ denotes oestradiol, P progesterone, $E_{25}$ and $E_{50}$ oestradiol at 25 $\mu$g/kg and 50 $\mu$g/kg body weight respectively. Vertical lines denote standard errors of the mean. The numbers in brackets indicate the number of individuals in each group.
RESULTS

LH-RH stimulation without steroid

Five patients receiving successive LH-RH tests without steroid showed increased gonadotrophin secretion in both tests (Figs. 1 and 2). The mean LH response to LH-RH in the first test (LH-R₁) was 42.3 ± 4.5 (se) mIU/ml. The mean LH response to LH-RH in the second test (LH-R₂) was 46.6 ± 5.1 (se) mIU/ml. This difference was not statistically significant. The mean FSH response in the first test (FSH-R₁) was 3.9 ± 0.8 (se) mIU/ml and the mean FSH response in the second test (FSH-R₂) was 4.2 ± 0.5 (se) mIU/ml. This difference was not significant. Serum Oe was between 100 to 150 pg/ml for all patients and remained unchanged in each test. Serum P was less than 0.6 ng/ml in all cases.

Fig. 2.
FSH response to LH-RH before and after steroid administration. E₂ denotes oestradiol, P progesterone, E₂₅ and E₅₀ oestradiol at 25 μg/kg and 50 μg/kg body weight respectively. Vertical lines denote standard errors of the mean. The numbers in brackets indicate the number of individuals in each group.

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LH-RH stimulation before and after Oe₂

Oe₂ was administered to 9 subjects in doses ranging from 5 to 50 μg/kg body weight. The increases in circulating oestrogen measured ranged from 100 pg/ml with the lowest dose to 1000 pg/ml with the highest dose.

In this group of subjects the mean LH-R₁ was 80.2 ± 10.4 (sₑ) mIU/ml and the mean LH-R₂ was 28.9 ± 4.6 (sₑ) mIU/ml (P < 0.001) (Fig. 1).

The mean FSH-R₁ was 9.0 ± 1.6 (sₑ) mIU/ml and the mean FSH-R₂ was 3.0 ± 0.5 (sₑ) mIU/ml (P < 0.01) (Fig. 2).

A suppressive effect on pituitary responsiveness of administered Oe₂ occurred throughout the range of increased circulating levels of Oe. However, it was not possible to correlate the magnitude of the increase in circulating Oe with the degree of suppression of the second LH and FSH responses.

LH-RH stimulation before and after P

Aqueous progesterone was administered to 5 women in doses ranging from 3 mg to 0.2 mg/kg body weight. Administration of 3 mg/kg produced a circulating level of P of approximately 20 ng/ml 20 h later, while 0.2 mg/kg produced a level of 1.6 ng/ml 20 h later. Each subject in this group showed suppression of the second gonadotrophin response after P. The mean LH-R₁ was 188.4 ± 23.8 (sₑ) mIU/ml and the mean LH-R₂ was 106.1 ± 13.7 (sₑ) mIU/ml (P < 0.01) (Fig. 1). The mean FSH-R₁ was 26.2 ± 4.5 (sₑ) mIU/ml and the mean FSH-R₂ was 13.3 ± 1.5 (sₑ) mIU/ml (P < 0.02). Serum Oe was between 100 and 150 pg/ml for all patients in both tests.

LH-RH stimulation before and after Oe₂ + P

Oe₂ + P was administered to 6 women. Three were given Oe₂ at a dose of 25 μg/kg body weight with progesterone in oil as 1 mg/kg. Oe levels increased in these patients to 400–500 pg/ml at 20 h and P levels rose to between 30–40 ng/ml. Suppression of the second LH and FSH responses to LH-RH after administration of the combined steroids was seen in each patient in this group. The mean LH-R₁ was 80.2 ± 9.8 (sₑ) mIU/ml and the mean LH-R₂ was 58.2 ± 6.4 (sₑ) mIU/ml (Fig. 1). The mean FSH-R₁ was 18.0 ± 4.4 (sₑ) mIU/ml and the mean FSH-R₂ was 9.0 ± 1.5 (sₑ) mIU/ml (Fig. 2). Although a suppressive effect between the first and second gonadotrophin responses in this group was observed, the differences did not reach statistical significance (P = 0.06).

Three other subjects were given Oe₂ at a dose of 50 μg/kg, each with a progesterone dose of 1 mg/kg. Oe levels in these patients increased to 800–1000 pg/ml at 20 h and P levels rose to 30–40 ng/ml.

Augmentation of the second gonadotrophin responses was seen in each patient in this group. The mean LH-R₁ was 53.2 ± 7.1 (sₑ) mIU/ml and the
mean LH-R2 was 103.1 ± 9.0 (SEM) mIU/ml (P < 0.001) (Fig. 1). The mean FSH-R1 was 4.1 ± 0.7 (SEM) mIU/ml and the mean FSH-R2 was 12.8 ± 1.7 (SEM) mIU/ml (P < 0.001) (Fig. 2).

**DISCUSSION**

This study was designed to further investigate the role of sex steroids in the regulation of hypothalamic-pituitary secretory function in the human. Women with secondary amenorrhoea of probable hypothalamic origin and demonstrated lack of androgen excess were selected for study, since the hypothalamic pituitary ovarian axis is in a relatively steady state in this group lacking reproductive cyclicity. It is recognized that even with these criteria for selection, these patients constitute a somewhat heterogeneous group with different aetiologies, but presumably they follow a final common pathway of impaired synthesis or release of endogenous LH-RH. Variations in responses to LH-RH have previously been described within this clinical diagnostic category and are again observed in this study. However, it is demonstrated that patients treated without steroid show identical responses on successive days. It is therefore believed that the initial response to LH-RH constitutes a satisfactory control value for each respective patient.

In the rodent, oestradiol has been found to augment LH release induced by LH-RH (Arimura & Schally 1971), while P suppressed LH release induced by small doses of LH-RH (Arimura & Schally 1970) but did not affect LH release after higher doses of LH-RH (Debeljuk et al. 1972). A suppressive effect of combined oestradiol and P on LH-RH induced LH release has also been demonstrated in intact dioestrous rats and anoestrous ewes (Debeljuk et al. 1972).

We have previously demonstrated (Thompson et al. 1973) in an initial study that Oe2 and P can exert a direct suppressive effect on the pituitary response to exogenous LH-RH. Keye & Jaffe (1974) later demonstrated a dose dependent suppressive effect of oestradiol on the pituitary response to LH-RH in normal women tested on Day 2 of the menstrual cycle. Yen et al. (1974) have also demonstrated a direct inhibitory action of oestradiol on the pituitary responsiveness to LH-RH.

The present study demonstrates a suppressive effect of both oestrogen and progesterone on the pituitary LH and FSH responses to LH-RH in women with hypothalamic amenorrhoea. This effect seemed to be present at circulating blood levels of 400–1000 pg/ml of Oe and 1.6–20 ng/ml of P.

It has been postulated that in the normal human menstrual cycle, the pre-ovulatory rise of oestrogen increases the sensitivity of the pituitary to LH-RH (Yen et al. 1972). In addition, Malacara et al. (1972) have demonstrated an
increase in bioassayable circulating LH-RH at the time of the mid-cycle. Arimura et al. (1974) have demonstrated an increase in circulating immuno-reactive LH-RH at the time of the mid-cycle. Their results suggested that LH-RH secretion reaches its peak level at approximately the same time as the LH peak.

The results obtained in this study and by those of Keye & Jaffe (1974) and Yen et al. (1974) indicate that increased oestradiol may also inhibit the release of LH by the pituitary in response to LH-RH. Hence, it remains possible that the initial effect of increased oestrogen is to promote increased storage of LH in the pituitary gland. The hypothalamic effect of increased LH-RH may then occur because of rising oestrogen levels or because of lack of a shortloop feedback effect of LH on the hypothalamus. Falling levels of oestrogen after the pre-ovulatory peak may then permit maximal pituitary LH response to the elevated LH-RH.

The role of progesterone in the regulation of pituitary secretory activity in the human also remains enigmatic. The present study demonstrates a possible inhibitory effect in the presence of low or constant oestrogen and a possible stimulatory effect in the presence of increased or rising oestrogen. Leyendecker et al. (1972) have previously shown an apparent stimulatory and inhibitory effect of P on LH release in a limited number of experiments. The small pre-ovulatory rise in circulating progesterone (Yussman & Taymor 1970; Abraham et al. 1972) may therefore also play a vital role in the maintenance of the LH surge by increasing the pituitary responsiveness to LH-RH at this time.

The effect of combined Oe_2 + P appears to be variable depending on the relative doses of Oe_2 and P. Those patients receiving the lower dose of Oe_2 showed suppressed gonadotrophin responses to LH-RH while those receiving the higher dose of Oe_2 had augmentation of the responses to LH-RH after administration of combined steroids. These results again indicate that pituitary responsiveness to LH-RH in the human may be altered by variation not only in circulating oestrogen, but also by variations in progesterone and the oestrogen:progesterone ratio. In addition, the temporal relationships between changes in the concentrations of each of these hormones and variations in pituitary responsiveness to LH-RH require further clarification.

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